INSTITUT INTERNATIONAL DE CHIMIE SOLVAY

SEPTIÈME CONSEIL DE CHIMIE

tenu à l'Université de Bruxelles, du 22 au 27 septembre 1947.

LES ISOTOPES

RAPPORTS ET DISCUSSIONS

Publiés par les Secrétaires du Conseil
sous les auspices de la Commission scientifique de l'Institut.

R. STOOPS
Editeur
76-78, COUDENBERG, BRUXELLES

—

1948

INSTITUT INTERNATIONAL DE CHIMIE SOLVAY

SEPTIÈME CONSEIL DE CHIMIE

tenu à l'Université de Bruxelles, du 22 au 27 septembre 1947.

RAPPORTS ET DISCUSSIONS

SUR

LES ISOTOPES

Publiés par MM. les Secrétaires du Conseil
sous les auspices de la Commission scientifique de l'Institut.

R. STOOPS
Editeur
76-78, COUDENBERG, BRUXELLES

—

1948

INTRODUCTION

Institut international de Chimie Solvay

EXTRAIT DES STATUTS.

Article premier. — Il a été fondé, à Bruxelles, à l'initiative de M. Ernest SOLVAY et pour une période de trente années, à partir du 1er mai 1913, un *Institut international de Chimie*.

La durée avait été prorogée jusqu'en 1949. Après le décès de M. Ernest Solvay, survenu le 26 mai 1922, Mme Ernest Solvay et ses Enfants ont désiré assurer l'avenir de l'Institut pour un temps plus long que celui qui avait été prévu. Dans ce but, une convention a été conclue entre les prénommés et l'Université de Bruxelles; en vertu de cette convention, l'avoir actuel de l'Institut est remis à l'Université en même temps que la somme nécessaire pour qu'à l'échéance prévue de 1949 le capital d'un million primitivement consacré par M. Ernest Solvay à l'Institut international de Chimie se trouve reconstitué.

L'Université assumera la gestion de cette somme en se conformant à toutes les dispositions des présents statuts.

Art. 2. — Le but de l'Institut est d'encourager des recherches qui soient de nature à étendre et surtout à approfondir la connaissance des phénomènes naturels à laquelle M. Ernest Solvay n'a cessé de s'intéresser.

L'Institut a principalement en vue les progrès de la Chimie, sans exclure cependant les problèmes appartenant à d'autres branches des sciences naturelles, pour autant, bien entendu, que ces problèmes se rattachent à la Chimie.

Art. 3. — L'Institut international de Chimie a son siège social à l'Université libre de Bruxelles, qui met à la disposition de l'Institut les locaux nécessaires à la tenue des *Conseils de Chimie*.

Art. 4. — L'Institut est régi par une *Commission administrative* comprenant *cinq* membres, belges de préférence, et par un *Comité scientifique* international comprenant *huit* membres ordinaires

auxquels peut être ajouté un membre extraordinaire ayant les mêmes droits qu'un membre ordinaire.

. . . . . .

Art. 9. — Le Fondateur a manifesté le désir qu'avant tout l'Institut fasse preuve dans tous ses actes d'une parfaite impartialité; qu'il encourage les recherches entreprises dans un véritable esprit scientifique, et d'autant plus que, à valeur égale, ces recherches auront un caractère plus objectif. Il lui a semblé désirable que cette tendance se reflétât dans la composition du *Comité scientifique*. Par conséquent, s'il y avait des savants qui, sans occuper une haute position officielle, pourraient être considérés en raison de leur talent comme de dignes représentants de la Science, ils ne devront pas être oubliés par ceux qui désigneront les candidats aux places vacantes.

COMPOSITION DE LA COMMISSION ADMINISTRATIVE

(En date du 27 septembre 1947)

M. J. BORDET, Professeur honoraire et membre du Conseil d'administration de l'Université Libre de Bruxelles, *Président*.

M. P. ERCULISSE, Professeur à l'Université Libre de Bruxelles.

M. P. HEGER-GILBERT, Professeur et administrateur de l'Université Libre de Bruxelles.

M. E.-J. SOLVAY, Gérant à la Société Solvay et Cie, membre du Conseil d'administration de l'Université Libre de Bruxelles.

M. F.-H. van den DUNGEN, Professeur à l'Université Libre de Bruxelles, *Secrétaire-administrateur*.

7e CONSEIL DE CHIMIE
(22-27 septembre 1947)

LISTE DES PARTICIPANTS

A. Le Comité Scientifique.

MM. Paul KARRER, Directeur de l'Institut chimique de l'Université (Zurich), Président.
H.-J. BACKER, professeur à la Rijksuniversiteit (Groningue).
Niels BJERRUM, professeur au Kgl. Veterinaer og Landbohojs koles Kemiske Laboratorium (Kobenhavn).
Marcel DELEPINE, professeur au Collège de France (Paris).
C. N. HINSHELWOOD, professeur à Oxford.
P. PASCAL, professeur de Chimie générale à la Sorbonne (Paris).
Sir Robert ROBINSON, professeur à Oxford.
J. TIMMERMANS, professeur à l'Université Libre (Bruxelles), secrétaire.

Note. — MM. Delépine, Hinshelwood et Sir Robert Robinson n'ont pu prendre part au 7e Conseil.

B. Les Membres rapporteurs.

MM. F. JOLIOT-CURIE, haut commissaire à l'Energie atomique (Paris).
M. de HEMPTINNE, professeur à l'Université de Louvain.
Ch. INGOLD, professeur à University College (London).
F. A. PANETH, professeur à l'Université de Durham.
A. LANGSETH, professeur à l'Université de Copenhague.
D. RITTENBERG, professeur à Columbia University (New-York).
M. CALVIN, professeur à l'Université de California (Berkeley).
G. de HEVESY, (Stockholm).
K.-T. BAINBRIDGE, professeur à Harvard University (Massachussets).

C. Les Membres invités.

MM. E. HENRIOT, professeur à l'Université Libre de Bruxelles.
G. GUEBEN, professeur à l'Université de Liège.
J. GOVAERT, assistant à l'Université de Liège.
A. BERTHELOT, titulaire de la Chaire Francqui à l'Université de Gand.
R. COURRIER, professeur au Collège de France (Paris).
B. BRINKMAN, professeur à l'Université de Groningue.
E. BRINER, professeur à l'Ecole de Chimie (Genève).
A. H. V. ATEN, attaché à l'Université d'Amsterdam.
M. COSYNS, maître de conférences à l'Université de Bruxelles.

D. Les Membres Secrétaires.

MM. J. TIMMERMANS, professeur à la Faculté des Sciences.
E.-J. BIGWOOD, professeur à la Faculté de Médecine.
L. de BROUCKÈRE, professeur à la Faculté des Sciences.
R.-H. MARTIN, chargé de cours à la Faculté des Sciences.
JOUKOWSKI, chef de travaux à la Faculté de Médecine.
Mlle A. LACOURT, agrégé à la Faculté des Sciences.

E. Les Membres auditeurs, professeurs de l'Université Libre de Bruxelles.

MM. W. DE KEYSER, professeur à la Faculté des Sciences Appliquées.
R. DESCAMPS, chargé de cours à la Faculté des Sciences.
P. ERCULISSE, professeur à la Faculté des Sciences.
L. FLAMACHE, Maître de Conférences à la Faculté des Sciences appliquées.
A. JULIARD, chargé de cours à la Faculté des Sciences.
I. PRIGOGINE, chargé de cours à la Faculté des Sciences.

Allocution prononcée par le professeur P. Karrer, Président, en ouvrant le Conseil le 22 septembre 1947.

Mesdames, Messieurs,

Pour la première fois depuis la guerre et pour la première fois depuis 1937 le Conseil de Chimie Solvay se réunit aujourd'hui à une session à Bruxelles; c'est le septième Conseil depuis la fondation de l'Institut International de Chimie Solvay.

Je suis convaincu de parler au nom de tous les participants, si j'exprime tout d'abord ma profonde joie de voir aujourd'hui après les longues années douloureuses de la guerre votre pays hospitalier, la Belgique, de nouveau libre.

Que le Royaume puisse maintenant bénéficier d'une longue et heureuse paix. Nous sommes saisis d'admiration en constatant avec quelle énergie et quel labeur le peuple de ce pays s'est mis à réparer les graves dégâts et à surmonter les pertes que la guerre lui a causés. Le succès en est étonnant. La vie intellectuelle et le commerce ont repris leur marche, partout règne la confiance, et l'aspect extérieur des villes et des villages ne se différencie déjà plus beaucoup de celui d'avant-guerre. De tous les pays d'Europe qui durent prendre part au conflit, la Belgique s'est remis le plus vite de ses souffrances. Nous en félicitons ce pays et formons nos meilleurs vœux de prospérité pour son avenir.

C'est au prompt redressement de l'économie politique belge que nous devons la séance d'aujourd'hui du Conseil de Chimie Solvay. Le Congrès de Chimie Solvay est devenu une institution qui jouit d'un haut prestige chez les chimistes du monde entier et dont on ne se passerait qu'avec grand regret. Il a, depuis son existence, non seulement contribué à l'approfondissement des recherches scientifiques, mais réuni aussi les collègues du monde entier à des rencontres personnelles et pris part ainsi à l'affermissement de la solidarité internationale. J'espère que la session qui débute aujourd'hui se déroulera harmonieusement comme toutes les autres.

Malheureusement plusieurs collègues du Comité scientifique de l'Institut International de Chimie Solvay, qui faisaient partie du Comité en 1937, ne sont aujourd'hui plus parmi nous. Nous déplorons en premier lieu la perte de notre vénérable et cher ancien président, le professeur Swarts, décédé pendant la guerre. Plein d'enthousiasme, il présidait les séances à la fois avec énergie et courtoisie. Il a su réunir en une seule famille tous les collègues qui participaient au Conseil de Chimie Solvay, et a su veiller à ce que celui-ci se déroule d'une manière profitable à tous.

De même plusieurs autres collègues nous ont été enlevés par la mort; je déplore en particulier le décès du professeur Sir William Pope, le premier président du Comité scientifique, le décès du professeur Max Bodenstein et du professeur Jaeger de Groningue, qui a fait partie du Comité pendant de longues années.

Deux de nos collègues à l'Institut International de Chimie Solvay nous ont été enlevés par la mort. Ils participèrent aux travaux de tous les Conseils de Chimie Solvay et contribuèrent beaucoup à la réussite de ces congrès. Je pense à nos vénérés Collègues les professeurs G. Chavanne et A. Pinkus. Monsieur Chavanne fit partie de la Commission Administrative dès sa fondation et connaissait par conséquent à fond tous les travaux et les besoins de l'Institut International de Chimie Solvay. Monsieur Pinkus exerça les fonctions de secrétaire adjoint à plusieurs congrès. Il fut déporté en Pologne pendant la guerre et y mourut des suites d'une infection. Enfin, nous avons perdu également Monsieur Ch. Lefébure, secrétaire de la Commission administrative de l'Institut, qui, pendant tant d'années a rempli ses délicates fonctions avec un zèle et un tact qui ne se sont jamais démentis.

Je vous prie de vous lever pour honorer la mémoire de nos Collègues.

Comme successeurs de Messieurs Swarts et Bodenstein, le Professeur Sir Robert Robinson et le Professeur H.-J. Backer ont été nommés nouveaux membres de notre Comité; je souhaite la bienvenue à nos deux collègues en espérant qu'ils trouveront dans le travail du Comité la même satisfaction que leurs prédécesseurs.

Le Comité Scientifique m'avait demandé d'en prendre la présidence. Je le remercie de la confiance qu'il me témoigne par ce geste. Mais c'est avec grande hésitation que je me plie à cette décision, car je sais très bien que je ne dispose pas d'une qualité

qui, ici, est essentielle, la connaissance parfaite des deux langues officielles du Congrès. Je dois donc, à cet égard, faire appel à votre indulgence et j'espère que vous trouverez plus tard un président auquel ces exigences ne causent aucune difficulté.

En se retirant de ses fonctions, notre ancien secrétaire, notre vénérable collègue, le professeur H. Wuyts a causé un autre changement important au sein du Comité. Monsieur Wuyts s'est occupé pendant bien des années en parfaite connaissance de cause et avec le plus grand soin des affaires du Comité Scientifique et s'est acquis par son travail le plus haut mérite de l'Institut International de Chimie Solvay. C'est principalement à lui que nous devons l'organisation parfaite de tous les Congrès antérieurs de leur succès. Nous remercions notre collègue, Monsieur Wuyts, pour tout ce qu'il a fait et espérons aussi pouvoir nous adresser à lui par la suite, quand nous aurons besoin d'un conseil.

Comme successeur de Monsieur Wuyts, a été élu secrétaire, notre collègue Timmermans, qui s'était déjà familiarisé, en temps que secrétaire adjoint, avec son travail. C'est Monsieur Timmermans qui a déjà organisé la session d'aujourd'hui. Nous le remercions vivement pour tout ce qu'il a fait et qu'il fait à présent pour le Congrès.

Ainsi je termine en espérant que le septième Conseil de Chimie Solvay se déroulera de la manière la plus satisfaisante. Les intéressantes conférences, qui sont au programme, en donnent la garantie. Je souhaite la bienvenue à Messieurs les conférenciers qui ont bien voulu se rendre ici, je les remercie et leur certifie que nous suivrons leurs exposés avec le plus vif intérêt et la plus grande attention.

P. KARRER.

ACTIVITÉS DU SEPTIEME CONSEIL.

Le septième Conseil de Chimie s'est réuni à Bruxelles dans les locaux de l'Université du 22 au 27 septembre 1947, sur l'invitation de la Commission administrative de l'Institut International de Chimie Solvay.

Le Comité Scientifique de l'Institut qui avait pris l'initiative de convoquer ce Conseil s'est réuni les 22 et 27 septembre 1947.

Les rapports ont été présentés au cours des séances qui ont été tenues le 22 (après-midi), les 23 et 24 (matin et après-midi), le 25 (matin), le 26 (matin et après-midi), le 27 (matin).

Les membres du Conseil ont été reçus par le Conseil d'administration de l'Université le lundi 22 à midi. Le mardi 23 à 16 h. 30, le personnel scientifique des services de chimie de l'Université a été invité à prendre le thé avec les participants. Enfin, la famille Solvay et la Commission administrative ont offert un banquet le 26 septembre à 19 h. 30.

RAPPORTS
ET
DISCUSSIONS

Modes de formation, constitutions et filiations des isotopes, notamment des isotopes artificiels

Rapport de M. F. JOLIOT

INTRODUCTION

Les nombreuses études de la composition isotopique des atomes des éléments chimiques contenus dans l'écorce terrestre ont montré que cette composition, à de rares exceptions près (1), est constante dans des limites très voisines pour chaque élément quelle que soit l'origine de celui-ci et la combinaison moléculaire d'où il est extrait.

Le développement considérable de la physique atomique et nucléaire depuis les découvertes de la radioactivité et des radio-éléments il y a un demi-siècle, et l'élaboration des techniques concernant la séparation des noyaux isotopes, a permis la préparation d'éléments chimiques dont la composition isotopique est différente de celle de l'élément chimique naturel. La distinction par des méthodes physiques de l'élément chimique artificiel, du naturel, fut rendue possible, en dépit de l'identité rigoureuse de leurs propriétés chimiques et de la plupart de leurs propriétés physico-chimiques. Un immense champ d'applications du plus haut intérêt scientifique s'est ainsi ouvert aux chercheurs.

(1) Les plombs d'origine radioactive contiennent des proportions d'isotopes 206, 207, 208, variables et très différentes de celle du plomb extrait de la galène par exemple (isotope 206 en proportion très importante dans Pb extrait de la pechblende). Un autre exemple est fourni par le Sr extrait de certaines biotites contenant du rubidium : le mélange isotopique du strontium contient en proportion notablement plus élevée l'isotope 87 Sr en raison de l'accumulation de celui-ci provenant de la désintégration au cours des temps du 87 Rb. (O. Hahn a vérifié ce fait.)

Je voudrais seulement, dans ce rapport, tenter de dégager, des domaines déjà si vastes de la physique et de la chimie nucléaire, les faits principaux qui ont rendu possibles ces applications et leur prodigieux développement.

Ce fut d'abord, lorsque l'on reconnut que certains radioéléments naturels dérivés de la famille de l'uranium ou du thorium sont isotopes d'éléments chimiques ordinaires : thallium, plomb, bismuth, que Hevesy et Paneth, il y a trente-quatre ans, proposèrent d'employer ces radioéléments comme indicateurs très sensibles dans l'étude quantitative des propriétés physiques et chimiques des éléments inactifs correspondants. Les propriétés chimiques d'un radioélément sont indépendantes de sa nature radioactive. L'atome radioactif isotope d'un atome inactif ne se distingue de celui-ci que lorsqu'il se désintègre en émettant un rayonnement. (Dans le cas de l'isomérie l'émission du rayonnement ne s'accompagne pas du changement de nature chimique.) On connaît les résultats très intéressants qui furent alors obtenus par de nombreux auteurs qui appliquèrent la méthode des indicateurs radioactifs. La grande sensibilité de la méthode est due à l'extrême sensibilité des méthodes de détection et de mesure des rayonnements émis par les radioéléments naturels employés.

Déjà des considérations pratiques interviennent pour le choix du radioélément naturel parmi ceux qui lui sont isotopes pour un élément chimique donné.

Le radioélément (sauf les cas d'expériences très particulières) devra avoir la période de désintégration la plus longue et émettre toutefois un rayonnement aisément mesurable (suffisamment pénétrant et s'il y a lieu facilement distinguable du rayonnement émis par son dérivé). Il faudra évidemment disposer de quantités suffisantes de substances mères pour extraire la quantité nécessaire du radioélément choisi. Par exemple, dans le cas du bismuth, le RaE de période cinq jours émettant des rayons pénétrants d'énergie maximum dépassant 1 mégaélectron volt et donnant naissance au polonium de relativement grande période émettant des rayons α très absorbables, est préférable aux autres radioéléments isotopes AcC, ThC, RaC, tous de périodes beaucoup plus brèves. Toutefois, si l'on dispose au laboratoire comme substance mère d'une solution d'un sel de radium assez récemment purifié dans laquelle on extrait périodiquement le radon (comme c'est fréquemment l'usage), la quantité de RaE que l'on pourra extraire sera très faible et inuti-

lisable pour la méthode des indicateurs. Dans ce cas, on aura avantage à séparer le RaC en dépit de sa période brève de 19,7 minutes. Toujours avec cet exemple, on conçoit l'avantage qu'il y a, pour se procurer le RaE en quantité suffisante, d'utiliser comme substance mère une quantité importante de RaD dont la période est vingt-deux ans. Le RaD peut s'extraire de tubes de radium scellés ayant accumulé ses dérivés depuis de nombreuses années. On peut aussi l'extraire des résidus de lavage des récipients dans lesquels se sont désintégrées de fortes quantités de radon (traitement d'importants lots d'aiguilles ayant contenu du radon utilisées dans les hôpitaux pour la curiethérapie).

Dans l'exemple précédent, l'élément étudié est indiqué par le rayonnement émis par le radioélément isotope (indicateur). Il peut arriver qu'il soit indiqué par le rayonnement de son dérivé qui est de nature chimique différente, ceci parce que le rayonnement de l'indicateur n'est pas décelable (exemple : Pb indicateur est isotope de RaD dont le dérivé est le RaE). Il faudra dans ce cas, pour doser le plomb par le radium D dans une phase, mesurer l'activité du RaE en fonction du temps et ceci pendant une durée fonction des périodes des deux radioéléments. Les formules classiques qui se trouvent dans les traités de radioactivité permettent de déduire de ces mesures la quantité de l'indicateur, ici RaD, dans la phase étudiée.

Des considérations du même genre sont évidemment applicables aux éléments chimiques dont tous les atomes sont radioactifs : radium, polonium, etc.

Si j'ai insisté sur ces exemples, sans doute bien connus, c'est que les considérations auxquelles ils ont donné lieu sont applicables aux radioéléments artificiels employés comme indicateurs.

Jusqu'en 1934, les applications de la méthode des indicateurs radioactifs se limita à l'emploi d'un petit nombre de radioéléments naturels choisis parmi la quarantaine de ceux qui existent dans l'écorce terrestre.

La constitution, la filiation et les caractéristiques radioactives de ces atomes sont bien connues et figurent dans de nombreuses tables et il ne me semble pas nécessaire d'y revenir ici.

D'une façon générale, la physique nucléaire subit un nouvel et rapide essor à partir des années 1930-1932. Ce furent d'abord les découvertes de particules nouvelles : le neutron, l'électron positif, celle des atomes de deuterium, et de processus nouveaux de trans-

mutation, des radioéléments artificiels, et enfin l'élaboration de techniques nouvelles, générateurs à haute tension et cyclotron, permettant de produire des faisceaux extrêmement intenses et de grande énergie de rayonnements transmutants.

De grands progrès furent en outre réalisés dans la séparation et le dosage des atomes rares stables, deuterium, carbone 13, azote 15, oxygène 17, chlore 35 et 37. Simultanément se précisait la théorie du noyau et des phénomènes nucléaires. Un grand nombre de ces résultats furent aussitôt l'objet d'applications dans d'autres domaines de la science, notamment en chimie et en biologie.

Parmi ces applications, celle de la méthode des indicateurs radioactifs étendue aux radioéléments artificiels isotopes d'éléments chimiques couvrant alors déjà presque toute la classification de Mendeleeff, fut de beaucoup la plus importante.

La découverte de la fission de l'uranium et du thorium en 1939, qui a conduit à la construction de dispositifs, piles à uranium, dans lesquels les bipartitions des noyaux d'uranium s'entretiennent d'elles-mêmes, a prodigieusement élargi le champ des applications et facilité celles-ci. En effet, de nombreux nouveaux radioéléments sont le résultat de la bipartition et les quantités d'atomes produites ainsi ou par les transmutations des substances soumises au rayonnement émis pendant le fonctionnement de la pile sont extrêmement importantes; elles peuvent être pondérables.

A l'heure actuelle, on sait faire la synthèse d'environ 450 radioéléments artificiels et il existe, au moins un isotope radioactif pour chaque élément chimique ayant un nombre atomique compris entre 1 et 96.

En outre, avec les cyclotrons puissants et surtout avec la pile à uranium, on peut préparer par réaction nucléaire des isotopes inactifs rares en quantité suffisante pour donner lieu à des applications (1).

Les méthodes de séparation des isotopes rares stables et leur dosage quantitatif ont aussi progressé pendant cette période et elles fournissent de puissants moyens d'investigation en physique nucléaire, chimie et biologie.

---

(1) Par exemple avec un cyclotron puissant, des deutérons accélérés irradiant une cible en or forme du radioor de nombre de masse 198 (l'or est composé d'une seule sorte d'atomes de nombre de masse 197). Le radioor se désintègre et forme les atomes de mercure stable de nombre de masse 198. Les quantités produites de mercure 198 pur furent suffisantes pour remplir un tube à décharge formant un étalon spectroscopique parfait.

CHAPITRE PREMIER.

CONSTITUTION, RÉPARTITION ET STABILITÉ DES NOYAUX ATOMIQUES.

Chaque noyau d'atome d'un élément chimique est caractérisé par un ensemble de grandeurs physiques, parmi lesquelles nous citerons le nombre de charge ou numéro atomique, le nombre de masse, la masse exacte, le rayon, le spin ou moment cinétique, les moments magnétique et quadripolaire (s'il y a lieu). Il faut ajouter enfin le genre de statistique (Bose-Einstein ou Fermi) à laquelle il obéit et indiquer s'il est normalement stable ou radioactif et le type de radioactivité). On connaît les méthodes physiques permettant de mesurer ces grandeurs, mais c'est seulement pour un relativement petit nombre de noyaux qu'on a pu les mesurer toutes — certaines d'entre elles avec une très grande précision (masse exacte). On a été amené à la conclusion que les noyaux sont composés de protons et de neutrons auxquels on a donné le nom de nucléons. Le nombre de masse du noyau est alors le nombre total de nucléons A et le nombre de charge est le nombre de protons Z qu'il contient.

On suppose qu'il n'existe pas de groupements particuliers de ces nucléons préformés comme par exemple des hélions composés de $2n + 2p$ plus fortement liés entre eux que les autres nucléons non groupés. Les difficultés d'interprétation de plusieurs résultats expérimentaux avec ce modèle de noyau sans groupement pourraient sans doute être levées en reprenant un modèle nucléaire comprenant des groupements comme par exemple des hélions, ceux-ci n'étant pas nécessairement préformés en nombre maximum.

A est encore l'entier le plus voisin de la masse exacte de l'atome en choisissant pour unité de masse (1) le $1/16^e$ de la masse exacte de l'atome neutre de l'isotope le plus abondant de l'oxygène naturel.

(1) Une unité de masse I U M vaut $1{,}660 \times 10^{-24}$ grammes et équivaut à la matérialisation totale d'une énergie de $1{,}493 \times 10^{-3}$ ergs ou 931 millions d'électron volts (931 Mev).

La référence choisie par les physiciens $^{16}_{8}O = 16{,}00000$ est différente de celle choisie par les chimistes. Par décision prise en 1940 par la Commission des poids atomiques de l'Union Internationale de Chimie la masse atomique du mélange isotopique des atomes d'oxygène 16, 17, 18 comme il se présente dans la nature, est posée rigoureusement égale à 16. La détermination des proportions relatives des trois isotopes et la détermination précise des masses exactes de chacun d'eux permet de faire la correspondance entre ces deux unités.

Voici à titre d'exemple les valeurs de certaines des grandeurs caractéristiques de l'atome de lithium $^{7}_{3}Li$:

$$A = 7\left(\begin{matrix}\text{Masse exacte}\\ \text{de l'atome}\end{matrix}\right) = 7.01819 \pm 0{,}00010 \text{ unités de masse}$$

$Z = 33$ Spin nucléaire $= \frac{3}{2}$ h.

Moment magnétique $\mu = +\,3{,}250 \pm 0.016$ magnétons nucléaires (1)

La masse d'un noyau est toujours inférieure à la somme des masses des nucléons (pris à l'état libre) qui le composent et la différence, qu'on appelle le défaut de masse, de l'ordre de quelques millièmes de la masse totale, est égale à l'annihilation de la masse qui serait due au dégagement d'énergie de la réaction fictive consistant à prendre les nucléons libres et à les réunir pour former le noyau considéré. Il en résulte que le défaut de masse que l'on exprime en unité de masse a pour valeur, avec cette unité, l'énergie de liaison des nucléons qui constituent le noyau. L'énergie de liaison des nucléons est au moins des millions de fois plus élevée que celle qui correspond aux liaisons des atomes dans les molécules.

Le problème de la physique de l'atome a été de trouver comment les électrons se meuvent et se distribuent sous l'action de forces dont on connaissait la nature (forces coulombiennes). Dans le cas de la physique nucléaire, on ignorait la nature des forces, l'on savait seulement que les forces principales s'exerçant entre les nucléons sont attractives. A ces forces attractives s'exerçant entre neutrons neutrons, neutrons protons et protons protons, se superposent les

---

(1) 1 magnéton nucléaire $\frac{e\,h}{2M_pc}$ vaut $5{,}02 \times 10^{-24}$ gauss/cm^3.

$h = \frac{h}{2\pi}$ e = charge de l'électron;

Mp = masse du proton; c = vitesse de la lumière.

Tables de J. Mattauch et S. Flügge, *Kernphysikalische Tabellen* (1942).

forces répulsives coulombiennes entre protons. Celles-ci, qui jouent en général un rôle de deuxième ordre, deviennent suffisamment importantes dans le cas des noyaux les plus lourds pour permettre leur fission sous certaines conditions. On a pu déduire de l'ensemble des expériences, la nature des forces attractives entre nucléons et la façon dont elles dépendent de la distance entre ceux-ci, de l'orientation des spins des nucléons et d'autres quantités encore. D'une façon générale comme en physique atomique, l'emploi de la mécanique quantique se justifiait. Le fait que l'énergie de liaison, déduite de la connaissance des masses exactes des noyaux, est approximativement proportionnelle au nombre total des nucléons contenus dans le noyau, suggéra que les forces nucléaires ont des propriétés de saturation analogues à celles que présentent les forces de liaison chimiques, en particulier les forces de liaison homopolaires. Elles ont le caractère des forces d'échanges; ici le proton échange ses coordonnées avec celles d'un neutron et réciproquement. Le parcours des forces nucléaires est de l'ordre de dimension des nucléons. Il résulte de la nature des forces que le volume du noyau doit être approximativement proportionnel au nombre des nucléons; et la densité de la matière nucléaire approximativement constante, de l'ordre de 10^{14} gr/cm^3. Le rayon R peut se calculer par $R = r_0 A^{1/3}$ avec $r_0 = 1{,}45 \times 10^{-13}$ cm (rayon nucléaire élémentaire).

On connaît actuellement 317 noyaux différents composant les 89 éléments chimiques identifiés avec certitude dans la nature.

Il semble bien qu'aucune expérience indubitable permette d'affirmer l'existence dans la nature des éléments :

$$Z = 43 \qquad 61 \qquad 85\ (1) \text{ et } > 92\ (2).$$

Sur ces 317 noyaux, 44 sont radioactifs : ${}^{40}_{19}K$ ${}^{87}_{37}Rb$ ${}^{148}_{62}Sm$ ${}^{176}_{71}Lu$, et 40 à partir de l'élément $Z = 81$ jusqu'à $Z = 92$.

Examinons la répartition de ces noyaux, en fixant notre attention sur A et Z de chaque noyau. Parmi les représentations possibles choisissons celle consistant à représenter un noyau existant dans la nature par un point en portant en abscisse A et ordonnée Z (fig. 1).

La valeur moyenne du nombre de protons en fonction du nombre de nucléons est représentée par la ligne moyenne passant au travers

---

(1) Karlin et Bernert, *Naturwiss.* **31**, 298, (1943) et **31**, 492, (1943).

(2) Hulubei et Cauchois, *Comptes rendus*, **224**, 1265 (1947).

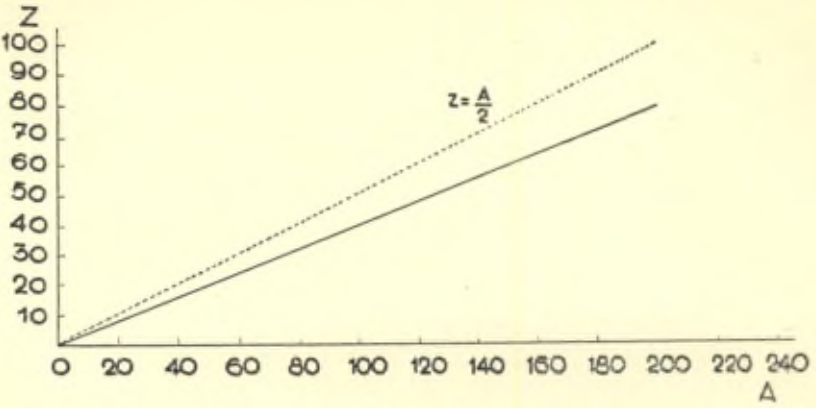


Fig. 1.

des points représentatifs. D'une valeur moyenne $Z = \frac{A}{2}$ pour les noyaux légers elle passe progressivement à des valeurs de plus en plus petites $Z = \frac{2}{5} A$ pour $A = 200$.

L'excès moyen de neutrons sur le nombre de protons, de nul, pour les noyaux légers, augmente à mesure que le noyau contient plus de nucléons. Il atteint déjà 40 au voisinage de $A = 200$. On représente souvent cet excès de neutrons $I = A - 2Z$ appelé nombre isotopique en fonction de A.

On constate une différence de répartition des noyaux suivant que A est pair ou impair. Ainsi pour un A pair il existe des noyaux ayant plusieurs valeurs de Z, mais celles-ci étant toujours paires (exceptions $A = 2, 6, 10, 14$). Pour A impair il n'existe qu'une seule valeur de Z avec une égale probabilité que Z soit pair ou impair (exceptions $A = 113, 115, 123, 187$, des noyaux ayant deux valeurs consécutives de Z existant).

On observe en outre (1) pour les noyaux stables que toutes les valeurs $A - Z$ (nombre de neutrons) sont représentées entre 1 et 126 à l'exception de 9 valeurs: 19, 21, 35, 39, 45, 61, 89, 115, 123.

On n'a pas identifié les noyaux stables $Z = 43, 61, 85$.

Ces observations furent extrêmement utiles pour dégager des conclusions concernant les forces nucléaires.

On peut comprendre très qualitativement pourquoi le nombre de noyaux que nous observons dans la nature ou que nous pourrions

(1) F. L. Breusch, *Experientia*, vol. II 9 (1946).

construire en imaginant de faire réagir à volonté des protons et des neutrons, est limité.

S'il y avait trop de protons, les forces de répulsion électrostatique s'exerçant à grande distance disloqueraient le noyau; s'il y avait trop de neutrons, le principe d'exclusion de Pauli, qui dit que l'on ne peut sur une même orbite avoir deux particules identiques dans le même état, forcera une partie des nucléons à se placer sur des orbites d'énergie si élevée que les forces nucléaires attractives et de court parcours ne seront plus suffisantes pour maintenir les nucléons en excès dans le noyau.

Une théorie générale des noyaux permettrait en particulier de calculer la masse exacte de chaque noyau dont on donne A et Z, et d'obtenir ainsi des renseignements précieux sur la stabilité des noyaux vis-à-vis de diverses transformations. Diverses tentatives furent faites, en particulier celle de Hartree (1) qui fait appel à un modèle statistique du noyau où tous les nucléons se meuvent indépendamment les uns des autres; ces tentatives furent insuffisantes.

Weizsäcker (2) a traité semi-empiriquement le problème en introduisant dans l'expression de la masse exacte les formes théoriques de chaque effet intervenant dans l'énergie de liaison totale ainsi que des coefficients empiriques à déterminer numériquement à l'aide des données les plus précises des masses de noyaux connus.

Weizsäcker et Bethe et Bacher (3) donnent l'expression suivante à la masse exacte d'un atome A, Z :

$$(1)\quad Mex = NMn + ZMp \underbrace{- \alpha A}_{1} \underbrace{+ \beta \frac{(N-Z)^2}{A}}_{2} \underbrace{+ \gamma A^{2/3}}_{3} \underbrace{+ \frac{3}{5}\frac{e^2}{r_0 A^{1/3}} Z^2}_{4}$$

N = nombre de neutrons;
e = charge de l'électron;
r_0 = rayon nucléaire élémentaire;
Mn et Mp = masse exacte du neutron et de l'atome d'hydrogène;
1 = énergie moyenne de liaison d'un nucléon;
2 = diminution de l'énergie de liaison due à l'excès de neutrons sur le nombre de protons;
3 = diminution d'énergie de liaison due aux nucléons à la surface (non saturation des forces);
4 = diminution de l'énergie de liaison due à la répulsion électrostatique.

(1) Hartree, *Proc. Camb. Phil. Soc.*, **24**, 89 (1928); — Heisenberg, *Rapport VII^e Conseil Physique Solvay* (1934).

(2) Weizsäcker, *Zeit, f. Phys.*, **96**, 431 (1935).

(3) Bethe et Bacher, *Review of Modern Physics* **8**, 165 (1936).

Cette expression permet d'interpréter qualitativement la courbe (fig. 2) représentant le facteur de condensation (packing fraction) en fonction de A. Le minimum, en particulier, est dû aux variations relatives de l'importance des effets de surface et de répulsion électrostatique sur la diminution de l'énergie de liaison en fonction de A.

Appliquée brutalement, cette expression indique pour chaque valeur donnée de A une variation parabolique de Mexacte (Mex) en fonction de Z (fig. 3). Le noyau stable est celui dont la valeur de Z (nombre entier) est la plus voisine de celle de la valeur de $Z = Z_A$ (non nécessairement entière) qui rend minimum la valeur de Mex.

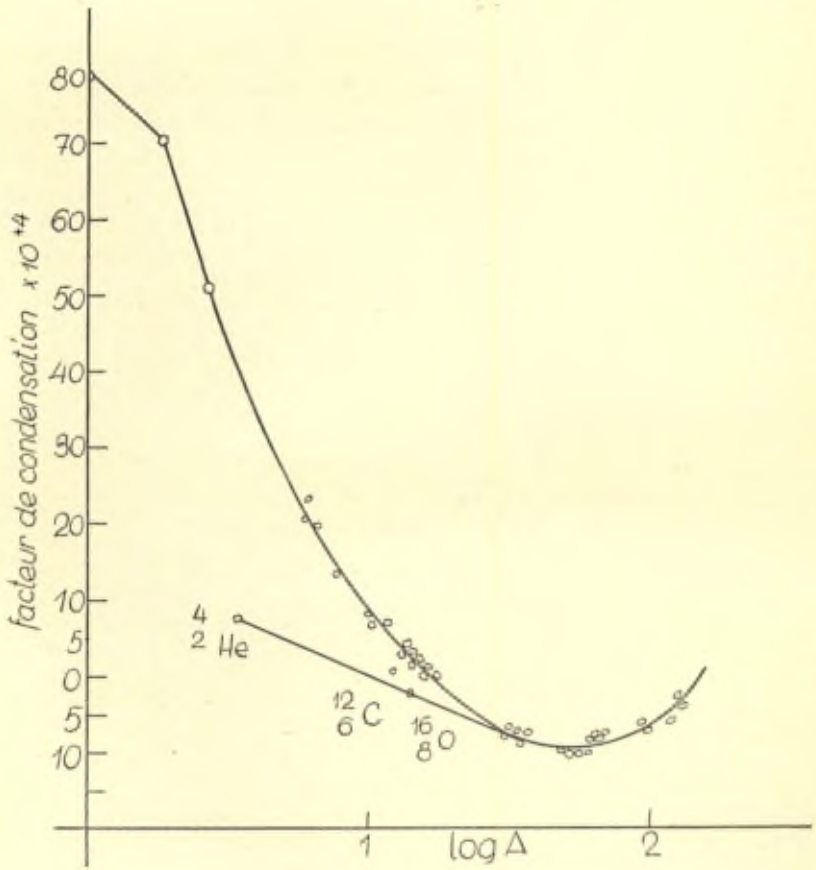


Fig. 2.

Seuls les cas où il existe deux valeurs de Z (nécessairement de part et d'autre de Z_A et d'écart entre elles, forcément d'une unité) pour lesquelles les masses exactes sont égales (ou très voisines) permettent l'existence de deux noyaux de même A (isobares) et de Z différents d'une unité.

La probabilité de cette égalité des masses exactes (ou d'avoir des valeurs très voisines) doit être d'autant plus élevée que le paramètre de la parabole, fonction de A, est plus petit. Mais en général, à chaque valeur de A correspond une seule valeur de Z pour la stabilité. Comme nous l'avons fait remarquer, il existe effectivement dans la nature des isobares dont les Z sont écartés d'une unité, mais la formule de Weizsäcker ne permet pas d'expliquer :

1° Que cette sorte d'isobares correspond à des *valeurs de* A *impaires* 113, 115, 123, 127;

2° Qu'il existe d'autres sortes d'isobares dont les Z diffèrent généralement de 2, parfois de 3 unités avec *toujours* A *pairs*;

3° Que la masse exacte d'un noyau de A pair et Z impair est plus grande que celle des isobares (même A) de Z pairs l'encadrant, ce qui a pour conséquence que pour les noyaux à A pairs les valeurs de Z des isobares sont paires (exceptions : 2, 6, 10, 14 nucléons).

4° Que pour les noyaux ayant des valeurs de A impaires il existe à peu près autant de Z pairs que d'impairs.

Ces constatations montrent que si tous les effets principaux intervenant pour fixer la masse exacte de l'atome figurent bien dans la formule, c'est dans les coefficients empiriques que doit intervenir l'influence de la parité de A.

D'après la répartition des noyaux, à chaque valeur de A impaire doit correspondre une seule parabole, tandis qu'à chaque valeur de A paire doivent correspondre deux paraboles de même paramètre B_A (fonction de A) décalées de δ_A, la supérieure (fig. 3) correspondant à Z impair et l'inférieure à Z pair (1).

D'où provient l'influence de la parité de A sur la prévision des masses exactes et par conséquent de la stabilité des noyaux?

Rappelons d'abord que la condition de stabilité la plus importante pour le problème qui nous préoccupe, est celle qui concerne la

(1) Les valeurs du paramètre B_A des paraboles et δ_A ont été d'abord calculées demi-empiriquement par Bohr et Wheeler et ensuite empiriquement par Irène Curie. Ce dernier auteur a, en outre, déterminé la courbe de variations de Z_A en fonction de A.

Bohr et Wheeler, *Phys. Rev.*, **56** , 427 (1939).

Irène Curie, *Journ. de Phys. et Rad.*, VI, 15 (1945).

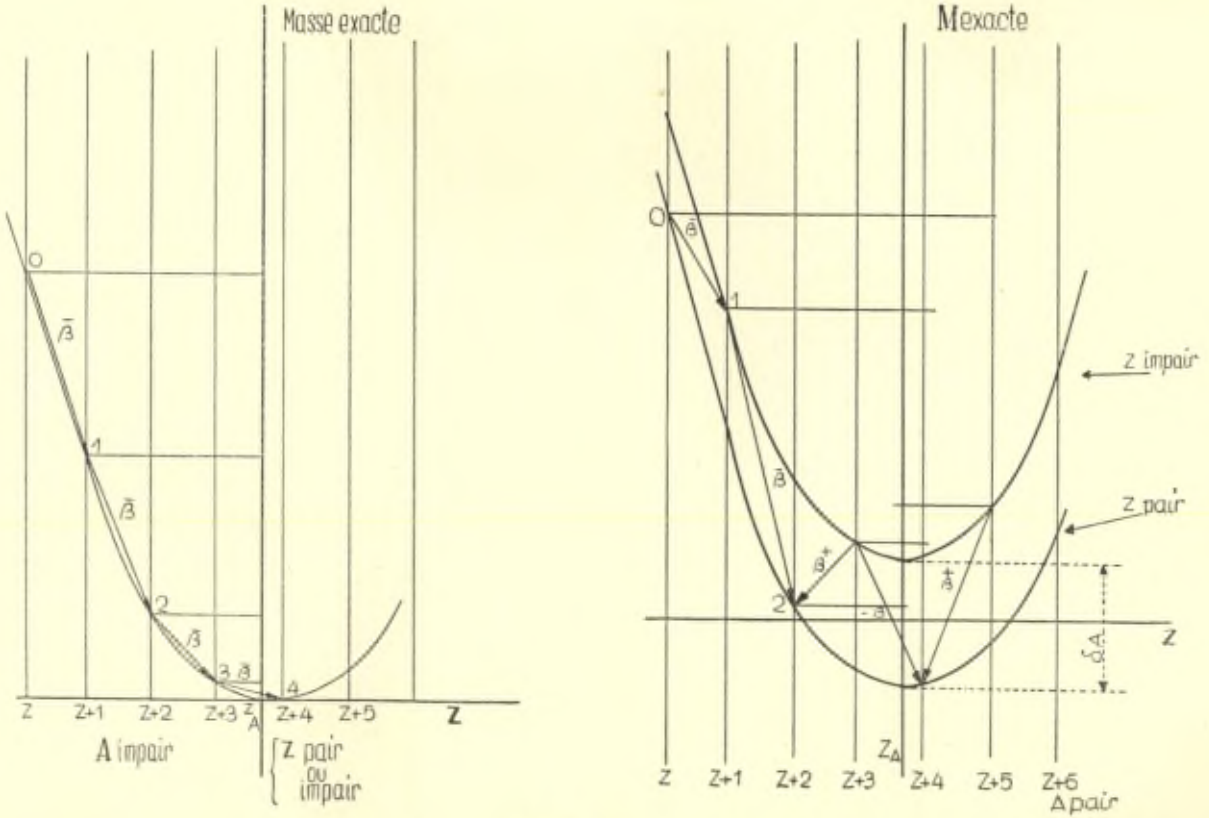

Masse exacte
O
1
2
3
4
Z
Z+1
Z+2
Z+3
Z+4
Z+5
Z
A impair
Z pair
ou
impair
Mexacte
Z impair
Z pair
Z
Z+1
Z+2
Z+3
Z+4
Z+5
Z+6
A pair

Fig. 3.

transformation spontanée dans le noyau soit d'un neutron en proton (émission d'un négaton et d'un neutrino), soit d'un proton en neutron (émission d'un positon et d'un neutrino), soit de la capture d'un électron K ou L par le noyau avec émission par celui-ci d'un neutrino. En d'autres termes très généraux : stabilité vis-à-vis de l'émission ou de l'absorption d'un électron par le noyau, phénomènes s'accompagnant du passage de Z à Z + 1 ou Z—1. Remarquons en outre qu'il y a une grande probabilité pour que les transformations β correspondent à des périodes de désintégration petites devant l'âge de la terre (exception K et Rb) et par conséquent que ces noyaux radioactifs, s'ils ont existé, ont depuis longtemps disparu de l'écorce terrestre.

Segré aux États-Unis et Daudel en France ont pensé indépendamment que la probabilité par unité de temps (constante de capture) de la capture L doit dépendre du nombre d'électrons présents sur la couche L. Pour un atome léger elle doit dépendre de l'état d'ionisation de celui-ci. Les expériences faites jusqu'ici par Segré pour mettre en évidence cet effet n'ont pas donné de résultats positifs. Les expériences avec $^{7}_{4}$ Be sont aussi en cours à Paris. Il n'en reste pas moins vrai que ces considérations sont d'une grande importance car elles peuvent conduire à ouvrir un champ nouveau de recherches où certains phénomènes nucléaires peuvent être influencés par l'état chimique de l'atome.

Pour que les phénomènes de désintégration β^- ou β^+ ou capture K puissent se produire, il faut avant tout que la transformation soit énergétiquement possible et c'est la connaissance des masses exactes qui est nécessaire pour prévoir ces désintégrations. Si l'on utilise les masses exactes des atomes on aura les conditions suivantes pour :

Désintégration β^- si $^{A}_{Z}M > {}^{A}_{Z+1}M$ + neutrino

Désintégration β^+ $^{A}_{Z}M > {}^{A}_{Z-1}M$ + 2 électrons + neutrino

Capture K $^{A}_{Z}M > {}^{A}_{Z-1}M$ + neutrino + énergie d'extraction K.

La connaissance des paraboles correspondant à chaque valeur de A permettra de prévoir les désintégrations précédentes possibles et d'estimer la valeur des énergies de désintégration auxquelles on doit

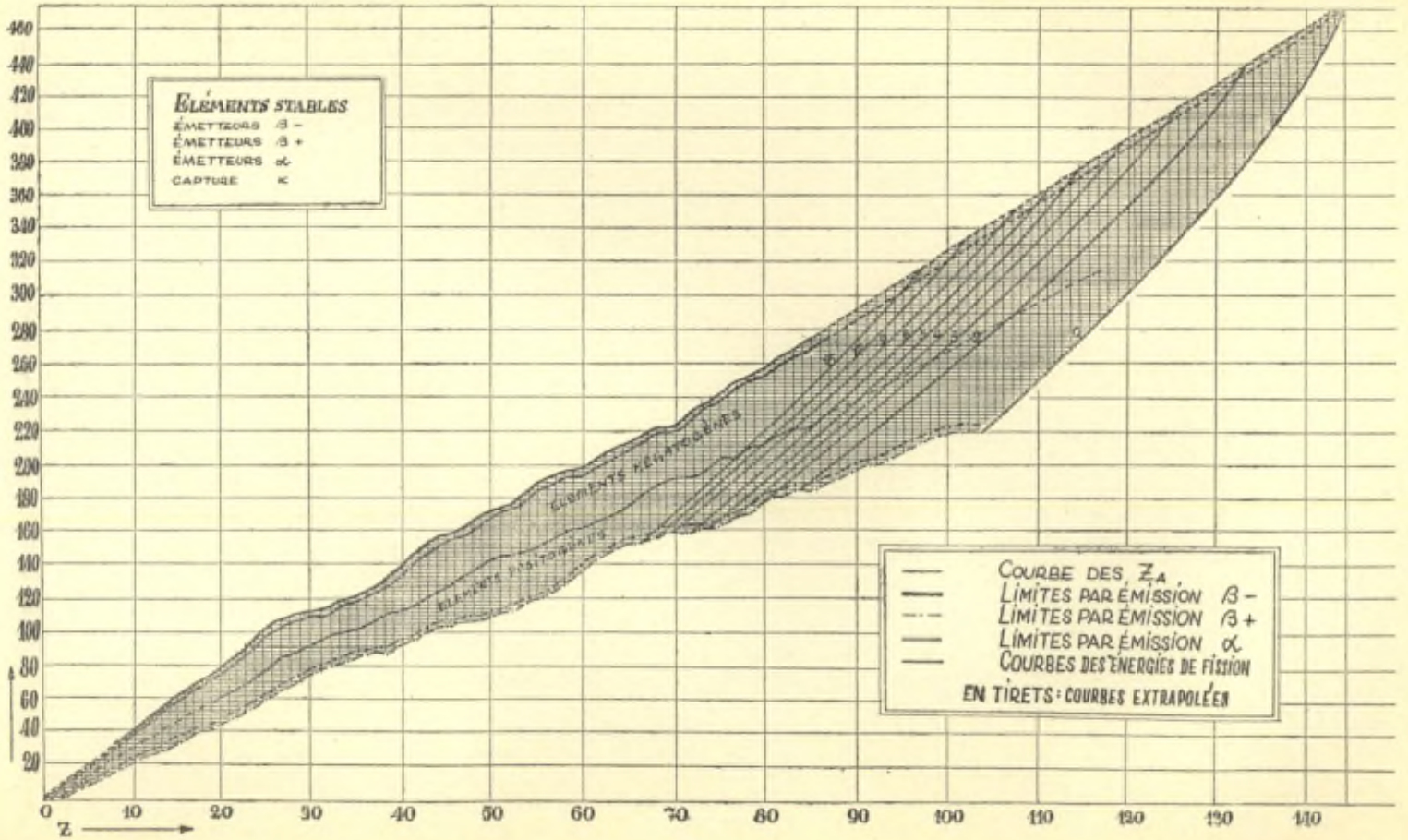

ÉLÉMENTS STABLES
ÉMETTEURS β −
ÉMETTEURS β +
ÉMETTEURS α
CAPTURE K
ÉLÉMENTS RADIOACTIFS
ÉLÉMENTS INSTABLES
COURBE DES Z_A
LIMITES PAR ÉMISSION β −
LIMITES PAR ÉMISSION β +
LIMITES PAR ÉMISSION α
COURBES DES ÉNERGIES DE FISSION
EN TIRETS : COURBES EXTRAPOLÉES
A
Z
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140
20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460

Fig. 4.

s'attendre (1). Elle permet en outre de prévoir les filiations possibles et le nombre de leurs termes. Le fait qu'à A pair doivent correspondre deux paraboles a pour conséquence que les énergies de désintégration β^- des noyaux successifs seront alternativement grandes et petites en décroissant dans leur ensemble lorsqu'on s'approche du terme final ayant un Z le plus voisin de Z_A ; tandis que si A est impair les énergies de désintégrations successives décroîtront régulièrement. Cette remarque est importante lorsqu'on étudie une filiation d'éléments radioactifs dont on ignore la valeur de A. Atteindre, en examinant les énergies limites des spectres continus, des termes successifs, la parité de A permet souvent de fixer la valeur de A lorsque, pour d'autres raisons, on hésite entre deux valeurs de cette grandeur. Enfin à titre d'exemple, on peut se demander l'étendue du domaine du plan (A, Z) dans lequel les chimistes nucléaires pourront pratiquement isoler les atomes dont ils font la synthèse. Ce sont encore les résultats précédents, joints aux relations empiriques de Sargent entre la période de désintégration β et l'énergie de désintégration, qui permettent d'estimer la place et l'étendue de ce domaine. Il faut évidemment fixer la période de désintégration au-dessous de laquelle il est pratiquement impossible d'identifier et d'isoler chimiquement le radioélément. Guental et Daudel (2) ont choisi 1 sec en se basant sur le fait, déduit du diagramme de Sargent, qu'un radioélément ayant une énergie de désintégration β dépassant 19 Mev a peu de chance de posséder une période dépassant la seconde. Un tel radioélément échapperait juste au domaine isolable étudié. Ils se trouvent ramenés à la détermination des Z et A des éléments qui correspondent à une transition nucléaire d'énergie inférieure à 19 Mev. La courbe ci-jointe (fig. 4) reproduit Z_A dans les cordonnées A, Z ainsi que les limites du domaine d'existence des noyaux isolables, négatogènes, positogènes, de capture K et stables. Des courbes analogues sont relatives au domaine des désintégrations α possibles et aux énergies nécessaires pour provoquer la fission. En particulier, la courbe d'énergie zéro qui correspond à la fission spontanée, limite vers les grandes valeurs de A et Z les noyaux isolables possibles. Bien entendu, il ne s'agit là simplement que d'indications assez approximatives, mais qui peuvent déjà être utiles. Elles seront d'ailleurs susceptibles d'être précisées avec les progrès des déterminations des masses exactes et de la structure nucléaire.

(1) Bohr et Wheeler, *Phys. Rev.*, **56**, 427 (1939).

(2) P. Guental et R. Daudel, *Revue Scient.*, **2**, 109 (1946).

Examinons maintenant la stabilité des noyaux vis-à-vis de l'émission de particules lourdes comme les neutrons, protons, alpha, etc.

Pour qu'une émission de ce genre puisse avoir lieu, il faut d'abord qu'elle soit énergétiquement possible, c'est-à-dire encore que la masse du noyau initial soit plus élevée que la somme des masses du noyau résiduel et de la particule émise à l'état libre.

On pourra donc prévoir les désintégrations possibles en calculant les masses exactes à l'aide de la formule de Weizsäcker. On déduit en particulier de ces calculs que les noyaux sont plus instables vis-à-vis d'une émission α que vis-à-vis de l'émission d'une particule plus légère. (La particule α a un très grand facteur de condensation). L'émission d'une particule α n'est énergétiquement possible qu'à partir d'une valeur de A voisine de 120, toutefois, à l'exception de $^{148}_{62}$Sm (période 1.7×10^{11} ans), il faut atteindre $^{211}_{83}$Ac C' pour observer effectivement l'émission. L'énergie qui serait communiquée à la particule α pour les éléments plus légers est trop faible pour leur permettre de traverser la barrière d'énergie potentielle avec une probabilité appréciable.

L'instabilité vis-à-vis d'une particule $^{12}_{6}$C commencerait avant 120, mais la hauteur de la barrière d'énergie potentielle empêcherait totalement la sortie de cette particule.

La réponse qualitative à l'importante question posée plus haut sur l'influence de la parité de A sur la stabilité des noyaux a été donnée par Young (1), commentée par Bethe et Bacher (2).

Les paires de neutrons ou de protons peuvent former des couches complètes, dans le sens des couches électroniques en physique atomique.

Deux neutrons ou deux protons peuvent former une couche complète d'état quantique donné (eu égard à leur mouvement) parce que ces particules ont un spin et que pour chaque paire de nucléons identiques les spins sont antiparallèles. Cette disposition étant imposée par le principe d'exclusion de Pauli.

Chaque état de mouvement orbital d'un proton ou d'un neutron a son énergie propre, différente de l'énergie des autres mouvements. Une couche de deux neutrons étant complète, un nouveau neutron devra se placer sur l'orbite d'énergie supérieure la plus voisine et sera de ce fait moins lié que les deux précédents (même considération pour les protons).

(1) Young, *Phys. Rev.*, **48**, 913 (1935).

(2) H.-A. Bethe et R.-F. Bacher, *Rev. of Mod. Phys.*, **8**, 100 à 105 (1936).

Considérons, comme l'ont rapporté Bethe et Bacher, un noyau standard caractérisé par A et Z pairs et construisons les noyaux en introduisant successivement des neutrons ou des protons.

A et Z étant pairs le nombre N de neutrons est pair. Les nucléons et protons occuperont par paire de nucléons identiques, les niveaux successifs à partir des énergies minima. Les $\frac{Z}{2}$ et $\frac{N}{2}$, états de plus basses énergies correspondant aux paires de protons et de neutrons, seront occupés. Le premier état non occupé par les neutrons peut être d'énergie plus petite que celui non occupé par les protons ou le contraire.

Pour former un noyau contenant A + 1 nucléons, valeur impaire, on peut ajouter soit un neutron, soit un proton; on formera les noyaux ${}^{A+1}_{Z}M$ et ${}^{A+1}_{Z+1}M$

(a) Si la première couche libre à laquelle on ajoute le neutron est d'énergie plus basse que celle de la première couche libre à laquelle on ajoute le proton, ce sera ${}^{A+1}_{Z}M$ qui sera stable et non ${}^{A+1}_{Z+1}M$ qui doit avoir une masse exacte plus élevée.

La probabilité que la première couche libre à laquelle on ajoute un proton soit d'énergie plus basse est la même en première approximation. En conséquence : pour les A impairs nous devons nous attendre à observer autant de noyaux stables ayant Z pairs et impairs.

Si c'est le noyau ${}^{A+1}_{Z}M$ qui est stable (addition d'un neutron à ${}^{A}_{Z}M$), ajoutons un deuxième neutron formant ${}^{A+2}_{Z}M$ contenant une nouvelle couche complète de deux neutrons. Si à ${}^{A+1}_{Z}M$ on ajoute un proton pour former ${}^{A+2}_{Z+1}M$, ce noyau aura une masse supérieure à ${}^{A+2}_{Z}M$ puisque l'énergie de la couche vide de proton est supérieure à celle des neutrons; ceci sera vrai à fortiori si à ${}^{A}_{Z}M$ on ajoute successivement deux protons formant ${}^{A+2}_{Z+2}M$.

Dans l'éventualité (a) on a l'ordre des masses exactes croissantes suivant :

$^{A+2}_{Z}M$, $^{A+2}_{Z+1}M$, $^{A+2}_{Z+2}M$ et $^{A+2}_{Z+2}M$ peut se transformer par absorption d'électron (β ou capture K) en $^{A+2}_{Z+1}M$ et celui-ci de la même manière en $^{A+2}_{Z}M$ c'est-à-dire en un noyau à nombre de nucléons et de protons pairs. Le noyau à nombre de nucléons pairs et de protons impairs est instable.

Si la première couche vide d'énergie la plus basse au début avait été celle des protons, on serait arrivé à un ordre inverse pour les masses des noyaux :

$^{A+2}_{Z+2}M$, $^{A+2}_{Z+1}M$, $^{A+2}_{Z}M$, et $^{A+2}_{Z+2}M$ aurait été le noyau stable de nombre de charge encore pair.

Toutefois cette explication est insuffisante pour interpréter les nombreux cas d'isobares stables ayant A et Z pairs; les valeurs de Z diffèrent habituellement de deux unités. Dans ces cas il faut expliquer que la masse de $^{A+2}_{Z+1}M$ doit être supérieure à la fois à $^{A+2}_{Z}M$ et $^{A+2}_{Z+2}M$.

La contradiction avec le raisonnement précédent n'est qu'apparente. Il faut, pour la lever, faire intervenir l'influence sur la masse du noyau, des attractions entre paires de nucléons de même nature ajoutées. Ces attractions s'exercent si les nucléons sont sur une même orbite et elles agissent dans le sens d'une augmentation de l'énergie de liaison c'est-à-dire une diminution de la masse exacte du noyau. Or, il s'exerce aussi une grande force attractive entre neutrons et protons, mais seulement si ceux-ci sont sur des orbites de même état énergétique. En conséquence, cette force attractive entre protons et neutrons ajoutés n'agira sur l'énergie de liaison que si les nombres de protons et de neutrons existant déjà dans le noyau sont égaux. En conséquence, cet effet de diminution de masse est notable pour $^{A+2}_{Z}M$ et $^{A+2}_{Z+2}M$ et négligeable pour $^{A+2}_{Z+1}M$ si ce dernier ne réalise pas la condition $A = 2Z$ et

il peut ainsi arriver que la masse $^{A+2}_{Z+1}M$ soit plus grande que $^{A+2}_{Z}M$ et $^{A+2}_{Z+2}M$ qui sont alors tous les deux stables. Le cas $A = 2Z$ est réalisé pour les noyaux légers $^{2}_{1}H$ $^{6}_{3}Li$ $^{10}_{5}B$ $^{14}_{7}N$; pour les noyaux plus lourds, déjà se fait sentir l'action répulsive des charges des protons qui impose pour la compenser l'addition d'un excès de neutrons et il ne peut plus exister de noyaux stables ayant A pair et Z impair.

CHAPITRE II.

A. — CHIMIE NUCLÉAIRE.

Nous ne nous sommes préoccupés jusqu'ici que de la constitution, de la répartition (stabilité et instabilité) des noyaux tels qu'ils se présentent dans la nature. Nous savons actuellement, de multiples manières, effectuer la synthèse d'un nombre considérable de noyaux dont la plupart sont nouveaux. Ce nombre est supérieur à celui des noyaux stables et instables existant dans l'écorce terrestre

Depuis la première transmutation artificielle d'un élément chimique en un autre élément chimique, l'azote en oxygène, réalisée en 1919 par Rutherford, un nombre considérable de réactions furent obtenues en employant des projectiles corpusculaires de divers types bombardant des cibles constituées par des atomes des divers éléments chimiques. A ces réactions s'ajoutèrent les réactions de fission des divers éléments lourds naturels : uranium, protactinium, thorium, ionium et même de synthèse comme le plutonium.

Je ne puis développer ici l'historique des découvertes successives et de l'élaboration des techniques qui ont fait rapidement progresser la chimie nucléaire.

Parmi les découvertes qui suivirent la réalisation de la première transmutation artificielle par les rayons α émis par les radioéléments naturels, celles du neutron et de ses propriétés, de l'électron positif et de la radioactivité artificielle eurent les plus grandes conséquences. Simultanément se développèrent les techniques d'accélération des

noyaux légers (protons, deutérons, hélions ou particules α) par les générateurs à très haute tension et le cyclotron.

Cette dernière technique permit en particulier de produire des faisceaux de très grande intensité de projectiles (courant de dizaines de microampères) ayant des énergies pouvant atteindre des valeurs considérables (dizaines de millions d'électrovolts).

La chimie nucléaire, comme la chimie moléculaire, consistant à faire rencontrer les réactifs, ici les noyaux, on doit s'attendre à de faibles rendements en raison, d'une part, de la petitesse des dimensions géométriques (ou encore des longueurs d'ondes effectives associées de L. de Broglie) et, d'autre part, dans le cas des projectiles chargés d'énergie pas trop élevée, de l'action de la barrière d'énergie potentielle. Jusqu'à la production des réactions nucléaires s'entretenant elles-mêmes (réacteur à uranium) les quantités d'atomes produits dans les réactions nucléaires étaient en général extrêmement faibles, impondérables. (Certes, déjà avec de très gros cyclotrons les quantités produites pour certaines réactions étaient à la limite du pondérable.)

Le neutron est un projectile de choix, n'étant pas chargé. Le rendement des réactions provoquées par les neutrons peut devenir très grand lorsque leur vitesse devient très faible, de l'ordre de grandeur de la vitesse d'agitation thermique des atomes des substances peu absorbantes qu'ils traversent avant de réagir : hydrogène de l'eau, paraffine, eau lourde, graphite, béryllium, etc.). Enfin des phénomènes de résonance peuvent intervenir.

Le rendement d'une réaction peut s'exprimer à l'aide de la section efficace. Pour que la réaction envisagée se produise il faut d'abord une rencontre du projectile avec un noyau de la cible. Ensuite plusieurs réactions peuvent se produire, dont celle que nous envisageons. Pour simplifier, assimilons les noyaux de la cible à des points et considérons N projectiles arrivant avec une vitesse v sur la cible contenant n noyaux par centimètre cube (fig. 5). Par seconde on observe N' rencontres dont Q provoquent la réaction envisagée. On considère une image géométrique de la rencontre en associant à chaque projectile, une surface S perpendiculaire à la vitesse. S sera définie par la relation N.S.v.n. = N', N.S.v étant le volume balayé par seconde par les N projectiles, volume contenant N.S.v.n. noyaux tous rencontrés. S est la section efficace de rencontre. Nous pouvons maintenant définir, toujours de ce point de vue géométrique, une

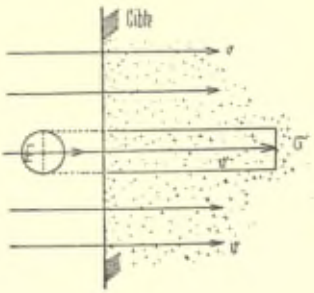


Fig. 5

surface σ attachée à chaque projectile, telle que $N \sigma n v$ représente le nombre Q de rencontres par seconde s'accompagnant de la *réaction envisagée;* on aura :

$$\sigma' = \frac{N'}{N.v.n.}$$

Cette surface fictive ou section efficace du projectile pour la réaction envisagée s'exprime en centimètres carrés.

Les valeurs de σ' sont très variables suivant les réactions (σ' aurait avantage à être exprimé avec une unité égale à 10^{-24} cm^2) (1).

La section efficace peut être introduite d'autre manière; en particulier elle intervient dans la loi d'absorption du projectile (avec production de la réaction étudiée); σ n étant le coefficient linéaire d'absorption par le processus considéré.

Suivant la conception moderne due à Niels Bohr, la particule incidente rencontrant un noyau peut s'y incorporer, et former un noyau composé dont l'existence est éphémère.

En raison du court parcours et de l'intensité des forces nucléaires, la particule incidente ne peut pas passer à travers le noyau sans perdre de l'énergie dès qu'elle arrive à la surface du noyau, la communiquant d'abord aux premiers nucléons à travers lesquels elle passe. Les nucléons communiquent à leur tour de l'énergie aux voisins de telle sorte que l'énergie incidente se distribue à l'ensemble des nucléons et non comme on l'envisageait auparavant à un seul constituant du noyau. Toutes ces particules ont une énergie accrue, mais

(1) Une commission mixte provisoire des Unions Internationales de Chimie et de Physique a proposé en 1947 de donner le nom de « Rutherford » avec le symbole « Rud » à cette unité 10^{-24} cm^2. Mais cette question est encore à l'étude.

insuffisante pour que l'une ou plusieurs puissent sortir immédiatement du noyau composé.

Après un temps suffisamment long comparé au temps qui serait nécessaire à la particule incidente pour traverser le noyau sans freinage, l'énergie peut, par accident, se concentrer suffisamment sur l'une des particules pour lui permettre de quitter le noyau. La particule sortante peut être de même nature ou différente de la particule incidente. Le noyau peut rester excité et émettre ensuite une radiation ou même parfois une autre particule. Le noyau composé, peut être considéré comme dans un état quasi-stationnaire. L'étude théorique de ces états et de leurs transitions à d'autres correspondant à la séparation d'une particule du composé permettra de prévoir les réactions et leur probabilité. Le processus correspond à une double transition.

Le résultat final de la collision dépend de la libre compétition entre tous les processus de désintégration et d'émission de radiations du noyau composé, avec conservation de l'énergie et de l'impulsion des nombres de masse et de charge. La probabilité d'émission de radiation γ est assez grande en raison de la vie relativement grande du composé. On aura une petite probabilité de chocs élastiques et une grande probabilité de chocs inélastiques suivis de désintégrations ou d'émission de radiations. On sait comment Niels Bohr développa cette conception et calcula la distribution des niveaux d'énergie correspondant aux divers états du composé en choisissant pour modèle de noyau celui d'une goutte liquide.

Le schéma ci-dessous donne une image de la distribution des

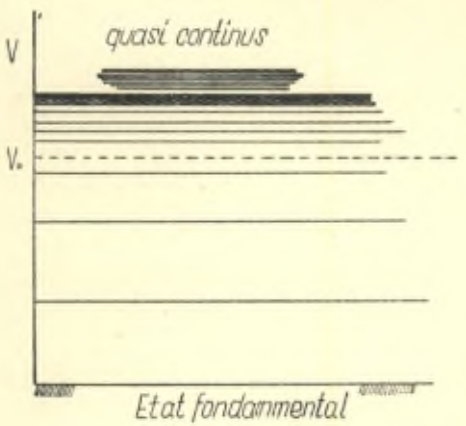


Fig. 6

niveaux. La théorie de Bohr a permis de comprendre les phénomènes de résonance correspondant à la capture des particules. Si l'énergie cinétique de la particule est telle que l'énergie d'excitation du noyau composé devient égale ou très voisine de celle d'un niveau, la probabilité de sa formation est beaucoup plus grande que si elle tombe entre deux niveaux. On conçoit l'importance de l'étude expérimentale de ces résonances pour la détermination des emplacements des niveaux et de leur largeur.

***

Pour simplifier on écrit le schéma général d'une réaction nucléaire de la façon suivante :

$$A + P \rightarrow C \rightarrow B + Q$$

A = noyau initial; C = noyau composé;
B = noyau final; P = particule incidente;
Q = particule émise.

On appelle teneur en énergie d'un processus la différence de la somme des masses exactes (exprimée en énergie) des noyaux réagissants avec la somme des masses exactes des noyaux formés. Cette teneur est positive ou négative, correspondant à une réaction exo ou endo énergétique.

Des processus secondaires dus au noyau résiduel B peuvent exister. Ce noyau après le départ de la particule Q peut être laissé dans un état excité. Si l'énergie d'excitation est inférieure à l'énergie de dissociation d'une particule, le noyau revient à l'état fondamental en émettant un ou plusieurs photons γ. A chaque énergie d'excitation de B correspond un groupe de particules Q de même énergie. Si l'énergie d'excitation est supérieure à l'énergie de dissociation une nouvelle particule peut être émise.

On aura les processus successifs suivants (^{x} signifie noyau excité):

$$A + P \rightarrow C \rightarrow B^x + Q \quad \text{puis} \quad B^x \rightarrow D + S$$

d'où finalement :

$$A + P \rightarrow C \rightarrow D + S + Q$$

On a mis en évidence de tels processus. Exemple :

$$^{11}_{5}B + {}^{1}_{1}H \rightarrow {}^{12}_{6}C^x \rightarrow 3\,{}^{3}_{4}He$$

ou encore :

$$^{9}_{4}Be + {}^{4}_{2}He \rightarrow {}^{13}_{6}C^x \rightarrow 3\,{}^{4}_{2}He + {}^{1}_{0}n$$

Radioactivité artificielle. — Isomérie.

Le noyau B peut être radioactif. On a alors effectué la synthèse d'un radioélément qui, dans la majorité des cas, n'existe pas dans la nature, c'est un « radioélément artificiel ». Ou bien il se transforme par radioactivité β^-, ou β^+, ou par capture d'un électron K ou L, en un autre radioélément artificiel ou en un atome stable existant dans la nature. Dans le premier cas on a une filiation entre radioéléments se terminant par un atome stable. L'émission des positons par un radioélément positogène s'accompagne de celle de rayons X de 500.000 eV, si les positons sont arrêtés dans la matière contenant le radioélément (rayonnement d'annihilation des positons). Cette remarque est importante, en particulier pour les applications de ces éléments.

Les radioéléments artificiels, du point de vue de leurs propriétés radioactives (période de désintégration, spectre continu des rayons, etc.) se comportent comme les radioéléments naturels (1).

Unité de radioactivité.

Récemment une commission mixte des constantes radioactives des Unions Internationales de Chimie et de Physique a proposé de redéfinir l'unité de radioactivité comme il suit :

Le curie est la quantité d'un radioélément subissant exactement 3,6000... $\times 10^{10}$ transformations radioactives par seconde, valeur très voisine des déterminations les plus précises du nombre de rayons alpha émis par un gramme de radium sans dérivés radioactifs.

L'abréviation du curie est Cur. Si l'on désire introduire une unité décimale pour le nombre de transformations radioactives par seconde on peut désigner par néocurie, nCur, 10^6 transformations par seconde. On a proposé aussi pour désigner le nombre de transformations nucléaires par unité de temps le mot « mutance ». L'unité de mutance proposée est le curie. Mais il faut encore attendre que ces propositions soient acceptées par une nouvelle réunion des unions scientifiques.

Isomérie nucléaire.

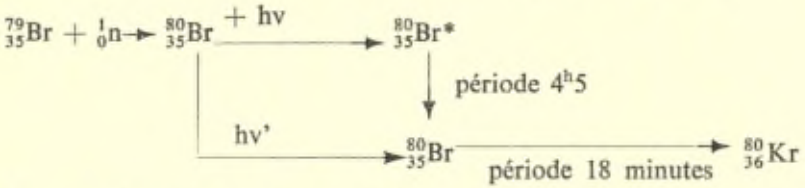
La vie moyenne des atomes radioactifs, même lorsqu'elle est très petite, est beaucoup plus grande que la durée des processus nucléaires

(1) On a réussi à faire la synthèse de radioéléments naturels. Exemple :

$^{210}_{83}$RaE par deux réaction $^{209}_{83}\text{Bi} + ^{1}_{0}\text{n} \rightarrow ^{210}_{83}\text{RaE} + \gamma$

et $^{209}_{83}\text{Bi} + ^{2}_{1}\text{D} \rightarrow ^{210}_{83}\text{RaE} + ^{1}_{1}\text{H}$

de formation de B et, dans la grande majorité des cas, beaucoup plus grande que la vie moyenne des états excités de B. Toutefois, il arrive que la vie moyenne d'un état excité de B (état de faible énergie) est du même ordre que celle de l'atome radioactif B. Le retour de B* à l'état fondamental s'effectue par émission d'un rayonnement γ en majeure partie converti en électrons K extraits de la couche de l'atome (1). On observe l'émission d'une raie d'électrons monocinétiques avec une période. Les noyaux B* et B sont isomères (même A et même Z). Voici un exemple classique d'isomérie artificielle :



On connaît actuellement un grand nombre de cas d'isomérie, certainement plus de quarante.

Tous les cas ne sont pas du type précédent, on en connaît où les deux isomères sont radioactifs β, enfin, d'autres où l'un des deux isomères est stable et l'autre correspond à l'état excité de vie moyenne longue.

Nous avons vu dans le chapitre précédent les conditions d'instabilité des noyaux, comment les divers processus de désintégrations β^-, β^+ et capture K peuvent être en compétition (voir les paraboles Fig. 3). Lorsque la capture K est en compétition avec la désintégration β^+, celle-ci est plus probable pour les éléments légers, tandis que la capture K est plus probable pour les éléments lourds. Il y a des radioéléments pouvant aussi émettre des β^-, des β^+, ou β^- et capture K.

Concernant la synthèse des radioéléments on peut dire d'une façon générale que si le processus conduit à augmenter le nombre de neutrons dans le noyau irradié, l'élément formé, s'il est radioactif, est probablement négatogène tandis qu'une augmentation du nombre de protons donnera de préférence un élément positogène.

On a de grandes difficultés à observer des radioéléments à vie très courte ou très longue $\frac{1}{50}$ sec $<$ période $<$ centaines d'années) et

(1) En réalité, on a émission γ ou bien transfert direct de l'énergie d'excitation à l'atome qui se traduit par extraction d'un électron K qui est émis.

aussi les radioéléments émetteurs de rayons β de très faible énergie ou ceux subissant la capture K.

B. — TYPES DE RÉACTIONS NUCLÉAIRES PROVOQUÉES.

Les projectiles employés sont les suivants :

α hélions, n neutrons, p protons, d deuterons, γ gamma, ${}^{3}_{1}H$ tritons, ε électrons.

Un type de réaction peut être caractérisé par la particule incidente et la ou les particules émises; ainsi (α, p) désigne une réaction provoquée par les rayons α (hélion) avec émission de protons.

La réaction est précisée en plaçant devant la parenthèse le symbole du noyau irradié et ensuite le symbole du noyau formé.

Exemple : ${}^{14}_{7}N$ (α. p) ${}^{17}_{8}O$.

Voici d'abord les principaux types de réactions provoquées :

(α, n) (p, n) (d, 2n) (d, n) (d, α) (d, p) (n, γ) (n, α) (n, p) (n, 2n) (γ, n).

Mais d'autres types beaucoup moins fréquents furent observés :

(α, p)	(p, γ)	(d, γ)	(n, p2n)	(t, ${}^{3}_{2}He$)	(ε, n)
(α, d)	(p, 2n)	(d, t)	(n, n)	(t, p)	
(α, pn)	(p, p)	(d, 3n)			
(α, 2n)		(d, 4n)			
(α, p2n)					
(α, p3n)					
(α, p4n)					
(α, γ)					

On peut distinguer deux types principaux suivant que la particule émise est un photon ou une particule matérielle. Dans le premier cas, il s'agit d'une capture radiative ou simple capture, dans le second cas, si la particule émise est de nature différente de celle de la particule incidente, il s'agit d'une transmutation.

Les réactions du type (n, n) ou (p, p) ... laissent le noyau initial dans l'état excité (diffusion inélastique). Enfin les projectiles précédents α, p, n, d, γ sont capables de provoquer la fission des noyaux dont nous dirons quelques mots plus loin.

Tous les types de réaction doivent satisfaire à la conservation de l'énergie et de l'impulsion.

Les tables de Mattauch et Flügge déjà citées (page 16) et celles plus

récentes (tables de Glenn Seaborg (1)) contiennent la liste des processus nucléaires, et en particulier ceux qui donnent des radioéléments artificiels, avec les caractéristiques de leur rayonnement et de leur période.

Le plus grand nombre de réactions provoquées jusqu'ici l'ont été en employant les projectiles neutrons rapides et surtout lents et thermiques, et deutérons (porteurs de neutrons, processus Oppenheimer Phillips) et α de grande énergie.

Discutons quelques-unes de ces réactions parmi les plus importantes :

Projectiles protons. — La réaction la plus probable est (p, n). Ce n'est que dans le cas où l'énergie du proton est insuffisante pour l'émission d'un neutron que d'autres réactions ont lieu. La plus grande probabilité d'émission de neutrons est due à l'absence de barrière d'énergie potentielle pour cette particule.

La réaction (p, 2n) se produit si l'énergie du proton est suffisamment grande pour laisser le noyau final après la réaction (p, n) avec une énergie d'excitation supérieure à celle de l'énergie de dissociation d'un neutron.

La réaction (p, γ) prédominera sur (p, n) et à fortiori sur (p, p) si l'énergie du proton fournit un composé d'énergie d'excitation inférieure à l'énergie de dissociation du neutron.

Projectiles alpha. — Conclusions analogues à celles avec les protons (α, n) est la plus probable.

Projectiles deuterons. — Les réactions suivent en général les mêmes lois que les réactions par protons, mais le deutéron n'étant pas un noyau très condensé conduit à une énergie d'excitation U très élevée du noyau composé. Pour les noyaux au voisinage de A = 20, U = 16 MeV + E_d' (E_d énergie cinétique relative du deutéron), tandis que pour neutrons, protons et α on a respectivement :

$$U = 10 \text{ MeV} + E_n$$
$$U = 9 \text{ MeV} + E_p$$
$$U = 9 \text{ MeV} + E\alpha$$

C'est une des raisons du grand rendement des réactions provoquées par les deutérons.

(1) Glenn Seaborg, *Rev. of Mod. Phys.*, **16**, 1, (1944).

Les réactions (d, n), (d, 2n) obéissent aux mêmes lois que celles qui régissent (p, n), (p, 2n).

Pour la réaction (d, p) qui devrait être moins probable que (d, n) les rendements observés sont assez voisins en raison du processus Oppenheimer Phillips (1), qui est dû à une forte polarisation du deutéron (grande distance entre neutron et proton et faible énergie de liaison) au contact de celui-ci avec le noyau. Le deutéron se brise, le neutron qui est à la surface du noyau est capturé, tandis que le proton est repoussé par le champ électrique nucléaire.

La réaction (d, γ) a un rendement beaucoup plus faible, car l'énergie d'excitation du composé étant très grande, c'est l'émission du neutron avec grande énergie qui est la plus probable.

Processus photonucléaires. — Pour que la réaction se produise il faut que l'énergie du photon qui excite le noyau soit plus élevée que la plus petite énergie d'extraction d'une particule matérielle. Les plus probables sont (γ, n). Les réactions (γ, p) qui ont été observées sont moins probables.

Projectiles neutrons. — La probabilité de formation du composé est très grande. Les réactions (n, n) sont plus probables que (n, p), (n, α), (n, 2n) qui nécessitent une plus grande énergie de neutrons.

Les réactions (n, α) ont un faible rendement dans le cas des éléments lourds, à cause de la barrière d'énergie potentielle; mais, si l'énergie du neutron devient très grande, alors (n, α) devient plus probable pour les noyaux très lourds que pour les moyennement lourds, car noyaux très lourds sont déjà instables vis-à-vis des désintégrations α.

La capture radiative (n, γ) est beaucoup plus probable que n, p et n, α si l'énergie des neutrons est très faible (phénomène de capture par résonance pour neutrons lents et section efficace de capture radiative proportionnelle en général à $\frac{1}{v}$, v vitesse du neutron pour les vitesses d'agitation thermique ou voisines).

***

On a réussi par les techniques d'accélération à donner aux projectiles chargés des énergies dépassant les plus hauts sommets des barrières d'énergie potentielle (uranium) et permettant aussi de provoquer de plus en plus de réactions nouvelles avec les mêmes

(1) Oppenheimer Phillips, *Phys. Rev.*, 48, 500 (1935).

noyaux. L'examen des tables de réactions nucléaires est suggestif à cet égard.

D'une façon générale, le rendement pratique de synthèse d'un noyau par les particules chargées augmente toujours avec l'énergie de celle-ci, car la particule qui est freinée par le phénomène d'ionisation en traversant la cible épaisse conservera plus longtemps une énergie supérieure ou voisine du sommet de la barrière d'énergie potentielle.

Fissions.

La découverte du phénomène de fission des noyaux lourds et la réalisation des réactions nucléaires en chaîne a encore augmenté considérablement le nombre des réactions nucléaires provoquées et, en conséquence, le nombre des radioéléments artificiels.

Voici un exemple de fission :

Sous l'action d'un neutron thermique le noyau ^{235}U peut se scinder en deux fragments, de préférence de masses inégales, qui, en raison de la grande teneur en énergie de la réaction, seront projetés avec de très grandes vitesses.

Des neutrons rapides accompagnent en général la bipartition et les deux fragments formés possèdent un grand excès de neutrons vis-à-vis de celui qui existe normalement dans les noyaux stables de même nature chimique.

Cet excès de neutrons tendra à diminuer par des désintégrations β^- successives. Parfois au début après une ou quelques désintégrations β^- de période assez brève, un neutron pourra s'évaporer (neutron différé).

Les fragments constituent ainsi des têtes de filiations radioactives parfois d'un grand nombre de termes. En voici un exemple concernant la fission de $^{235}_{92}U$.

$$^{143}_{54}Xe \xrightarrow[1\text{ sec}]{\beta^-} {}_{55}Cs \xrightarrow[\text{Courte}]{\beta^-} {}_{56}Ba \xrightarrow[\text{Courte}]{\beta^-} {}_{57}La \xrightarrow[\text{Courte}]{\beta^-}$$

$$ {}_{58}Ce \xrightarrow[275\text{ jours}]{\beta^-} {}_{59}Pr \xrightarrow[17\text{ min}]{\beta^-} {}_{60}Nd \text{ Stable.}$$

La répartition des éléments formés lors de la bipartition précédente a été représentée par C. D. Coryell et publiée par le *Journal of the Chemical Society*, 68, 2411 (1946). Les nombres de masse des

fragments se répartissent entre 72 et 162 et les fragments légers se répartissent autour de la valeur la plus probable A = 95, les fragments lourds autour de Z = 140.

Les proportions relatives des éléments de fission sont données ainsi que les périodes, la nature et l'énergie des rayonnements émis par les radioéléments de fission. Un grand nombre des termes de filiation formés à partir des fragments de fission s'identifie avec des radioéléments formés par des réactions nucléaires des divers types envisagés précédemment. Exemple :

Le $^{95}_{40}Zr$ est contenu dans les produits de fission (radioactivité β^- accompagnée de γ). Le noyau identique se forme aussi par des réactions $^{94}_{40}Zr$ (n, γ) $^{95}_{40}Zr$; $^{94}_{40}Zr$ (d, p) $^{95}_{40}Zr$ et par désintégration β^- de $^{95}_{41}Nb$ qui se forme par : $^{97}_{42}Mo$ (d, α) 95Nb, $^{97}_{42}Mo$ est stable.

On a pu identifier au total 196 radioéléments dans les produits de fission de ^{235}U sous l'action des neutrons. La liste commence par $^{72}_{30}Zn$ et se termine par $^{158}_{63}Eu$.

Les valeurs des périodes s'étalent entre la fraction de seconde et des durées très longues. En particulier l'élément $^{99}_{43}$ isotope de l'élément n'existant pas dans la nature, Z = 43 a une très longue période de l'ordre de 10^6 ans. Une partie de ces radioéléments avait déjà été préparée par les réactions de types divers.

La fission de l'uranium ne s'effectue pas toujours en deux fragments. C'est ainsi que ^{235}U fissionné par neutrons thermiques peut se diviser en trois ou quatre morceaux (1). Elle fut en outre provoquée par d'autres projectiles α, p, d, γ.

De même: les éléments Pa, Th, Io subissent la fission avec des neutrons rapides et il faut ajouter les transuraniens dont le plutonium.

Récemment (2), avec des rayons α de 400 MeV du très gros cyclotron de Berkeley, on a réussi à fissionner.

	Bi	— Pb	— Tl	— Pt	— Ta
Z	83	82	81	78	73

83, 82 et 81, 78 se fissionnent avec les deutérons de 200 MeV;
83 se fissionne même avec des deutérons de 50 MeV.

---

(1) Tsien-San-Tsiang, R. Chastel, M^me^ Ho Zah-Wei et Vigneron, *Comptes rendus Acad. des Sciences*, **223**, 986 (1946); **223**, 1119 (1946).

(2) I. Perlman, R.-H. Goeckermann, D.-H. Templeton et J.-J. Howland, *Phys. Rev.*, **72**, 352 (1947).

Enfin, autre résultat remarquable (1), l'arsenic et le sélénium irradiés par des α (400 MeV) ou par des d (200 MeV) peuvent être pulvérisés en 20 ou 30 morceaux.

L'énergie d'excitation due à la capture de la particule α est si élevée que jusqu'à 14 protons peuvent être expulsés et des neutrons de 100 MeV peuvent être émis.

L'emploi de moyens aussi puissants nous promet sans doute de nouvelles surprises.

***

La théorie du phénomène de la fission a d'abord été ébauchée par Frisch et Lise Meitner et développée par Niels Bohr et Wheeler (2). Dans les noyaux les plus lourds, la répulsion des charges des protons s'oppose à un grand degré à l'effet des forces nucléaires de court parcours. L'effet de forces électrostatiques est relativement plus important que l'effet de surface. La tension superficielle tend à donner comme forme stable la sphère, forme qui est la moins stable vis-à-vis des forces électrostatiques. Le noyau sera stable lorsque la somme de l'énergie potentielle électrostatique et l'énergie de surface présente un minimum pour la forme sphérique.

Lorsque le nombre de particules augmente, la charge positive augmente, le minimum disparaît d'où instabilité du noyau. Cette condition dépend de Z et de A.

Si Z et A sont suffisamment grands, ou plus exactement, si une fonction de Z et de A, $\frac{Z^2}{A}$ dépasse une certaine valeur, le noyau se brise spontanément. C'est le phénomène de fission spontanée (observé pour $^{238}_{92}U$ qui subit la bipartition avec une période de 10^{16} ans, c'est-à-dire environ 5 bipartitions par seconde par kilo d'uranium-élément naturel).

L'excitation d'un noyau qui dans l'état fondamental a un $\frac{Z^2}{A}$ inférieur à la valeur critique, peut provoquer la fission de celui-ci. En effet, l'énergie d'excitation communiquée au noyau par la capture d'une particule provoque des mouvements de la matière nucléaire analogues au mouvement d'un fluide sous l'action de la tension superficielle. En raison des déformations du noyau celui-ci peut se diviser de préférence en deux fragments comme une goutte liquide.

(1) P.-R. O'Connor, I. Perlman, G.-T. Seaborg et R.-C. Thomson, *Bulletin of the American Physical Society*, **22**, p. 5 (1947).

(2) N. Bohr et Wheeler, *Phys. Rev.*, **56**, 427 (1939); — Frisch et Lise Meitner.

Bohr et Wheeler calculèrent théoriquement la valeur de $\left(\frac{Z^2}{A}\right)_{\text{limite}}$ au-dessus de laquelle le noyau se divise si la matière subit une très petite déformation.

Examinant ensuite les conditions de stabilité pour une grande déformation du noyau ils déterminèrent l'énergie d'excitation (énergie de fission Ef) nécessaire pour la fission du noyau de Z et A donnés; $\left(\frac{Z^2}{A}\right)_{\text{lim}}$ a pour valeur calculée 47,8.

On peut calculer l'énergie minima de la particule incidente pour provoquer la fission en retranchant de l'énergie de fission particulière au noyau composé l'énergie de liaison de la particule capturée dans ce noyau. On peut ainsi expliquer que $^{235}_{92}U$ subit la fission avec des neutrons thermiques et non $^{238}_{92}U$ et prévoir l'énergie minima des neutrons capable de provoquer la fission de ^{234}U, Pa, Io, Th, etc.

On prévoit aussi la variation de la section efficace de fission avec l'énergie de la particule incidente.

Transuraniens.

L'étude des radioéléments formés dans l'uranium sous l'action des neutrons ou d'autres projectiles (de grande énergie) α; p, d, γ, a permis de mettre en évidence la formation de noyaux de $Z > 92$ appelés transuraniens. C'est ainsi que furent découverts les nouveaux éléments neptunium, plutonium, américium, curium de Z 93, 94, 95, 96. Symboles Np, Pu, Am, Cm (1).

Ces éléments du point de vue chimique se comportent comme une série analogue à celle des terres rares, la nouvelle série commence à l'actinium.

D'après Seaborg on peut représenter par le schéma ci-contre :

57 La	58 Ce	59 Pr	60 Nd	61	62 Sm	63 Eu	64 Gd	65 Tb	66 Dy	67 Ho	68 Es	69 Tm	70 Yb	71 Lu

Lanthanides

89 Ac	90 Th	91 Pa	92 U	93 Np	94 Pu	95 Am	96 Cm

Actinides

Schéma I

(1) On trouvera des détails sur les transuraniens dans plusieurs articles, dont G.-T. Seaborg, *Chemical and Engineering News*, **25**, p. 358 (1947); *Science*, **104**, p. 379 (1947), etc.

les deux séries d'éléments, lanthanides d'une part, actinides d'autre part. Les 14 éléments suivant le Lanthane correspondent à l'addition de 14 électrons successifs dans la couche interne 4f, tandis que les éléments de la série des actinides correspondent à l'addition d'électrons successifs dans la couche interne 5f. D'après un article de Seaborg, cet auteur a donné le nom Américium à l'élément 95 (6 électrons dans la couche 5f) par analogie avec le nom Europium donné à l'élément correspondant des lanthanides (6 électrons dans la couche 4f), et Curium à l'élément 96 (7 électrons dans la couche 5f) pour rappeler le nom des auteurs de la découverte des premiers éléments radioactifs par analogie avec Gadolinium (7 électrons dans la couche 4f) du nom de Gadolin. On peut aussi remarquer que le Curium 242, dont $A = 4n + 2$, fait partie de la série du Radium découvert par les Curie.

Voici quelques réactions où ces éléments se forment, les périodes de décroissance sont indiquées.

$$\text{I}\quad {}^{239}_{92}\text{U} \xrightarrow[23\text{ mi}]{\beta^-} {}^{239}_{93}\text{Np} \xrightarrow{\beta^-} {}^{239}_{94}\text{Pu} \xrightarrow[24000\text{ ans}]{\alpha} {}^{235}_{92}\text{U} \longrightarrow \text{famille}$$

radioactive naturelle $A = 4n + 3$ (série actinium).

${}^{239}_{92}\text{U}$ se forme par réaction n, γ sur ${}^{238}_{92}\text{U}$

$$\text{II.}\quad {}^{237}_{92}\text{U} \xrightarrow[7\text{ j. }07]{\beta^-} {}^{237}_{93}\text{Np} \xrightarrow[2.25 \times 10^6\text{ ans}]{\alpha} {}^{233}_{91}\text{Pa} \xrightarrow{\beta^-} \text{nouvelle famille ra-}$$

dioactive n'existant pas dans la nature ($A = 4n + 1$) contenant l'élément Francium $Z = 87$ (1)

${}^{237}_{92}\text{U}$ provient de la réaction (n, 2 n) sur ${}^{238}_{92}\text{U}$.

$$\text{III}\quad {}^{238}_{92}\text{U} + {}^{2}_{1}\text{H} \longrightarrow {}^{238}_{93}\text{Np} + 2n \qquad \text{et}$$

$$ {}^{238}_{93}\text{Np} \xrightarrow[2\text{ j}]{\beta^-} {}^{238}_{94}\text{Pu} \xrightarrow[50\text{ ans}]{\alpha} {}^{234}_{92}\text{U} \longrightarrow \text{famille radioactive}$$

naturelle, $A = 4n + 2$ (famille du radium)

---

(1) M^lle M. Perey, *Journ. de Phys. et de Rad.*, **10**, 435 (1939).

$A = 4n$

$^{232}_{90}Th$

$\alpha \downarrow 2\times10^{10}$ ans

$^{228}_{88}MsTh1$

$\beta \downarrow 9.74$ ans

$^{228}_{89}MsTh11$

$\beta \downarrow 8.9$ heures

$^{228}_{90}RaTh$

$\alpha \downarrow 2.75$ ans

$^{224}_{88}ThX$

$\alpha \downarrow 5.25$ jours

$^{220}_{86}Tn$

$\alpha \downarrow 78$ sec.

$^{216}_{84}ThA$

$\alpha \downarrow 0.23$ sec.

$^{212}_{82}ThB$

$\beta \downarrow 15.3$ heures

$^{212}_{83}ThC$

$\beta \downarrow$ 87 min. — 87 min. $\downarrow \alpha$

$^{212}_{84}ThC'$ — $^{208}_{81}ThC''$

$\alpha \downarrow 4\times10^{-7}$ sec. — 4.5 min. $\downarrow \beta$

$^{208}_{82}Pb$ ou (ThD)

$A = 4n+1$

$^{237}_{92}U$

$\beta \downarrow 10$ jours

$^{237}_{93}Np$

$\alpha \downarrow 3.23\times10^{6}$ ans

$^{233}_{91}Pa$

$\beta \downarrow 39$ jours

$^{233}_{92}U$

$\alpha \downarrow 2.36\times10^{5}$ ans

$^{229}_{90}Th$

$\alpha \downarrow 7 \times 10^{3}$ ans

$^{225}_{88}Ra$

$\beta \downarrow 20$ jours

$^{225}_{89}Ac$

$\alpha \downarrow 14.5$ jours

$^{221}_{87}Fr$

$\alpha \downarrow 7$ min.

$^{217}_{85}At$

$\alpha \downarrow 3\times10^{-2}$ sec.

$^{213}_{83}Bi$

$\beta \downarrow 66$ min.

$^{213}_{84}Po$

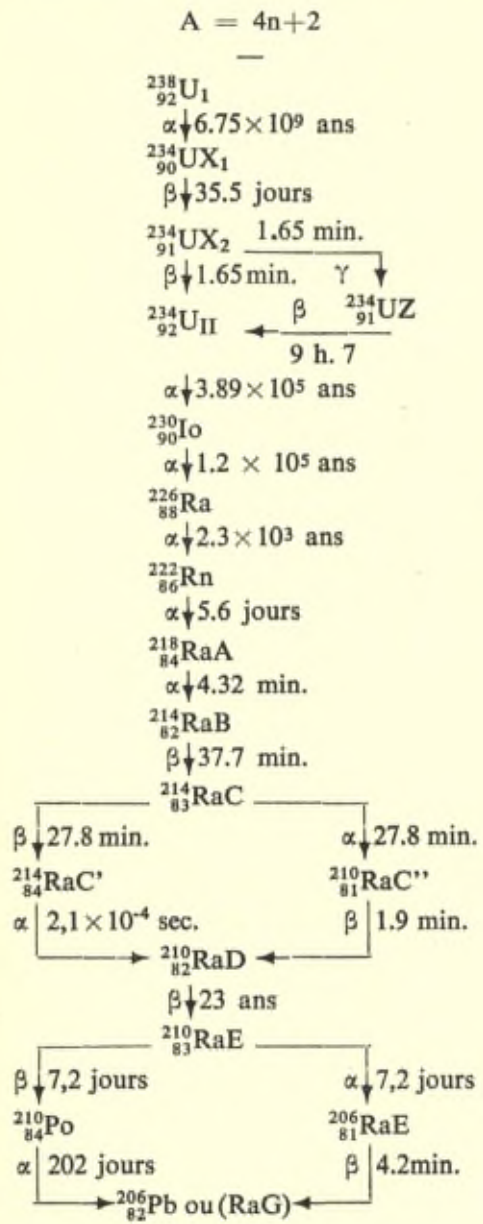
$\alpha \downarrow 10^{-5}$ sec.

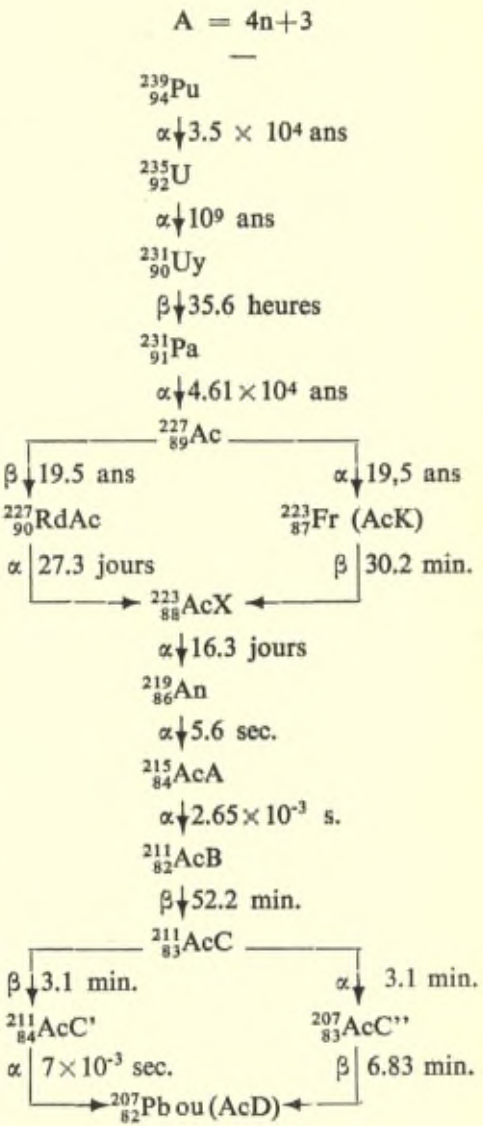
$^{209}_{82}Pb$

$\beta \downarrow 4.7$ heures

$^{209}_{83}Bi$

rayonnements et vies moyennes





IV $^{238}_{92}U + {}^{4}_{2}He \longrightarrow {}^{241}_{94}Pu + {}^{1}_{0}n$ et $^{241}_{94}Pu \xrightarrow[\text{longue}]{\beta^-} {}^{241}_{95}Am \xrightarrow[500\text{ ans}]{\alpha}$

$^{237}_{93}Np \xrightarrow{\alpha}$ (famille 4n + 1)

V $^{239}_{94}Pu + {}^{4}_{2}He \rightarrow {}^{242}_{96}Cm + {}^{1}_{0}n \rightarrow {}^{242}_{96}Cm \xrightarrow[5\text{ mois}]{\alpha} {}^{238}_{94}Pu \xrightarrow{\alpha} {}^{234}_{92}U$

(famille du radium).

Ces isotopes et d'autres isotopes des transuraniens ont été obtenus par irradiation de l'uranium avec des deutérons (20 MeV) et alpha (40 MeV) de divers types :

(d, n) (d, 2n) (d, 3n) (d, 4n) (α, p) (α, p2n) (α, p3n) (α, p4n)

par exemple : Np isotopes 234, 235, 236, 241.

Il existe $^{242}_{95}Am$ et $^{242}_{96}Cm$.

Signalons la réaction $^{231}_{91}Pa$ (d, n) $\rightarrow {}^{234}_{93}Np \xrightarrow[4\text{ j. }4]{} {}^{234}_{92}U$ capture K.

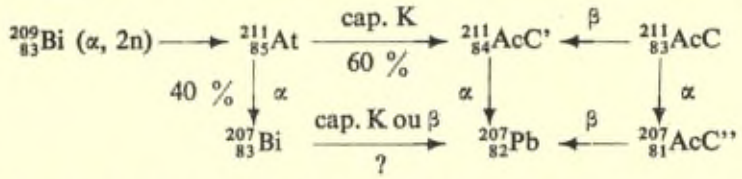
Éléments Z = 43, 61, 85, 87.

La synthèse de l'élément 43, n'existant pas dans la nature, a été faite par Segré et Perrin. C'est le nuclide $^{99}_{43}$ qui fut isolé par Segré et Saeborg parmi les produits de fission de l'uranium; sa période de désintégration est très longue, elle est d'environ 10^6 ans. Après avoir identifié les principales propriétés chimiques de cet élément le nom de Technétium lui fut donné.

L'élément 61 n'existant pas dans la nature a aussi été mis en évidence parmi les produits de fission. Deux nuclides $^{147}_{61}$ et $^{149}_{61}$ de périodes respectives de 3,7 ans et 47 heures furent identifiés. On ne lui a pas encore donné un nom définitif.

Par la réaction (α, 2n) sur $^{209}_{83}Bi$, le nuclide $^{211}_{85}$ émetteur de α fut préparé et le nom d'Astatine lui fut donné. Ce nuclide, qui par son numéro atomique se trouve dans la région des familles radioactives naturelles, a une valeur de A = 211 nombre de masse, de la forme 4n + 3. Il devrait appartenir à la famille de l'actinium. A ma connaissance, ce nuclide n'a pas été identifié dans cette famille.

Toutefois $^{211}_{85}$ par capture K donne $^{211}_{84}$AcC' de la famille de l'actinium. Le schéma est le suivant :



Un autre noyau du même élément chimique Astatine $^{217}_{85}$At fut trouvé dans la quatrième famille radioactive dont la tête est $^{237}_{92}$U de synthèse. $^{217}_{85}$At émet les rayons α avec une période de 3.10^{-2} secondes. De même il a été mis en évidence un Francium $^{223}_{87}$Fr ou AcK (Mlle Perey), émetteur de β^-, dans la famille de l'actinum $4n + 3$ et $^{211}_{87}$Fr dans la famille $A = 4n + 1$.

Les éléments de cette famille sont reportés sur le schéma ci-contre en compagnie de ceux des trois autres familles. Pendant longtemps les radioactivistes ont recherché à la mettre en évidence. Comme le tableau ci-joint le montre, cette famille se comporte, du point de vue de la nature des désintégrations de certains de ses termes, différemment des trois autres familles connues. En particulier l'élément terminal stable de la famille est le bismuth 209 au lieu d'un plomb; elle comporte un radium émetteur de rayons β.

Production des radioéléments dans les piles.

On sait comment les réactions de fission peuvent s'entretenir d'elles-mêmes au sein d'une pile à uranium. Dans les barres d'uranium de la pile s'accumulent les produits de fission en quantité importante (pondérable). Ceux-ci sont périodiquement séparés de l'uranium des barres. La pile constitue donc une source très importante de radioéléments de fission.

Pendant le fonctionnement de la pile, celle-ci est le siège d'un rayonnement de neutrons d'une prodigieuse intensité. En introduisant dans la pile des substances à transformer absorbant des neutrons (en quantité insuffisante pour arrêter le développement des réactions de fission) on produit des radioéléments artificiels en quantités considérables. En définitive, l'emploi des piles à uranium, même d'énergie faible, permet de produire des radioéléments artificiels répondant aux besoins d'un grand nombre d'applications.

Un article très détaillé paru dans *Science* (1) traite de la production

(1) « Announcement from Headquarters », Manhattan Project, Washington D. C.; *Science*, 103, 697 (1946).

des indicateurs radioactifs et de l'organisation de leur distribution alors réservée aux recherches américaines. Un tableau indique en particulier les radioéléments produits par la réaction (n, γ) dans des piles et les quantités maxima que l'on peut approximativement se procurer.

En voici quelques exemples :

	^{24}Na	14 h. 8	100 millicuries
	^{32}P	14 j. 3	500 millicuries
	^{35}S	87 jours	1 millicurie
	^{42}K	12 h. 4	1 curie
	^{55}Fe	4 ans	100 millicuries
	^{59}Fe	44 jours	1 millicurie
par réaction n. $\gamma \rightarrow$	^{131}I	8 jours	100 millicuries

Un radioélément de très grande importance pour les applications biologiques est le ^{14}C de période 25.000 ans. Sa production avec les cyclotrons déjà puissants, par la réaction $^{14}_{7}N + {}^{1}_{0}n = {}^{14}_{6}C + {}^{1}_{1}H$ a un rendement trop faible pour permettre aisément les applications.

La réaction (n, p) avec les neutrons rapides des piles permet de rendre disponibles, pour les recherches particulières, des quantités de 1 millicurie de ^{14}C.

Les produits qui peuvent être fournis ont d'assez grandes activités spécifiques (millicurie par gramme de matière) :

^{24}Na	250 mCur/gr
^{32}P	72 mCur/gr
^{42}K	20 mCur/gr
^{131}I	2,5mCur/gr

On remarquera que l'on peut, pour certaines expériences, préférer employer des indicateurs radioactifs préparés par le cyclotron qui permet de les produire avec de très grandes activités spécifiques (possibilité de les produire par des réactions donnant un radioélément de nature chimique différente de celle des atomes de la cible), par exemple :

$$^{130}_{52}Te + {}^{2}_{1}H \longrightarrow {}^{131}_{53}I + {}^{1}_{0}n$$

On trouvera ci-après la liste des 90 radioéléments artificiels qui sont produits dans les piles à uranium du Laboratoire de Clinton (U. S. A.) et régulièrement disponibles jusqu'ici seulement pour les recherches aux U. S. A.

Antimoine	122	Europium	154	Potassium	42
	124		155	Praséodyme	142
	125	Fer	55		143
Argent	108		59	Rhénium	186
	110	Gallium	72		188
	111	Germanium	71	Rhodium	105
Argon	37		77	Rubidium	86
Arsenic	76	Hafnium	199	Ruthénium	97
	77	Indium	114		103
Baryum	131	Iode	131	Samarium	153
Bismuth	210	Iridium	192	Scandium	46
Brome	82		194	Selenium	75
Cadmium	109	Lanthane	140	Sodium	24
	115	Mercure	197	Soufre	35
Calcium	45		203	Strontium	89
Carbone	14		205	Tantale	182
Cérium	141	Molybdène	99	Technetium	97
	143	Néodyme	147		99
Cœsium	131		149	Tellure	127
	134	Nickel	59		129
Chlore	36	Or	198		131
Chrome	51		199	Thallium	206
Cobalt	60	Osmium	185	Titane	51
Cuivre	64		191	Tungstène	185
Elément, 61	147		193		187
	149	Palladium	103	Yttrium	90
Etain	113	Phosphore	32	Zinc	65
	121	Platine	197		69
	123		199	Zirconium	95
		Polonium	210		

Très récemment, par décision du Président des Etats-Unis, des radioéléments ont été rendus disponibles, sous certaines conditions fixées et contrôlées par la Commission de l'Energie Atomique américaine, pour les savants des diverses nations, et destinés aux applications biologiques et médicales.

Je voudrais, pour terminer, envisager très brièvement quelques considérations pratiques concernant les radioéléments artificiels.

Je rappelle que l'on sait actuellement faire la synthèse de 450 radioéléments et qu'il existe au moins un isotope radioactif pour chaque élément de nombre de charge compris entre 1 et 96. Les radioéléments isotopes des éléments chimiques n'existant pas dans l'écorce terrestre, ont été formés (43, 71, 85) et l'on a fait la synthèse des éléments nouveaux 93, 94, 95, 96. Un même radioélément artificiel

peut souvent être formé par un grand nombre de réactions nucléaires. Le ^{32}P par 6 réactions différentes, ^{70}Ga par 8, etc.

Pour préparer un radioélément artificiel destiné à une application, on choisira la réaction ayant le plus grand rendement (en fonction évidemment de la nature et de l'intensité des sources de projectiles dont on dispose). En outre, on s'attache à choisir la réaction qui permet d'obtenir la plus grande activité spécifique (rapport du nombre d'atomes radioactifs au nombre total de ceux de l'isotope stable qui l'accompagne). C'est ainsi que chaque fois que le radioélément dérivé peut se produire par réaction (n, γ) il y a avantage à choisir cette réaction à grand rendement si la méthode de séparation Szilard et Chalmers est possible. Il y a aussi souvent avantage à utiliser la réaction (d, p) qui a un grand rendement. En effet, le parcours des deutérons dans la cible est relativement faible, $1/10^e$ à quelques dixièmes de millimètres et tous les atomes radioactifs sont concentrés dans cette couche mince qu'il suffit de gratter pour obtenir une activité spécifique suffisamment élevée. Exemple :

$$^{30}_{15}P + ^{2}_{1}H \longrightarrow ^{32}_{15}P + ^{1}_{1}H$$

on obtient facilement un atome de radiophosphore pour 10^8 atomes de phosphore inactif avec un cyclotron de moyenne puissance.

Très souvent on choisit une réaction du plus haut rendement possible qui donne le radioélément désiré à partir d'une cible de nature chimique différente. Exemple :

$$^{130}_{52}Te + ^{2}_{1}D \rightarrow ^{131}_{53}I + ^{1}_{0}n \qquad \text{ou}$$
$$^{32}_{16}S + ^{1}_{0}n \rightarrow ^{32}_{15}P + ^{1}_{1}H$$

La séparation de l'iode du tellure ou du phosphore du soufre, est aisée et permet d'atteindre avec un cyclotron moyen des activités absolues suffisantes et spécifiques très élevées.

Les radioéléments isotopes de 50 éléments chimiques différents ont déjà été utilisés pour des applications en biologie, chimie et physique.

Enfin, il ne suffit pas de consulter les tables de radioéléments artificiels pour en déduire si une recherche nécessitant un radioélément artificiel particulier est possible ou non (autrement dit il ne suffit pas d'examiner si le radioélément existe ou non). En effet des facteurs importants concernant la préparation et les caractéristiques radioactives de l'élément interviennent. Certains radioéléments sont faciles à préparer, d'autres très difficiles. Certains émettent des rayonnements facilement mesurables, d'autres difficilement. Dans tous les cas, la consultation du physicien nucléaire et du chimiste sera nécessaire.

Paris, le 19 septembre 1947.

Discussion du rapport de M. Joliot

M. Paneth. I should like to add a few remarks to the part of Professor Joliot's report dealing with the chemistry of the newly produced elements.

Professor Joliot mentioned that a fourth radioactive series is now known whose members have atomic masses corresponding to the formula (4n + I). Those interested in this new series may like to learn that two short notes giving preliminary information have been published in the first August issue of the *Physical Review*. The authors of one note are Professor Seaborg and his collaborators in Chicago, of the other, former members of the British-Canadian Atomic Energy team in Montreal. The general results of both groups are in perfect agreement, and I hope that permission will soon be given for a more detailed description. The Canadian work was almost completely done in 8 or 9 months starting in November 1944, but secrecy regulations did not allow immediate publication. The high accuracy of the results obtained by the Canadian team in such a short time shows how much can be achieved nowadays by collaboration between competent chemists and physicists if they make full use of modern laboratory techniques. By way of contrast it is interesting to remember how many years ik took to complete the three old radioactive series.

Among the remarkable pecularities of this new series is the fact that its radium isotope emits beta rays and its actinium isotope alpha rays. As a consequence, there is no emanation represented in this series, but isotopes of the very rare elements 87 (francium) and 85 (astatine). Moreover, the stable end-product is the ordinary element bismuth and not a kind of lead as in the three other series.

As to the name of this series, both teams working on it agreed that it should take it from its longest-lived member neptunium 237. Thus a « neptunium series » (4n + I) will take its place beside the thorium series (4n), the uranium-radium series (4n + 2) and the uranium-actinium series (4n + 3).

Professor Joliot has already mentioned the name for the elements 43 (technetium), 85 (astatine) and 87 (francium). Quite recently a name was also proposed for element 61, the artificially produced rare earth; Professor M. L. Pool from Ohio State University recommends « cyclonium ». It is, however, doubtful whether Professor Pool's claim on the discovery of element 61 will be accepted, and I would rather recommend to abstain from using the name « cyclonium » until this question is settled.

Concerning the trans-uranium elements, Professor Joliot, in his report, says : « Ces éléments du point de vue chimique se comportent comme une série analogue à celle des terres rares, la nouvelle série commence à l'actinium ». This is the opinion expressed by Professor G. Seaborg, who played such an important part in the discovery and chemical investigation of the transuranium elements; but I am doubtful, whether we should follow him in his views on the «actinide series ». The idea that a group similar to that of the rare earths should begin « with or shortly after » uranium was expressed by Bohr as soon as 1922; he based his surmise on considerations about the probable electronic configuration of these elements. But Professor Seaborg goes further by starting already with actinium and assuming an atomic structure for the elements from actinium to curium similar to those from lanthanum to gadolinium.

If I interpret the views of theoretical physicists correctly, nothing certain is known yet about this point; but even if it could be convincingly shown that there is such a similarity in the electronic configuration, I would still hold that the question whether a « rare earths group » begins with actinium ought to be decided by chemists on chemical evidence. Now it seems to me that such chemical evidence is clearly against Professor Seaborg's view as it is expressed in the table of the Periodic system published by him. In this table, the elements thorium, protactinium and uranium have lost their positions as homologues of hafnium, tantalum and tungsten, and are grouped as homologues of cerium, praseodymium and neodymium, in order to express that they are forming the beginning of a new « rare earths group ». However, for a chemist the meaning of the somewhat vague expression « rare earths group » can only be that they all have 3 as their most characteristic valency, just as all elements of the old rare earths group are tervalent, in addition to the valency state 2 or 4 in a few cases. But who would consider 3 as the typical valency of uranium? In my opinion, the

hexavalency of uranium (in UF_6 for instance) shows that its place below tungsten is quite natural; in the same way as protactinium, with its maximum valency of 5, fills the place below tantalum; and thorium (maximum valency 4) that below hafnium. So the normal group regularities of the periodic system are very well observed in this region. There exist, of course, some similarities also between these elements and their horizontal neighbours, but this is in perfect agreement with their character as so-called « transition elements » in one of the long periods of the table; to call them on the basis of these similarities « rare earths » seems to me much exaggerated. The four neptunium, plutonium, americium and curium apparently behave so similarly to each other that it may be justified to call them a rare earths group; but to break the connection between actinium, thorium, protactinium and uranium with their homologues in the table, seems to me sacrificing the valuable chemical information which is given to us in the periodic table in favour of a non-chemical, and moreover doubtful, theoretical speculation.

It is hardly necessary to emphasize that the names proposed for the last two of the trans-uranium elements need not depend on the motivation given for them on the basis of this supposed correspondence of the two rare earths groups. If their discoverers, Seaborg and his group, recommend the names americium and curium, I am sure that all chemists will gladly accept their proposal, even if there may be some slight embarassment caused by having to call the lower valency states of element 96 « curious salts » (in French : « les sels curieux »). There will certainly be found a way to overcome this small linguistic difficulty, and the choice of names in honour of the country of their discoverers, and of Pierre and Mme Curie, will no doubt be generally applauded.

M. Bainbridge. — M. Joliot in his very interesting talk included mention of the special group of four pairs of stable isobares, the members of each pair differing by only one unit in Z. They are Cd — In 113, In—S 115, Sb—Te 123 and Re — Os 187. The energy region in which the members of a pair can co-exist as stable nuclei is very narrow. It is equal to the proper energy of two neutrinos plus the K level energy. I believe that some investigators in Switzerland have demonstrated that K-electron capture does occur in some cases so that the higher Z member of a pair transmutes. The periods of half life, are extremely long so that the observation of these radioactive changes is near the limit of observation by the methods used.

Recently Feenberg (1) has considered in detail the effect of compression in the Weizsäcker equation. This added term allows a somewhat closer fit to the experimentally observed packing fraction curve.

Since the discovery of artificial radioactivity by F. Joliot and I. Curie the number of these radioactive isotopes has grown to 450 or more. There remains the possibility of many more formed from (α, n,) (α, 2n) and (He^3, n) reactions. These isotopes should appear in the area on the right fringe of the A — Z versus Z stability curve. The higher activities obtained from (n, γ), (d, p) and (d, n) reactions have had the effect from leaving the (α, n) and (α, 2n) reactions explored to a lesser extent than the others.

M. Joliot. — « Le terme supplémentaire introduit par Feenberg traduisant un effet de compression contient-il aussi un paramètre empirique (fait-il intervenir les parités de A ou Z)? ».

M. Bainbridge. — It is essentially an added empirical factor which Feenberg has supported theoretically. A and Z appear as they do in the Coulomb surface and « symmetry » terms of the Weizsäcker equation.

---

(1) E. Feenberg, *Phys. Rev.*, **71**, 468a (1947).

Some Results of Mass-Spectrum Analysis

by

Kenneth T. BAINBRIDGE

I.

ISOTOPE STUDIES BY ION DEFLECTION METHODS

Two of the fundamental properties of atomic species are A, the mass number which defines the number of protons and neutrons in the nucleus, and M, the accurate isotopic weight, which gives a measure of the total energy released in the formation of the nucleus from Z protons and N = A — Z neutrons.

The mass number, A, of isotopes of an element Z, their relative abundance, and the isotopic weights of atoms are basic in the development of chemistry and physics.

The demonstration of the multiple isotopic nature of nearly all the chemical elements and the accurate determination of the isotopic weights of atoms were made brilliantly and rapidly by Aston. Extension of his work and wide applications of mass spectrographs and mass spectrometers have been made in physics, chemistry, geophysics, and biology. It is the object of my paper to report on the results of some of these researches.

The instruments developed for the study of isotopes by deflection of ion beams in various combination of electric and magnetic fields fall into two classes. Photographic recording is used where high resolving power, high dispersion, and high accuracy are paramount in the comparison of relative isotopic weights. Where accurate measurement of the relative abundance of isotopes is the prime consideration, magnetic spectrometers using electrical recording are preferable. The simplicity, wide range, and accuracy of electrical ion measurements as compared to photometric measurements favor the electrical method for all except those elements from which it is difficult or nearly impossible to obtain monenergic ion sources.

Photographic plate recording mass spectrographs.

Before describing the results of mass spectographic research, there should be a brief description of the instruments and their principles of operation. Ions heterogeneous in mass and velocity are collimated by a system of two slits, then introduced into an electric field which acts as a combination lens and prism (Figure 7). Ions of a wide range of masses are dispersed into an energy spectrum from which a band is selected by a third slit for further deflection in a magnetic field. The velocity dispersion in the first field is compensated by the velocity dispersion in the second field so that by proper choice of the intensities of the two fields and their geometrical position and extent, ions of the same e/m values are brought to a focus at the same region of a photographic plate. Refocusing at the plate can be obtained both for ions which have diverged from the second slit and for ions of the same value of e/m which emerge from the third slit with a spread in velocity over a small range which is controlled by the width of the third slit (H—1, C—1).

Double focusing spectrographs in which both direction and velocity focusing are attained were developed independently by Mattauch and Herzog, Dempster, and the author in 1934. Of these, Mattauch's (Figure 8) yields double focusing for all masses over the entire extent of the plate (M—1) (E—1). In this spectrograph, S_3 does not control the velocity spread and angular divergence independently, $\Delta v/v_{max} = \pm 1/3320$ and $\alpha_{max} = \pm 1/3320$ for a resolving power of 1750 when $S_2 = .008$ cm as was used. The one which I designed in England in 1934 (Figure 7) gives coincidence between the locus for velocity focusing and a linear mass scale over some 14 centimeters (B—1). Since the locus of the refocused divergent beams is at an angle of 5° to the other loci, the angle of divergence of the ion rays must be small. The dispersion is high, being 5 mm for 1% mass difference. For this instrument $\Delta v/v_{max} = \pm 1/500$ and $\alpha_{max} = \pm 1/2800$ for a resolving power of 15,000 when $S_2 = .0017$ cm. In the Dempster instrument, the plate and locus of foci for divergent beams coincide but the locus of the foci for velocity focusing is at an angle of 45° to the plate, so that in this case the extent of the velocity dispersion must be limited to secure good spectra (D—2, H—2).

More recently an instrument has been built by Jordan using a crossed electric and magnetic field velocity selector followed by a

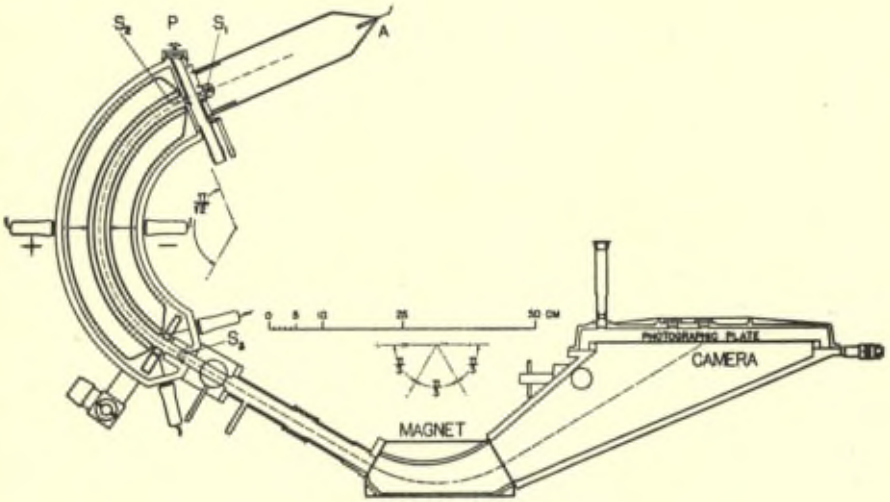


Fig. 7.
High Resolving Power Mass Spectrograph.
K. T. Bainbridge, E. B. Jordan,
Physical Review **50**, 282 (1936).

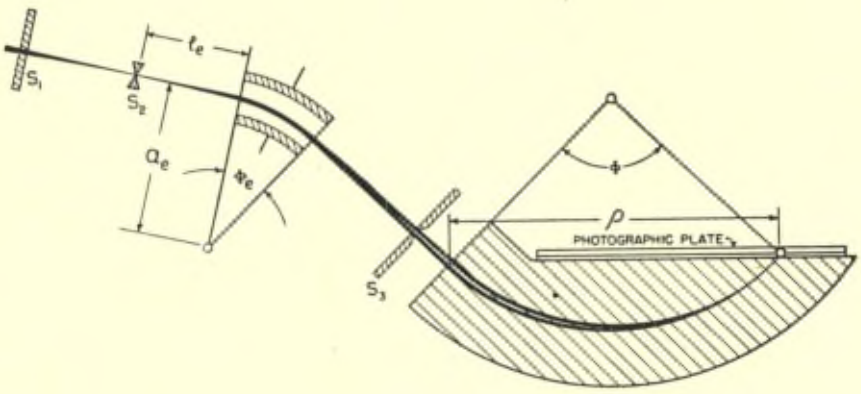


Fig. 8.
High Resolving Power Spectrograph with Double Focusing for All Masses
J. Mattauch, R. Herzog, *Z. Phys.* **89**, 786 (1934);
H. Ewald, *Z. für Naturforschung* I, 131 (1946).

60° magnetic analyser (J—1). This follows the general design principles suggested by Brueche and Scherzer (B—4) in their criticism of an earlier instrument used by the author (B—2). The resolving power and dispersion are high but the velocity and direction refocusing loci suffer from the same lack of coincidence as occurs in the instrument operated by the author and Jordan. The preferential selection of ions which has been noted should be easy to avoid while making mass comparisons for which the instrument was specifically designed (J—2). This type of selection is more difficult to prevent if one desires to measure the relative abundance of the isotopes of different elements.

The three mass spectrographs with which Aston contributed so much to the development of our knowledge of isotopes, refocused ions with a small spread in energy but did not refocus divergent beams of ions at the plate (H—2). The actual locus of the foci for divergent beams was a considerable distance in front of plate in each case so that very fine slits widely separated were necessary to minimize the effect of this lack of refocusing.

Many mass spectrographs have been described which have not been built (H—3, K—1). Had they been constructed, the results in many cases would be disappointing because no account has been taken in the design of a focusing effect, which can have serious results in diminishing the intensity of the ion beams incident on the plate or receiving slit. In those cases in which a beam of ions enters or leaves the boundary of a magnetic field at an angle ε to the normal, there is a divergent or convergent focusing effect in the z direction, i. e., perpendicular to the pole faces, for which the focal length is given by the relation :

$$f = \frac{\rho}{\tan \varepsilon}.$$

ρ is the radius of curvature of the ion in the magnetic field and ε is positive increasing towards the center of curvature (C—3, L—1). By proper use of the ε focusing condition one can, of course, partially compensate for the usual divergence of the beam in the z direction and obtain increased intensity at the receiving plate.

Magnetic spectrometer for ion analysis.

In the magnetic spectrometer, monenergic ions are produced by electron impact or by thermal ionization (Figure 9). These ions are accelerated in the electric field produced by a voltage V between

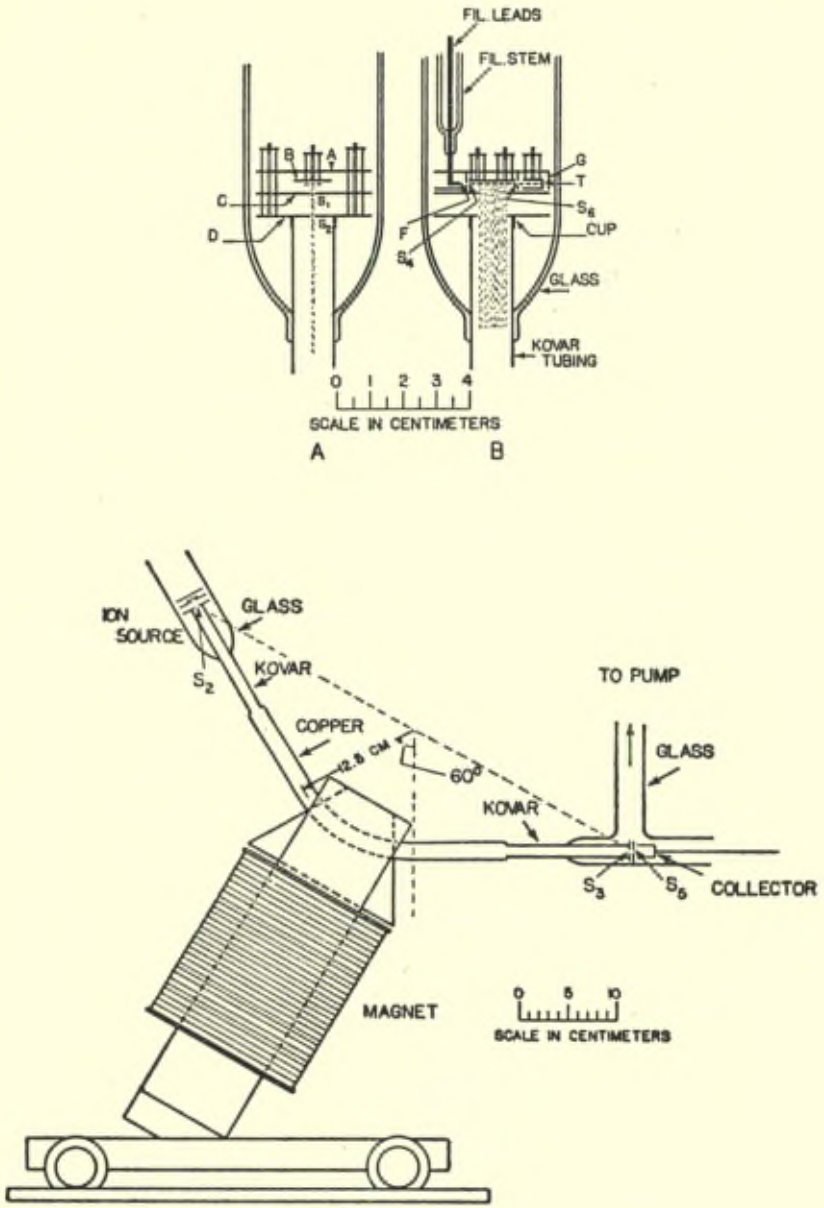


Fig. 9.
Magnetic Spectrometer,
Sector Type,
A.-O. Nier, *Rev. Sci. Inst.* **11**, 212 (1940).

plates 1 and 2, and describe an orbit of radius R in the magnetic field H;

$$R = \frac{144}{H}\sqrt{MV} \text{ cm}$$

for single charged ions for a magnetic field of H gauss and ion masses of M isotopic weight units ($0^{16} = 16$). The receiving slit is at a fixed position corresponding to a fixed value of R so that successive ions of mass M are brought to the receiving slit by varying V, MV = const. for a fixed value of H or by varying H, M/H^2 constant with V fixed. As the ions are all of the same energy within very close limits only «direction» focusing is necessary to produce an image of the source slit at the receiving slit. Such a result was first achieved under equivalent conditions by Classen for beta rays (C—2). It was Dempster who first applied the magnetic focusing technique to positive ion rays (D—1). Bleakney devised the present type of electron impact ion source and many of the technical advances which have contributed greatly to the reliability of this type of instrument (B—3). Tate, Smith (T—1), and particularly Nier (N—1), introduced further refinements and simplifications, so that today most of the many instruments in the U. S. A. were either built by Nier or directly follow his latest designs. The value of the Metcalf and Thomson FP—54 electrometer tube has also been very important in this development (M—2).

As magnetic spectrometers have many applications in chemistry, physics, and biology, some suggestions may be in order for their further improvement.

Improvement of the focus and resolving power in magnetic spectrometers.

A further angle focusing refinement, which may be of interest to others, is being incorporated by the author in a spectrometer which is now under construction.

The width of the ion beam at the receiving slit is reduced by proper shaping, of the pole piece boundaries so as to produce second order focusing. Once the image spread, $\sim \alpha^2 R$, of the refocusing of a divergent beam is eliminated, then most of the image width is due to the object slit width. Poor resolution due to gas collision scattering of ions has been eliminated in modern instruments by differential pumping methods and operation at very low gas pressures (10^{-6} — 10^{-4} mm of Hg). Poor resolving power attributable to inhomogeneity

in energy of the ions produced at the source has been made negligible by the use of high accelerating potentials, 1200 to 2500 volts.

For the usual 60° or 90° deflection type spectrometers, second order refocusing can be achieved if the following relation is fulfilled :

$$r_H = r_O \cot^2 \Theta (\cot \Theta - \alpha)$$

where r_H is the radius of the boundary of the field, r_O is the radius of curvature of the median ray, Θ is the half angle of deflection and α is the half angle of divergence, as illustrated in Figure 10.

To a good approximation, in the case of 90° focusing and symmetrical object and image, one needs only to make the radius of curvature (1) of the entrance and exit edges of the pole equal to the radius of curvature of the ion beam, r_O. Under these conditions, the image width will be equal to the source width. If the usual straight pole edges are used, the image width equals the object slit width plus $\alpha^2 r$.

The advantages of circular pole boundaries have not been previously utilized. Stephens suggested a graphical solution for one pole boundary to correct the focusing of electrons by solenoidal fields (S—1). Aston used circular pole pieces for simplicity under conditions where the requirements for angle focusing were not satisfied and had been obviated by extreme collimation of the incident beam. Herzog's theory omits second order terms and so gives the same focusing formulae for circular and straight edged poles (H—1). Cartan may have worked out the theory but definitely does not include it in his published work (C—1).

When the major contribution to the beam width at the receiving slit is produced by the initial divergence of the ion beam, then optimum resolving power is obtained for the asymmetrical arrangement of Figure 11 (S—1).

If the initial divergence of the ion beam is small, α small, or if the effects of angular divergence have been minimized as suggested above, then the resolving power is improved if the construction of Figure 12 is followed. Here a *reduced* image of the object slit is focused at the receiving slit (C—1). The usual design construction with straight pole edges is symmetrical about the center of curvature of the ions, Figure 10.

If a divergent beam of ions is used, the resolving power of all of

---

(1) The effective radius is between one and one and a half gap widths greater than the geometrical pole radius.

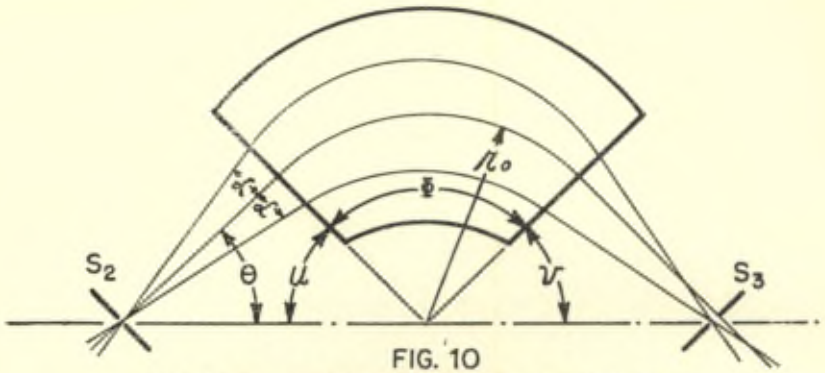


FIG. 10

MAGNETIC SPECTROMETER, SECTOR TYPE,
SYMMETRICAL, $\frac{\cos u}{\cos v} = 1$

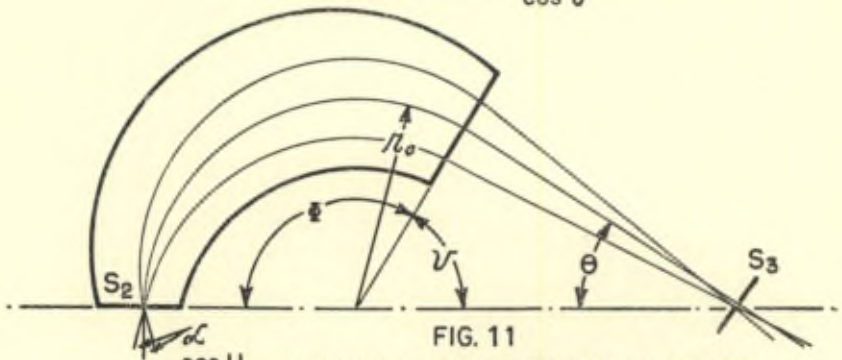


FIG. 11

$\frac{\cos u}{\cos v} = 2$ FOR MAXIMUM RESOLVING POWER
WHEN WIDTH $S_2 \ll \alpha^2 \rho_o$

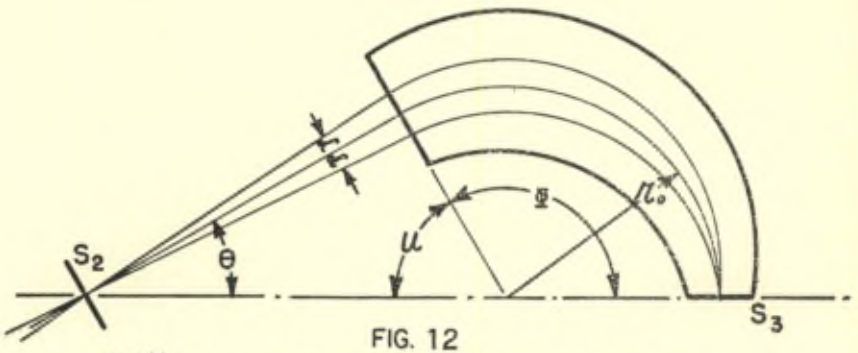


FIG. 12

$\frac{\cos u}{\cos v} < 1$ FOR INCREASED RESOLVING POWER
WHEN $\alpha^2 \rho_o \ll S_2$

these possible spectrometers or of those already built can be improved by proper profiling of the pole boundary.

Where only one pole edge is available for correction of the ion trajectories,

$$r_H = r_O \, (\cos^2 \Theta) \, (\cos \Theta - \alpha \sin \Theta)/(\sin^3 \Theta + 1).$$

The exact formulae for r_H have been worked out, but in practice the approximate solutions given above are satisfactory.

The Cycloidal Path Spectrometer.

Dr. Hipple has informed me that he is constructing a large mass spectrometer of the crossed electric and magnetic field type which has perfect focusing properties (B—5). The focusing is a property of M/e only and is not dependent on the velocity or direction of the ions entering the analyser in this type of instrument. The path of the ions is cycloidal.

B-1 K. T. Bainbridge, E. B. Jordan, *Phys. Rev.*, **50**, 282 (1936).
B-2 K. T. Bainbridge, *J. Franklin, Inst.* **215**, 509 (1933).
B-3 W. Bleakney, *Phys. Rev.* **34**, 157 (1929) et **40**, 496 (1932).
B-4 E. Brueche, O. Scherzer, *Geometrische Electronenoptik*, p. 304, J. Springer (1934).
B-5 W. Bleakney, J. A. Hipple Jr., *Phys. Rev.* **53**, 521 (1938).
C-1 L. Cartan, *J. de Physique* **8**, 453 (1937).
C-2 J. Classen, *Physik Z.* **9**, 762 (1908).
C-3 M. Cotte, Thèse, p. 40, Paris (1938).
D-1 A. J. Dempster, *Phil. Mag.* **31**, 438 (1916).
D-2 A. J. Dempster, *Proc. Am. Phil. Soc.* **75**, 755 (1935).
E-1 H. Ewald, *Z. für Naturforschung* **1**, 131 (1946).
H-1 R. Herzog, *Z. Phys.* **89**, 447 (1934).
H-2 R. Herzog, V. Hauk, *Z. Phys.* **108**, 609 (1938).
H-3 R. Herzog, V. Hauk, *Ann. d. Phys.* **33**, 89 (1938).
J-1 E. B. Jordan, *Phys. Rev.* **57**, 1072a (1940).
J-2 E. B. Jordan, *Phys. Rev.* **60**, 710 (1941).
K-1 A. Klemm, *Z. für Naturforschung* **1**, 137 (1946).
L-1 L. S. Lavatelli, PB — 52433, U. S. Dept. of Commerce, O. Tech. Services, MDDC, Report 350.
M-1 J. Mattauch, R. Herzog, *Z. Phys.* **89**, 786 (1934).
M-2 G. F. Metcalf et B. J. Thompson, *Phys. Rev.* **36**, 1489 (1930).
N-1 A. O. Nier, *Phys. Rev.* **52**, 933 (1937) et **53**, 282 (1938); *Rev. Sci. Inst.* **11**, 212 (1940) et **18**, 398 (1947).
S-1 W. E. Stephens, *Phys. Rev.* **45**, 513 (1934).
T-1 J. T. Tate, P. T. Smith, *Phys. Rev.* **46**, 773 (1934).

II.

ISOTOPIC WEIGHTS

The accurate determination of isotopic weights is of great importance.

The use of these accurate values in combination with the energy data from nuclear reactions makes it possible to check experimentally the equivalence of mass and energy, $\Delta E = \Delta MC^2$. One can determine the energy released in nuclear reactions and in fission from the mass change in the reactions, if one assumes the equivalence of mass and energy. Conversely a measurement of the energy change is a measure of the mass difference between the reacting nuclei and their products. In cases where ΔE and ΔM are both measurable, the Einstein relation is fulfilled within the present limits of accuracy of the measurements.

Accurate isotopic masses are a measure of the total binding energy of nuclei, assuming that protons and neutrons are the basic components.

In the theoretical consideration of intra-nuclear forces and the structure of nuclei the masses of n, H^1, D^2, T^3, He^3 and He^4 have been of particular value.

The determination of chemical combining weights of elements from relative abundance measurements of their isotopes and from isotopic weight data has been important in developing the present atomic weight table.

The Isotopic Weights of H^1, D^2, C^{12}.

In measuring isotopic weights, 0^{16} is taken as the standard of reference and equals 16 exactly. Since it is often impossible to refer other atoms to 0^{16} directly, molecular ions of H and D with C^{12} and O, such as OD_2, C_2, CH, etc., form secondary reference standards. Hence the importance of accurate values for the masses of H, D, and C^{12}. Also, the masses of H and D taken in combination with the energy required for the photo-disintegration of the deuteron, yield the most accurate value for the mass of the neutron. The masses of H and D also enter into the determination of the masses of He^3 and T^3, whether by disintegration methods now or by mass-spectrographic methods later when He^3 and T^3 become available. He^4 is related to D and O through the (2 D^2 — He) doublet.

The isotopic weights of H^1, D^2 and C^{12} relative to 0^{16} are obtained from the three mass spectrograph doublets

$$(2H - D) = a$$
$$(3\ D - C/2) = b$$
$$(CH_4 - O) = c$$

Solution of these equations yields :

$$H = 1 + 3/8\ a + 1/8\ b + 1/16\ c$$
$$D = 2 - 1/4\ a + 1/4\ b + 1/8\ c$$
$$C = 12 - 3/2\ a - 1/2\ b + 3/4\ c$$

All of the determinations of the doublets a, b, and c are listed in Table I, and the ones used in the present solution for the isotopic weights are underlined. The values listed under Mattauch and Bönish, 1938, are those obtained by a least squares solution involving a set of directly measured doublets and additional check doublets.

The values of the isotopic weights and their probable errors are :

$$H^1 = 1.008\ 128\ 3 \pm .000\ 002\ 8$$
$$D^2 = 2.014\ 718\ 6 \pm .000\ 005\ 5$$
$$C^{12} = 12.003\ 856 \pm .000\ 019$$

The doublet values were weighted objectively and the general procedure in the calculations for errors by internal consistency followed Birge's usage (B—1). The doublets not used were rejected on the basis of Wright and Hayford's criterion, according to which an individual value should be rejected if its divergence from the weighted value is greater than five times the probable error of the weighted value. The high $CH_4 - O$ value $364.9 \pm .8 \times 10^{-3}$ isotopic weight units is at the boundary for rejection, but is retained.

That the measurements of the oxygen-methane doublet have not been very satisfactory may be seen by inspection of Table I. This emphasizes the need for further work by other methods, particularly the direct comparison of $(C^{+++} - O^{++++})$, or $(C/3 - O/4)$ in the nomenclature of this paper. The doublets $(C - Ti^{48}/_4)$ and $(O - Ti^{48}/_3)$ furnish another approach, but measurements which have been made to date are not accurate enough to compare with the doublets *a*, *b*, and *c*. The doublets $(CD_2 - O)$and $(3\ D - C/2)$ would yield values for C and D in terms of O. If, however, the difficulties

TABLE I

Fundamental H. D. C. O Doublets.
M in 10^{-4} Mass Units.

Reference	a (2 H — D)	b (3 D — C/2)	c (CH_4 — O)
1 '27 Aston	—	—	350
2 '35 Aston	14.2	419.5	374
3 '36 Aston	15.2 ±0.4	423.6 ±1.8	360.1 ±2.4
4 '36 Bainbridge, Jordan	**15.3 ±0.4**	—	—
5 '36 Jordan, Bainbridge	—	—	369 ±2
6 '37 Bainbridge, Jordan	—	**421.9 ±0.5**	**364.9 ±0.8**
7 '37 Mattauch	—	—	366.9 ±0.6
8 '38 Mattauch, Bönish	**15.380 ±0.021**	**422.35 ±0.21**	**363.81 ±0.29**
9 '39 Asada, Okuda, Ogata, Yoshimoto	—	—	**364.2 ±0.35**
10 '40 Jordan (J-2)	—	—	**363.2 ±0.35**
Most probable values :	15.380 ±0.021	422.28 ±0.19	363.69 ±0.21

TABLE II

Reaction Energy Values.

No.	Reaction	Q Mev	Reference
1	Li^6 (d, α) He^4	22.20 ± 0.04	S-2
2	Li^7 (p, α) He^4	17.28 ± 0.03	S-2
3	Be^9 (p, α) Li^6	2.115 ± 0.04	A-2
4	Be^9 (d, α) Li^7	7.093 ± 0.022	G-1
5	Li^6 (p, α) He^3	3.945 ± 0.06	P-1
6	D^2 (d, n) He^3	3.243 ± 0.016	W-2
7	D^2 (d, p) T^3	3.98 ± 0.02	M-1
8	Li^6 (n, α) T^3	4.86 ± 0.04	L-3
9	Be^9 (p, d) Be^8	0.547 ± 0.006	
10	D^2 (γ, p) n'	— 2.1862 ± 0.0042	Page 66
11	$T^3 = He^3 + \beta$	0.012 ± 0.002	Page 70 and (W-1)
12	B^{11} (p, α) Be^8	8.60 ± 0.11	M-1
13	B^{10} (d, α) Be^8	17.76 ± 0.08	M-1
14	B^{10} (d, p) B^{11}	9.14 ± 0.06	M-1
15	B^{11} (d, α) Be^9	8.13 ± 0.12	M-1
16	$Q_1 + Q_3, Q_2 + Q_4$ (weighted average)	24.3556 ± 0.031	
17	$Q_{12} - Q_9, Q_{15}$ (weighted average)	8.088 ± 0.081	

in the CH_4 — O determination are related in some way to the behavior of the molecular ion CH_4, as Aston has suggested without proof (A—1), one might not gain in accuracy by going to the CD^2 — O doublet.

A direct measurement of helium relative to oxygen has been made by the author, using the doublet of the molecular ion of helium to doubly ionized oxygen or, (2 He — 0/2) (B—2). This doublet in combination with the (2 D — He) and (3 D — C/2) doublets provides another way of determining the isotopic weight of C.

The Isotopic Weight of the Neutron

An accurate value of the mass of the neutron relative to the hydrogen atom H^1 is obtained by the combination of the mass-spectrograph doublet (2 H — D) and the energy balance, $Q\gamma$, in the photo-disintegration of the deuteron (C—1).

$$n - H = -(2\ H - D) - Q\gamma$$

where $(2\ H - D) = .001\ 538\ 0 \pm .000\ 002\ 1$

and $-Q\gamma = 0.002\ 347\ 9 \pm 0.000\ 004\ 5$

mass units or $2.1862 \pm 0.0042 \times 10^6$ electron volts.

$$n - H = 0.0008100 \pm .0000050 \text{ m. u.}$$

or $0.7542 \pm 0.0047 \times 10^6$ e. v.

The isotopic weight of the neutron is then $n = 1.0089383 \pm 0.0000057$.

The value of $Q\gamma$ used is the weighted value from the five determinations summarized below.

Threshold Energy for the Photo-Disintegration of the Deuteron.

	Reference (S-1)	$-Q\gamma$ Mev	Probable Error
'38	Stetter, Jentschke	2.189	0.022
* '39	Rogers, Rogers	2.174	0.050
'40	Kimura	2.189	0.007
'42	Myers, Van Atta	2.183	0.012
'45	Wiedenbeck, Marhoefer	2.185	0.006
	Final weighted value	**2.1862**	**0.0042**

(*) Note added in proof. This value should not be included as the probable error in its determination was greater than that stated by the authors because of the effect of multiple scattering. (Bethe, *Phys, Rev*,, **70**, 821, 1946). The final weighted value is 2.1863 for the four remaining measurements.

The differences between the final weighted value and its probable error and the values published by Stephens are due chiefly to different treatment of the same data. The present most probable values were obtained by weighting the data according to error theory, while Stephens rejected the second and fifth entries in the table and directly averaged the results.

Experimental Tests of the Equivalence of Mass and Energy.

The first experimental test of the relationship $\Delta E = \Delta MC^2$ was made by the author in 1933 using mass spectrograph measurements of H, D, Li^6, and Li^7 in combination with the ΔE or Q values, « heats of reaction », obtained by Lewis, Livingston, and Lawrence, and by Cockroft and Walton for the reactions :

$$(1)\ Li^6 + D^2 = 2\ He^4 + Q_1$$
$$(2)\ Li^7 + H^1 = 2\ He^4 + Q_2$$

listed in Table II.

The difference of the measured doublets 2 (2 D — He^4) — (3 D — Li^6) should equal Q_1 in mass units if $\Delta MC^2 - \Delta E = Q$. The mass change in the reaction is 0.0238 ± 0.0004 using 1933 values throughout and is 0.02381 ± 0.0004 using the 1933 measurements for the doublet separations, which are altered only by applying the present accepted values of H and D to obtain the plate dispersions. The Q value available at that time was 23.0 Mev equivalent to 0.02570 mass units (L—1). The most probable Q value today is 22.20 ± 0.04 Mev corresponding to $.02385 \pm .00004$ mass units in better agreement with the mass value. The agreement where the differences of the two doublets are involved is much better than the individual doublet measurements, as there were systematic errors which tend to cancel in the subtraction.

The Li^7 reaction data are summarized in the following table.

	ΔM old	Mass units	ΔM new
Mass Spectrograph	0.018 1 $\pm$ .000 6	0.018 7	$\pm$ 0.000 6
Disintegration value	0.018 40 $\pm$ .000 06	0.018 56	$\pm$ 0.000 03

The new ΔM value is the mass spectrograph measurement of 1933 (B — 3) referred to the present values of H and He, 1.008128 and 4.003880 respectively. The old disintegration ΔM values are from Cockroft and Walton (C—2), and the present most probable value 0.01856 is that given by Smith (S—2). These results are mainly of historical interest as more accurate values for Li^7 and (2 D — He) have been obtained with improved instruments.

Since the earliest experiments there have been many researches which permit more accurate tests of the equivalence of mass and energy. No anomalous cases outside of the limits of error have appeared which could not be explained either by errors in the results themselves which subsequently become evident or by misinterpretation of the reactions involved. So the Einstein relation rests upon a firm experimental basis, not only from the results of mass analysis and disintegration results, but also from precise measurements of the energy of the radiation produced in the two-quanta annihilation of positive electrons.

Some examples of more recent mass-spectrograph and disintegration results are of interest.

The doublet ($C^{12}H - C^{13}$) has been measured as $0.0045 \pm .0001$ mass units (B—4). $(C^{12}H - C^{13}) - (2\,H - D^2) = 0.00297 \pm .0001$ which should equal the Q value in mass units of the reaction $C^{12} + D^2 = C^{13} + H^1 + Q$. The desintegration value is $2.91 + .05$ mass units (L—2). In a more recent determination, Figure 13, by H. Ewald (E—I), $C^{12}H - C^{13} = 4.410 \pm 0.008 \times 10^{-3}$ mass units. This is between the values $4.5 + 0.1 \times 10^{-3}$ given above (B—4) and $4.37 \pm 0.05 \times 10^{-3}$ measured by Mattauch (M—2).

Similarly the mass-spectrograph doublets $(N^{14}H^1 - N^{15}) - (2\,H - D^2)$ indicate an energy release Q of 8.57 ± 0.2 Mev in the reaction $N^{14} + D^2 = H^1 + N^{15}$ (J—1). The reaction energy for comparison is 8.55 ± 0.08 Mev as measured by Cockroft and Lewis (L—2), and 8.51 ± 0.1 Mev according to Holloway and Moore (H—1).

The simultaneous solution of reaction equations provides yet another test of the equivalence of mass and energy. The Q values for the first four reactions in Table II have been measured with great care at the same laboratory. If all of the mass changes in the reactions appear as kinetic energy of the product nuclei, $Q_1 + Q_3$ should equal $Q_2 + Q_4$. The sum $Q_2 + Q_4 = 24.373 \pm 0.037$ Mev differs from $Q_1 + Q_3 = 24.315 \pm 0.057$ Mev by only 0.058 Mev and the agreement is satisfactory.

The Isotopic Weights of T^3 and He^3.

An accurate knowledge of the masses of T^3 and He^3 is important for theory and for experiment. These isotopic weights are obtained indirectly by the combination of mass spectrograph results and disintegration studies. Detailed consideration discloses discordant figures for reaction energies which should be re-examined.

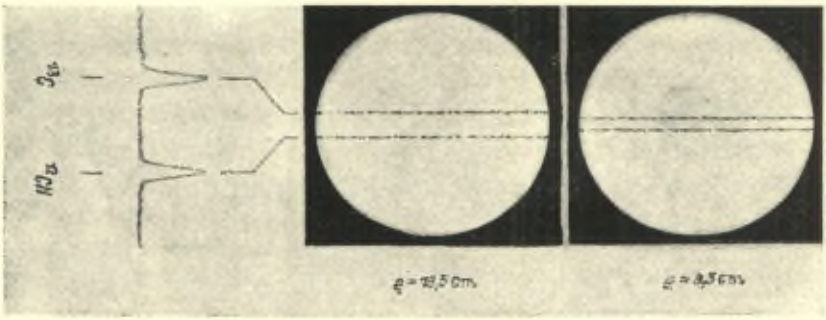


Fig. 13.

Microphotographs of the $C^{12}H$ — C^{13} doublet taken at two different positions on the plate. Enlarged 75 times.

H. Ewald, Reference (E—1).

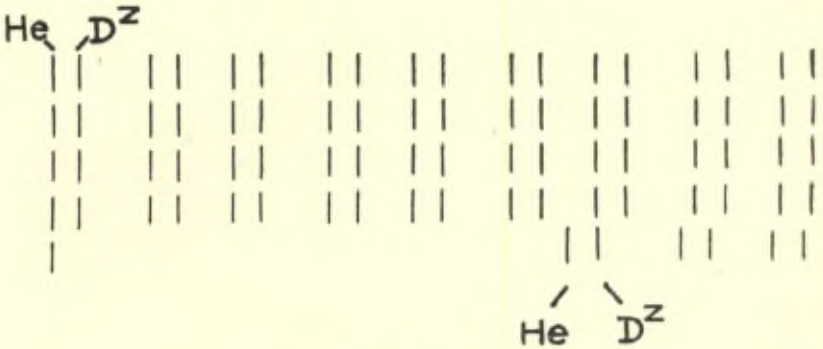


Fig. 14.

He^4 — D^2 doublets. Natural size.

K. T. Bainbridge and E. B. Jordan, Reference (B—6).

Using reactions (6), (7), and (10) of Table II for one solution and (5), (8) and (10) for another, one obtains :

a. $(T^3 - He^3) + Q_{10} + (2\ H - D^2) = Q_6 - Q_7$

b. $(T^3 - He^3) + Q_{10} + (2\ H - D^2) = Q_5 - Q_8$

All three terms on the left of the equations are very accurately known and their sum = —.7422 $\pm$ 0.0052 Mev.

$(T^3 - He^3) = Q_{11}$ which is equivalent to the maximum energy of the β rays emitted from T^3, 12 $\pm$ 2 Kev, assuming that the mass of the neutrino is zero.

$- Q_{10} = 2.1862 \pm 0.0042$ Mev, energy required for the photo-disintegration of the deuteron.

$(2\ H - D^2)$ is the hydrogen deuterium doublet separation equivalent to 1.4320 $\pm$ 0.0020 Mev.

The value — .7422 $\pm$ 0.0052 on the left side of the equations has to be compared with $Q_6 - Q_7 = 0.737 \pm 0.028$ for equation *a*, and $Q_5 - Q_8 = - 0.915 \pm 0.072$ for the right hand side of equation *b*.

If Q_6 is assumed to be correct, then :

$$He^3 = 3.017016 \pm 0.000032$$

and $$T^3 = 3.017029 \pm 0.000032 \text{ plus neutrino.}$$

The values are obtained from reactions (6) and (11) using the mass-spectrographic values for D^2 and H^1, and the value for *n* given earlier. $Q_5 - Q_8$ is now equal to — 0.7422 Mev, an increase of 0.173 Mev which may act mainly to decrease Q_8 although we are dealing with differences in Q values and both may change (1).

The Q values for reactions (5), (6), (7) and (8) should be redetermined with the greatest care. The best determination of the He^3 and T^3 weights can be provided by measurement of the doublets $(HD - He^3)$, $(HD - T^3)$ and $(3\ D^2 - T_2^3)$ by a mass spectrograph.

---

(1) T. Lauritsen in a letter has called attention to the existence of a resonance at 0.27 Mev for neutrons in the Li^6 (n,) T^3 reaction where Q_8 is 4.86 $\pm$ 0.04 Mev (L—3), and 4.97 Mev, with no stated errors, from the work of Rumbaugh, Roberts and Hafstad (*Physical Review* **54**, 657, 1938). Possibly 0.3% of the observed particles could be attributed to neutrons of about this energy. The true Q value then would be too high by an amount dependent on the method of determination of the particle range and on the energy spectrum of the neutrons (undetermined in the experiments).

The Isotopic Weight of the Neutrino.

The neutrino was postulated by Pauli as a neutral particle with half integer spin and zero rest mass, or at any rate a mass small compared to the electronic mass. The main requirements it must satisfy are the conservation of energy and angular momentum in the β-ray disintegration process.

Many attempts have been made to obtain evidence for the existence of the neutrino. The measurement of the (T^3 — He^3) doublet in a mass-spectrograph is suggested as a possible method for the determination of the value of the rest mass which from other experiments is estimated to be equivalent to 25 Kev or less. The (T^3 — He^3) doublet separation should equal the rest mass of the neutrino plus the mass equivalent of the maximum energy of the β-rays emitted by T^3. This is not likely to be a simple experiment, as for the worst case, that of zero neutrino mass, the complete separation of the T^3 and He^3 lines would require a resolving power of 250,000. A resolving power of 250,000 is 10 to 20 times greater than the highest achieved to date by any mass spectrograph. It is worth emphasizing that if the measurement can be made at all by the doublet method, any probable error in the determination should be much smaller than that obtainable by the combination of nuclear reactions to give T^3 — He^3.

The Isotopic Weight of He^4 from Mass Spectrographic and Nuclear Reaction Data.

The isotopic weight of He^4 and the (D_2^2 — He) doublet separation, Figure 14, furnish the main connecting links between the isotopic weight scale secured from mass spectrographic data and the isotopic weight scale secured from the Q data of disintegration experiments. The comparison of the two scales is necessary to provide a combined mass scale for general use (B—5, 0—1).

The Q values which were used are listed in Table II and the doublets in Tables I and III.

The first step in the comparison of the mass spectrographic and nuclear reaction isotopic weight scales is to solve for the weight of He^4 from mass spectrographic data alone.

The final weighted value for the isotopic weight of He^4 from mass spectrographic doublet measurements is 4.003851 ± 0.000032, corresponding to $255.87 \pm 0.32 \times 10^{-4}$ weight units for the (2D — He) doublet separation. This value was obtained by weighting the

following two values. $He^4 = 4.003847 \pm 0.000038$ from the weighted mean $255.90 \pm 0.36 \times 10^{-4}$ w.u. for the doublet (2 D — He) using Aston's value $255.1 \pm 0.8 \times 10^{-4}$ w.u. and the author's $256.1 \pm 0.4 \times 10^{-4}$ w.u. The author's direct comparison of the single charged molecular ion He_2^+ with doubly ionized oxygen gives a value for He^4 of 4.003860 ± 0.000060.

The second step in the comparison of the two scales is to obtain the isotopic weights of Li^7, Be^9, B^{10}, B^{11}. All available mass-spectrographic data were weighted objectively according to error theory. The doublets used are listed in Tabel III and Table I, and the isotopic weights are listed in Table IV*A*.

The next step is to obtain expressions for He^4 in terms of Li^7, Be^9, B^{10}, B^{11} and the Q values from disintegration experiments listed in Table IV.

These expressions are :

$2\ He^4 = Li^7 + H - Q_2$ from reaction (2).

$3\ He^4 = Be^9 + D + H - Q_{16}$ from reactions (2) and (4) and also from (1) and (3). Q_{16} is the weighted average of $Q_1 + Q_3$ and $Q_2 + Q_4$.

$4\ He^4 = B^{10} + 3\ D + Q_9 - Q_{13} - Q_{16}$ from the combination of reactions (9) and (13) with the equation above containing Be^9.

$4\ He^4 = B^{11} + 2\ D + H - Q_{16} - Q_{17}$ from reaction (15) and the equation above involving Be^9. Reactions (12) and (9) can be substituted for reaction (15). The weighted average Q_{17} has been substituted for Q_{15} and $Q_{12} - Q_9$.

When the mass-spectrographic values for Li^7, Be^9, B^{10}, and B^{11} of Table IV*A* are substituted in these expressions, four values of He^4 are obtained.

A fifth value for He^4, in this case an upper limit, can be obtained from disintegration data and the isotopic weight of D^2 if Be^8, which has not yet been found to occur as a stable isotope, is assumed to be unstable. The fifth value for He^4 is obtained from the combination of reactions (2), (4) and (9) and (1), (3) and (9), which is equivalent to (16) and (9). If Be^8 is unstable and can disintegrate into two helium nuclei with a total release in energy of Q_{Be}.

$$Be^8 \geqslant 2\ He^4 + Q_{16} - Q_9 - (D_2^2 - He^4)$$

or

$$(D_2^2 - He) \leqslant Q_{16} - Q_9$$

$Q_{16} - Q_9$ is equal to 0.025571 $\pm$ 0.000032 weight units so that He^4 must be greater than 4.003866.

The values obtained for He^4 and their sources are listed below in the second column. The probable errors are indicated below the He^4 figures.

Isotopic Weight of He^4

Source	All Doublets	Doublets 11, 12, 25 omitted
Mass Spectrograph alone	4.003 851	4.003 851
	$\pm$ 32	32
M. S. and Q values for Li^7	4.003 884	4.003 884
	53	53
M. S. and Q values for Be^9	4.003 959	4.003 916
	51	70
M. S. and Q values for B^{10}	4.003 950	4.003 917
	30	39
M. S. and Q values for B^{11}	4.003 891	4.003 896
	32	34
Q values and instability of Be^8. . .	> 4.003 866	> 4.003 866
	32	32

The values obtained from Be^9 and B^{10}, Column 2, are out of line with the other four. If one searches for a possible cause one finds that these high values arise chiefly from the use of the doublets (11), (12) and (25) indicated by an asterisk in Table III. Livingston and Hoffman have called attention to the inconsistency of doublet (12) with disintegration measurements (L—3). Doublets (11), (12) and (25) were rejected on account of the apparent presence of errors inconsistent with the assigned probable errors and the isotopic weights of Ne^{20}, Be^9, B^{10}, B^{11} were then recalculated. The results are listed in Table IV *B*. Discrepancies remain in the Q values considered earlier, $Q_1 + Q_3 \neq Q_2 + Q_4$. Their effect is small compared to the probable errors from other sources and can be neglected. The new values in the third column above for He^4, from six sources, agree within their probable errors.

The weighted average of these six values is $He^4 = 4.003881 \pm .000016$ from the combination of nuclear reaction and disintegration data. Since in a calculation of this sort the final probable error is likely to be less than the true value, the figure suggested for adoption is :

$$4.003880.$$
$$\pm .000030.$$

TABLE III

Mass Spectrograph Doublets.

No.	Doublets	$\Delta M \times 10^4$ Isotopic Weight Units		Observer
1	$(2\ D^2 - He^4)$	255.1	$\pm$ 0.8	A
2	—	256.1	0.4	B, J
3	—	**255.87**	**0.32**	Weighted value of 1, 2, 4
4	$(2\ He^4 - O^{16}/2)$	77.2	1.2	B
5	$(Li^7 - N^{14}/2)$	144.3	1	B, J
6	$(Be^9H - Ne^{20}/2)$	239.1	2	J, B
7	$(Be^9H - B^{10})$	69.6	2	J, B
8	$(B^{10} - Ne^{20}/2)$	168.4	1.5	A
9	—	167.5	1.5	J, B
10	—	**167.95**	**1.06**	Weighted value of 7, 8
11*	$(B^{10}H - B^{11})$	116.0	1.0	J, B
12*	$(B^{10}H_2 - C^{12})$	287.5	2.0	J, B
13	—	**287.43**	**1.15**	Weighted value of 11, 12, 14
14	$(B^{11}H - C^{12})$	171.4	1.0	J, B
15	$(CH_2 - N^{14})$	125.81	0.23	Mattauch, Bönish
16	—	125.7	0.60	Asada and others
17	—	125.6	0.15	(J-2)
18	—	**125.66**	**0.12**	Weighted value of 15, 16, 17
19	$(Ne^{20} - A^{40}/2)$	111.42	0.38	M
20	$(O^{16}D_2 - A^{40}/2)$	418.9	2.0	J, B
21	$(O^{16}D_2 - Ne^{20})$	307.48	2.0	From 19 and 20
22	—	308.3	4.0	A
23	—	306.5	2.0	J, B
24	—	**307.135**	**1.33**	Weighted value of 21, 22, 23
25*	$(CD_4 - Ne^{20})$	638.16	0.5	M

Note. — Under Column 4, Observers, A, Aston; B, Bainbridge; J, Jordan; M, Mattauch. Doublets are listed in Reference M-1.

A-1 F. W. Aston, *Nature* **143,** 797 (1939).

A-2 S. K. Allison, L. S. Skaggs and N. M. Smith Jr., *Physical Review* **57,** 550 (1940).

B-1 R. T. Birge, *Physical Review* **40,** 207 (1932).

B-2 K. T. Bainbridge, *Physical Review* **53,** 922a (1938).

B-3 K. T. Bainbridge, *Physical Review* **44,** 56; 57, 123 (1933).

B-4 K. T. Bainbridge and E. B. Jordan, *Physical Review* **49,** 883a (1936).
J. Mattauch and R. Herzog, *Naturwiss.* **25,** 747 (1937).
H. Ewald, *Z. f. Naturforschung* **1,** 131 (1946).

B-5 H. A. Bethe, *Physical Review* **47,** 633 (1935).

C-1 J. Chadwick and M. Goldhaber, *Proc. Roy. Soc.* **151A,** 479 (1935).

TABLE IV

Isotopic Weights.

A Obtained from Doublets Tables I and III	B Doublets 11, 12, 25 of Table III not used
Li^7 7.018 20	7.018 20
$\pm$ 10	10
Be^9 9.015 19	9.015 06
$\pm$ 15	20
B^{10} 10.016 287	10.016 16
$\pm$ 72	13
B^{11} 11.012 847	11.012 87
$\pm$ 78	10
N^{14} 14.007 536	14.007 536
$\pm$ 22	22
Ne^{20} 19.998 885	19.998 72
$\pm$ 53	13

C-2 J. D. Cockcroft and E. T. S. Walton, *Proc. Roy. Soc.* **137A,** 229 (1932).

E-1 H. Ewald, *Z. f. Naturforschung* **1,** 131 (1946).

G-1 E. R. Graves, *Physical Review* **57,** 855 (1940).

H-1 M. G. Holloway and B. L. Moore, *Physical Review* **58,** 847 (1940).

J-1 E. B. Jordan and K. T. Bainbridge, *Physical Review* **50,** 98 (1936).

J-2 E. B. Jordan, *Physical Review* **60,** 710 (1940).

L-1 G. N. Lewis, M. S. Livingston and E. O. Lawrence, *Bull. Am. Phys. Soc.* **8,** No. 4, 13 (1933).

L-2 M. S. Livingston and H. A. Bethe, *Rev. Mod. Phys.* **9,** 245 (1937).

L-3 M. S. Livingston and J. G. Hoffman, *Physical Review* **53,** 227 (1938).

M-1 J. Mattauch, *Nuclear Physics Tables,* Springer (1942).

M-2 J. Mattauch, *Phys. Rev.* **57,** 1155 (1940).

O-1 M. L. E. Oliphant, A. E. Kempton and E. Rutherford, *Proc. Roy. Soc.* **150A,** 241 (1935).

P-1 G. J. Perlow, *Physical Review* **58,** 218 (1940).

S-1 W. E. Stephens, *Rev. Mod. Phys.* **19,** 19 (1947).

S-2 N. M. Smith Jr., *Physical Review* **56,** 548 (1939).

W-1 R. J. Watts and Dudley Williams, *Physical Review* **70,** 640 (1946). 12 $\pm$ 2 Kev is the weighted average of all determinations when the 9.5 $\pm$ 2 Kev value is corrected as suggested by O'Neal.

W-2 D. H. Wilkinson, private communication. The listed value is from Wilkinson's value 3.23 $\pm$ 0.02 Mev and Bonner's value (M—1). The latter is corrected to conform to accurate mass values in the expression for Q, and for straggling. The final Bonner value is 3.27 $\pm$ 0.03.

III.

MEASUREMENTS OF THE RELATIVE ABUNDANCE OF ISOTOPES

There have been many applications of mass spectrometric analysis to scientific problems which depend for an answer upon the determination of the relative abundance of isotopes or upon the relative intensities of ion beams of different M/e values.

Some of these applications are listed below. In some cases important results have been obtained quite recently, and they are summarized later.

Measurements of the relative abundance of isotopes have been most important to chemistry and physics for the identification of the stable atomic species of which all matter is constituted.

Abundance measurements of the isotopes of lead in conjunction with chemical analyses of radioactive minerals have brought new precision to the determination of the geological age of the earth (N—1).

In the case of certain radioactive isotopes mixed with others, measurement of the relative abundance of the isotopes allows a determination of the decay constant of the radioactive isotope (N—2, C—1, N—3).

The identification of fission products, and of atomic species with large neutron capture cross-sections, is described in detail in Section IV.

Data on the abundance of isotopes and accurate values of the isotopic weights have been very useful in fixing values for chemical atomic weights.

Recently, J. A. Hipple summarized as follows the type of problems in which the mass spectrometer has been used advantageously for the analysis of gases (H—1) :

1. Tracer work in which stable isotopes are used;
2. The determination of small amounts of a particular gas in a gas mixture where stringent purity standards must be met;
3. Analytical work where mass spectrometric analysis is more rapid than ordinary methods;
4. Cases where only very minute quantities of gas are available and an accurate analysis is required. Thode has obtained absolute measurements of the relative abundance of the

isotopes of krypton and xenon to an accuracy of $\pm$ 1 % using a sample equivalent to 10^{-3} cm^3 of vapor at atmospheric pressure (T—1). Nier reports that $\sim 4 \times 10^{-3}$ cm^3 of CO_2 at N. T. P. is an amount sufficient for a C^{12}/C^{13} determination (N—4);

5. The continuous analysis of gases, such as may be needed in an experiment or an industrial process.

Dr. Rittenberg has given a summary of important applications of spectrometric analysis to problems in chemistry (R—1). More recently, with A. O. Nier, Thode and others, he has contributed to a symposium on the preparation and measurement of isotopic tracers (T—2).

The Relative Abundance of the Isotopes of Helium, Boron, Silicon, Bromine, Krypton, Molybdenum, Tellurium, Xenon and Tungsten.

HELIUM

Alvarez and Cornog showed that He^3, known only as a product of nuclear reactions, existed as a component of helium from the atmosphere and from gas wells (A—1). Recently, Aldrich and Nier measured two samples of helium concentrated from the atmosphere by the Air Reduction Sales Company and obtained a figure of 1.3×10^{-6} for the He^3/He^4 abundance ratio (A—2). Two samples of gas well helium from an unspecified locality gave an abundance ratio of 1.6×10^{-7}. These concentrations are too small to have any effect on the atomic weight. The impressive feature of this work is the wide range in intensity which could be measured even with the He^3 peak completely resolved from the contaminant HD. Also, this work opened up the possibility for measuring the effectiveness of various fractionation methods in concentrating He^3.

BORON

The first electrical measurement of the isotopes of boron was reported by Inghram (I—1) who analyzed BF_3^+, BF_2^+, BF^+ and B^+ ions from BF_3, and B^+ ions from $BO(CH_3)_2$. A Nier type 60° magnetic spectrometer was used. The B^{10} abundance is 18.82 $\pm$ 0.2 percent, and B^{11} is present to 81.17 $\pm$ 0.2 percent. These results

confirm Ornstein and Vreeswijk's figures of 18.4 and 81.16 percent obtained from the analysis of line spectra. Inghram's results are of higher accuracy and supplant the earlier work. From this data and from the isotopic weight figures given in Section II, the chemical atomic weight is 10.822, where the ratio of the physical isotopic weight scale to the chemical combining weight scale is taken as 1.000275. It is unfortunate that the exact source mineral, its location, and the chemical history of the boron were not specified, since boron may possibly differ in its isotopic composition, depending on the source of the material, as Nier and Gulbransen have demonstrated in the case of carbon (N—7).

SILICON

The relative abundance of the isotopes of silicon have been measured independantly by Ney and McQueen, Williams and Yuster, and by Inghram (N—5, W—1, I—1). The results obtained are in very excellent agreement.

Observer	Si^{28}	Si^{29}	Si^{30}
N, M	92.24 $\pm$ 0.10	4.69 $\pm$ 0.05	3.07 $\pm$ 0.05
W, Y	92.27 $\pm$ 0.09	4.68 $\pm$ 0.05	3.05 $\pm$ 0.03
I	92.28 $\pm$ 0.08	4.67 $\pm$ 0.05	3.05 $\pm$ 0.03
Weighted average	92.27 $\pm$ 0.09	4.68 $\pm$ 0.050	3.05 $\pm$ 0.03

The error of the average of the determinations is not reduced below the errors of the individual measurements as the observers consider that possible *systematic* errors of 1 percent may be present.

The chemical atomic weight from these data and the packing fractions is 28.086_6.

BROMINE

The ratio of the relative abundance of Br^{81} and Br^{79} is given by Williams and Yuster as 0.979 ± 0.004 from a large number of measurements of the Br_2^+, Br^+, and Br^{++} ions (W—1). Errors of measurement are halved by measuring the molecular ions, as the ratio of abundance Br^{81}/Br^{79} is equal to the square root of the measured ratio of the $(Br^{81}_2)^+$ and $(Br^{79}_2)^+$ peaks. That is, since N_{81} and N_{79} are the numbers of atoms of Br^{81} and Br^{79} in a sample, there will be :

$$\frac{N_{81}^2}{2(N_{79}+N_{81})}, \quad \frac{N_{79}N_{81}}{(N_{79}+N_{81})} \text{ and } \frac{N_{79}^2}{2(N_{79}+N_{81})} \text{ molecules respectively of the types } Br^{81}_2,\ Br^{79},\ Br^{81},\ Br^{79}_2.$$

The packing fraction for bromine, —7.4, is in doubt. It should be —6.4 to agree with the international value for the atomic weight of 79.916.

KRYPTON

The isotopes of krypton have been measured by Lounsbury, Epstein and Thode (L—1). Their work agrees with the earlier work of Nier (N—6) except for Kr^{80}. In this case an incorrect abundance figure of 2.01 atom percent resulted from use of the wrong shunt factor in computing the background correction for Kr^{80} (N—8).

Abundance of Kr Isotopes in Atom Percent.

A = Mass No.	Nier (old)	Nier (corrected)	Lounsbury, Epstein, Thode
78	0.35	0.346	0.342
80	2.01	2.261	2.228
82	11.53	11.50	11.50
83	11.53	11.50	11.48
84	57.10	56.95	57.02
86	17.47	17.43	17.43

As tabulations of the relative abundance of krypton isotopes must be changed, it is suggested that Thode's recently determined values be used for this element. The best values for Kr^{78} and Kr^{80} cannot be decided finally until checked by another investigation with particular emphasis on these isotopes. In order to control every step of the process, it would be desirable to concentrate the krypton from the air at the laboratory where the abundance measurements are to be made, rather than to depend upon commercially fractionated krypton, the composition of which might have undergone some selective fractionation.

It would be easier to incorporate new values, representing better experimental data, if the relative abundance of isotopes were tabulated with respect to the most abundant isotope taken as 100, rather than on the basis of atom percent abundance. The latter system requires that all figures be changed even if an error is found for only one isotope.

Molybdenum furnishes another example in favor of this method of tabulating results.

MOLYBDENUM

The results obtained by Williams and Yuster for molybdenum using the Mo^{+} and Mo^{++} ions from molybdenum hexacarbonyl are listed with those from other sources below.

Relative Abundance of Molybdenum Isotopes (W—1).

Isotope	Aston	Mattauch, Lichtblau	Valley	Williams, Yuster
92	61.7	61.0	61.8	66.6
94	43.5	34.3	39.0	38.0
95	67.4	64.2	66.8	66.1
96	77.4	66.1	68.9	69.5
97	41.7	34.2	40.0	39.8
98	100.0	100.0	100.0	100.0
100	42.6	33.8	38.4	40.5
Method	Photometric	Photometric	Electrometer 180° Spectrometer	Electrometer 60° Spectrometer

The two electrical determinations are in better agreement with each other than with either of the photometric measurements or than the photometric measurements are with each other. One percent accuracy has been claimed for the measurements by Valley and by Williams and Yuster but disagreements greater than this occur. The results of Williams and Yuster are accepted here because the stability of the vapor pressure in the ion gun was easier to maintain in their experiments and the resolving power greater than in those of Valley's. There would also have been less fractionation by molecular evaporation.

TELLURIUM

As tellurium had not been measured electrically prior to the experiments of Williams and Yuster, their results are the first for which $\pm$ 1 percent accuracy is claimed.

Abundance of Tellurium Isotopes.

Isotope	Williams and Yuster Relative Abundance		Williams and Yuster Atomic Percentage	Aston, Bainbridge, Dempster
120	0.256	$\pm$ 0.017	0.09	Faint
122	7.05	0.18	2.43	2.9
123	2.47	0.05	0.85	1.6
124	13.3	0.4	4.59	4.5
125	20.2	0.2	6.98	6.0
126	54.2	0.2	18.70	19.0
128	92.3	0.3	31.85	32.8
130	100.00		34.51	33.1

The packing fraction of tellurium has not been measured, and no comparison can be made of the physical atomic weight with the chemical atomic weight.

XENON

The isotopic composition of xenon has been re-determined by Lounsbury, Epstein and Thode (L—1) with results in excellent agreement with Nier's earlier values, which do not require any change (N—6).

Abundance of Xenon Isotopes in Atom Percent.

A = Mass No.	Nier	Lounsbury, Epstein, Thode
124	0.094	0.095
126	0.088	0.088
128	1.90	1.917
129	26.23	26.24
130	4.07	4.053
131	21.17	21.24
132	26.96	26.93
134	10.54	10.53
136	8.95	8.93

TUNGSTEN

Recently the isotopic composition of tungsten was determined independently by Williams and Yuster, and by Inghram, using electrical measurements in Nier-Bleakney type spectrometers, and photometrically by Mattauch and Scheld with the Mattauch double focusing mass spectrograph (W—1, I—1, M—1).

Relative Abundances of Tungsten Isotopes.

Isotope	Inghram	Mattauch, Scheld	Williams, Yuster
180	0.398	0.52	0.440
182	84.0	85.89	86.2
183	46.41	46.67	47.0
184	100.0	100.0	100.0
186	95.08	92.86	92.7

Mattauch has pointed out (M—1) that his results agree with those of Williams and Yuster but that systematic differences are apparent when a comparison is made with Inghram's data. If one takes the

ratio of the relative abundance from Williams' results to that from Inghram's measurements for each individual isotope, one obtains the following values :

Mass No. A_i	Ratio of abundance $= \frac{\text{Williams data for } A_i}{\text{Inghram data for } A_i}$
180	1.105
182	1.026
183	1.0127
184	1.000
186	.975

Except for W^{180}, the ratios in the second column are very nearly equal to $(184/A_i)^{2\cdot3}$. Mattauch has suggested a value of 2 for the exponent. The value to be assigned to the exponent is unimportant compared to the clear indication of systematic differences in the measurement of relative abundance values by practically identical 60° sector mass spectrometers.

The results of Williams and Yuster have been accepted here for the following reasons :

1. In their experiments, the ions followed the same trajectories independent of the M/e value, whereas the fundamental requirements for identical trajectories were not satisfied in all of Inghram's measurements (B—1);
2. Mattauch's measurements support the results of Williams and Yuster;
3. The resolving power for their instrument was greater than for Inghram's;
4. The stray and scattered ion background was less than in the case of Inghram's work.

Williams and Yuster varied V and kept H constant. This procedure satisfies the requirement for bringing ions of different M/e values over identical paths to the receiving slit in a sector type spectrometer which has a *fixed* auxiliary magnetic field at the ion gun (B—1). Inghram varied H and kept V fixed, so that in those cases where an auxiliary magnet of fixed intensity was used at the ion source the ion beams for successive M/e values could not follow identical paths to the receiving slit. Under these conditions, some preferential separation was to be expected at the source, where the ions follow cycloidal paths.

The Ratio of the Physical to the Chemical Atomic Weight Scale.

Chemical combining weights are referred to oxygen as a standard while physical isotopic weights use O^{16} and not the naturally occurring mixture of isotopes.

The abundance of O^{18} relative to O^{16} has recently been measured by H. G. Thode (T—3) for carbon dioxide and oxygen samples obtained from various sources, with the results shown in Table I taken from his paper.

TABLE I

Isotopic Abundance Ratios for Carbon Dioxide and Oxygen Samples obtained from various natural sources.

Source of Oxygen	Carbon Dioxide Ratio $\frac{\text{Mass } 44}{\text{Mass } 46}$	Oxygen Ratio $\frac{\text{Mass } 32}{\text{Mass } 34}$	Percent O^{18} calculated from oxygen analysis
Lake Ontario Water . . .	237	245.3	0.204
Atmospheric Oxygen . .	235	241.3	0.207
Tank Oxygen (Claude Process)	235	240.0	0.208
Swedish Magnetite Fe_3O_4	237.2	245.3	0.204
Lead Peroxide PbO_2 . . .	240.8	249.5	0.202
Lead Peroxide PbO_2 . . . (Oxygen by Thermal decomposition)	—	249.8	0.201

Precision : $\pm$ 0.3 percent.

The average of the carbon dioxide gas samples are about 3.3 percent higher in O^{18} content than the oxygen gas samples, a result in good agreement with that for tank CO_2, or CO_2 equilibrated with normal water.

Prior results for the relative abundance of the oxygen isotopes are given in Table II.

TABLE II

Ratio of Abundance of Oxygen Isotopes.

	O^{16}	O^{18}	O^{17}
1931 R. Mecke, W. Childs	630 $\pm$ 20	1	—
1932 F. W. Aston	536	1	.238
1934 W. R. Smythe	503 $\pm$ 10	1	—
1935 W. Bleakney, J.-A. Hipple	500 $\pm$ 20	1	—
1941 B. F. Murphy	500 $\pm$ 15	1	.204 $\pm$ .008
1941 R. T. Birge (most probable values)	506 $\pm$ 10	1	.204 $\pm$ .008
1944 H. G. Thode (from water)	490.6 $\pm$ 1.5	1	—

The ratio of the physical scale to the chemical scale is 1.000272 $\pm$ 0.000005 if Birge's adopted values are used (B—2). The Committee on Atomic Weights of the International Union of Chemistry utilizes 1.000275 $\pm$ 0.000009 (C—2). Thode's new results, with 0.204 for the O^{17}/O^{18} ratio, give a figure of 1.0002808 $\pm$ 0.0000014. Here the uncertainty is caused almost equally by the probable errors in the determination of O^{16}/O^{18} and O^{17}/O^{18}.

If the probable error of Thode's O^{16}/O^{18} abundance ratio is accepted, then on the basis of error theory no prior O^{16}/O^{18} ratio measurement should be included in the determination of the conversion factor. Any future measurements of the relative abundance of the oxygen isotopes should include the abundance of O^{17}. The final value of the conversion factor should be determined on the basis of measurements of the relative abundance of all three oxygen isotopes.

The Determination of the Half-Life Periods of U^{234} and C^{14}.

The period of half-life of a radioactive isotope in secular equilibrium with another isotope may be determined from the equilibrium relation $T_{II} = T_I N_{II}/N_I$ in which N_{II}/N_I is the ratio of the isotopic abundance of isotope II to isotope I. Chamberlain, Williams, and Yuster measured the value of T_{II} for U_{II}^{234} in terms of T_I for U_I^{238} where $T_I = 4.51 \times 10^9$ years (C—1, K—1). The value N_{II}/N_I was determined as 1/19700 $\pm$ 6% in a Nier-Bleakney type spectrometer. From this measurement T_{II} equals 2.29 $\pm$ 0.14 $\times$ 10^5 years.

A second method for the determination of T requires an absolute measurement of the number of nuclei disintegrating per second from a known number of nuclei N, $dN/dt = -\lambda N = -0.693\ N/T$.

In this experiment, U_3O_8 samples enriched in U^{235} and U^{234} were deposited on platinum foil in thin layers, and the number of alpha particles emitted per second, dN/dt, determined by electrical counting. Suitable corrections were made for the finite thickness of the source and for back scattering from the platinum foil. The abundance of U^{234} was measured in a mass spectrometer relative to U^{235} and U^{238} so that N and dN/dt for U^{234} alone could be obtained for the accurately weighed deposits when corrections were made for the effects of U^{235} and U^{238}. This value of T is 2.35 $\pm$ 0.14 $\times$ 10^5, and the mean value from these two measurements is 2.33 $\pm$ 0.10 $\times$ 10^5 years.

C^{14} is produced in the nuclear reaction N^{14} (n, p) C^{14} by neutron bombardment of an ammonium nitrate solution. Since the C^{14} obtained is contaminated with normal carbon, the abundance of C^{14} in the mixture must be determined by mass-spectrum analysis before a measurement of the half-life of C^{14} can be made using the relation $T = 0.693\ N/dN/dt$. Norris and Inghram measured 3.23 and 3.35 atom percent C^{14} in two samples of barium carbonate. These figures, together with the activity and sample weight measurements, yielded a value of $T = 5300 \pm 800$ years for C^{14} (N—3).

A-1 L. W. Alvarez, R. Cornog, *Physical Review* **56,** 379, 613 (1939).

A-2 L. T. Aldrich, A. O. Nier, *Physical Review* **70,** 983 (1946); **71,** 911 (1947)

B-1 W. Bleakney, *Am. Phys. Teacher* **4,** 12 (1936).

B-2 R. T. Birge, *Rev. Mod. Phys.* **13,** 233 (1941).

C-1 O. Chamberlain, D. Williams, P. Yuster, *Physical Review* **70,** 580 (1946).

C-2 Committee on Atomic Weights of the International Union of Chemistry, *J. Am. Chem. Soc.* **63,** 845 (1941).

H-1 J. A. Hipple, *J. App. Phys.* **13,** 551 (1942).

I-1 M. G. Inghram, *Physical Review* **70,** 653 (1946).

K-1 A. F. Kovarik, N. I. Adams, *Physical Review* **40,** 718 (1932); **54,** 413 (1938);—*J. App. Phys.* **12,** 296 (1941).

L-1 M. Lounsbury, S. Epstein, H. G. Thode, *Can. J. Research.* In press.

M-1 J. Mattauch, H. Scheld, private communication. Article scheduled for publication in *Z. f. Naturforschung.*

N-1 A. O. Nier, *Physical Review* **55,** 153 (1939).

N-2 A. O. Nier, *Physical Review* **55,** 150 (1939).

N-3 L. D. Norris, M. G. Inghram, *Physical Review* **70,** 772 (1946).

N-4 A. O. Nier, *Rev. Sci. Inst.* **11,** 212 (1940).

N-5 E.-P. Ney, J. H.McQueen, *Physical Review* **69,** 41 (1946).

N-6 A. O. Nier, *Physical Review* **52,** 933, (1937).

N-7 A. O. Nier, E. A. Gulbransen, *J. Am. Chem. Soc.* **61,** 697 (1939); B. F. Murphy, A. O. Nier, *Physical Review* **59,** 771 (1941).

N-8 A. O. Nier, private communication to H.-G. Thode.

R-1 D. Rittenberg, *J. App. Phys.* **13,** 561 (1942).

T-1 H. G. Thode, R. L. Graham, *Canadian J. of Res.,* A **25,** 1 (1947).

T-2 H. G. Thode, A. F. Reid, A. O. Nier, D. Rittenberg and others, *Preparation and Measurement of Isotopic Tracers* (1946);—J. W. Edwards, Ann. Arbor, Michigan.

T-3 H. G. Thode, Report No. MC-57, National Research Council. McMaster University, Hamilton, Ontario (April 29, 1944).

W-1 D. Williams, P. Yuster, *Physical Review* **69,** 556 (1946).

IV.

IDENTIFICATION OF RADIOACTIVE AND STABLE ISOTOPE PRODUCTS OF NUCLEAR REACTIONS

Hevesy and Pahl (H—1) in 1932 showed that samarium was α radioactive with a very long half-life, T $\sim 10^{12}$ yr for the element as a whole. The assignment of this radioactivity to Sm^{148} (T $\sim 1.5 \times 10^{11}$ yr) from among the seven possible isotopes was made by Wilkins and Dempster by an ingenious method (W—1). A mass spectrum of samarium was photographed in the ordinary manner and then a photographic plate sensitive to single α particles was laid against it, emulsion to emulsion, with fiducial marks relating their relative position. After sufficient time had elapsed for some alpha particles arising from the separated samarium atoms on the mass spectrum plate to be recorded, both plates were developed. From the location of the individual α tracks recorded, the nucleus responsible for the α activity was found to be Sm_{62}^{148}.

β Radioactive Nuclei.

In recent years this technique has been extended at the Metallurgical and Argonne Laboratories of the Atomic Energy Commission (formerly M. E. D.) to β radioactive nuclei produced in fission and in (n, γ) reactions. The published results are tabulated in Table I.

10^5 β-rays (R—1) are needed to produce a record on the autographic plate as compared to the small number of alpha particles needed in the pioneer experiment.

The much higher number of particles required for recording β-rays is compensated by the much greater specific activity of the element when produced by the high intensity slow neutron flux available in uranium reactors. As we shall see later, it should be possible to extend this technique to many radioactive substances produced by charged particle bombardment in cyclotrons (S—5).

This application of the mass spectrometer is of the greatest importance to radio chemistry and the nuclear study of artificially radioactive substances. Of some 500 radioactive isotopes, assignment to a specific isotope of the activity and characteristic T value is probable in about one-half of the cases. In about one-third of the cases, only the element is certain (S—1).

TABLE I

Half-life T	Assignment	Prior assignment Seaborg 1944 Table Reference S-1	Reference	Ion source and detection method
10 y	Kr 85	* No record New isomer of 4 h Kr 85	T-1	Electron impact, relative abundance.
55 d	Sr 89	* Same	H-2	Thermal ionization auto-radiograph. L-1.
30 y	Sr 90	* Sr 90 probable		»
57 d	Y 91	* Y 91 probable		»
1 y	Ru 106	* No record		»
13 h	Pd 109	Pd 107 or 109	R-1	Hot spark, auto-radiograph
35 y	Cs 137	* No record	H-2	Thermal ionization auto-radiograph. L-1.
40 h	La 140	* Same		»
28 d	Ce 141	* Same		»
14 d	Pr 143	* Pr 143 probable		»
275 d	Ce 144	* Element certain only		»
> 72 d	Sm 145	No record	I-5	»
3.7 y	Z61-147	* No record	H-2, I-6	»
49 h	Z61-149	* No record		»
~ 20 y	Sm 151	No record	H-2, I-5	»
9.4 h	Eu 152	Eu 152 probable	H-3, I-2	»
~ 5 y	Eu 152	No record	I-1, I-5	»
46 h	Sm 153	Element certain only	H-3, I-2	»
> 72 d	Gd 153		I-5	»
~ 5 y	Eu 154	Eu 154 probable	I-5, I-1	»
2-3 y	Eu 155	No record	H-2, I-5	» $\sigma = 14.000 \times 10^{-24}$ cm^2
15.4 d	Eu 156	No record	I-5	»
72 d	Tb 160	Same	I-5	»
2.5 h	Dy 165	Same	I-3	»
27.5 h	Ho 166	Ho 166 probable	I-1, I-2	»
~100 h	Yb 175	No record	I-2, I-3	»
short	Yb 177	Element certain	I-2, I-3	»
6.6 d	Lu 177	Element certain	I-3	»
60 d	Ir 192	Ir 192 or 194	R-1	Hot spark, auto-radiograph
19 h	Ir 194	Ir 192 ou 194		

* Fission product chain unit.

When a radioactive nucleus results from slow neutron capture, the determination of the isotope responsible is confined to a few possibilities. In the past, elimination of various possibilities and final assignment of an n—γ reaction for element Z was made by the cross checks provided by fast neutron (n, p) (n, α) reactions, and (p, n), (d, n), (α, n) reactions on adjacent elements with atomic numbers $Z + 1$, $Z + 2$, $Z - 1$, $Z - 2$. The starting points for these reactions are the known stable isotopes obtained from mass spectrograph research. Studies of (γ, n) and (n, 2n) reactions also aid in the identification of the nuclear species responsible for an (n, γ) capture leading to a radioactive isotope of element Z. The mass spectrometer method of analysis can be applied in many cases where for one reason or another the usual transmutation methods cannot be used.

In the case of a fission chain which does not contain a known radioactive isotope, the mass spectrometer analysis provides the only satisfactory method at present to determine the mass members of the chain. It is here (Table I) that a considerable part of the work has been done as very high activity fission product sources can be produced. Also, measurable quantities of stable end products are manufactured, Table II, (T—1, I—4).

In the studies reviewed so far the element is first made radioactive and then analyzed by a mass spectrometer and the auto-radiographic technique. At Berkeley and at Clinton Laboratories the procedure is reversed. Stable elements are first separated into their isotopic constituents and then the nuclear reactions of these separated isotopes are studied (S—2, M—1, P—1).

The 2.6 hr activity of Nickel, previously assigned to Ni^{63}, was shown to belong to Ni^{65}, using separated isotopes of copper, by the process Cu^{65} (n, p) Ni^{65} (S—2). A new activity of 1.75 hour half-life was assigned to Co^{61} produced from separated Ni^{61} by an (n, p) reaction, and from Ni^{64} by a (p, α) reaction (P—1). Also, Cd^{113} was demonstrated to be the main slow neutron absorbing isotope of cadmium, $\sigma \sim 24.000 \times 10^{-24}$ cm^2 (M—1).

APPARATUS

The mass spectrographs used to separate the isotopes for radioactive identification purposes are essentially standard types.

The majority of the results in Table II were obtained with a Nier-

Bleakney type 60° spectrometer (L—1, N—1, N—2). Special attention was paid to the ion optics at the source so that one ion of a particular e/m value was received at the plate out of forty formed at the source. The ions were produced by thermal ionization of oxides of the rare earth metals coated on a filament, from nitric acid solutions of the oxides. The efficiency of ionization was high, amounting to 16 per cent in the case of lanthanum. In addition to the high conversion efficiency of atoms to ions, this type of source has another useful characteristic. There is some difference in behavior in ion emission of different rare earth metals as a function of Z. Elements 60, 61, 62, 64 give copious emission of isotopic ions, for example Nd^+, and ions of sixteen units greater mass corresponding to NdO^+, etc. Europium, Z63, does not give oxide ions, and Holmium, Z67, only yields HoO^+ weakly compared to Ho^+. These differing emission characteristics can be of some help in the identification of various rare earths, and reduce the necessity for complete chemical separation prior to coating the filaments.

When auto-radiographic recording was the final aim of the experiments, a recording photographic plate was substituted for the usual slit system and amplifier, the location of the plate being determined by the ion optics theory of L. Cartan and R. Herzog (C—1, H—4).

Elements not amenable to the thermal ionization method can be ionized by the hot spark method introduced by Dempster or by the electron impact source used by Bleakney. The hot spark has been used with a double focusing type of spectrograph (L—2, R—1). The conversion of atoms at the source to ions at the collector is much less efficient in this case and only one ion is collected for 5×10^7 atoms consumed at the source (R—1), compared to one out of 250 or more for the thermal ionization source and Nier spectrometer case (L—1).

Source Intensity Requirements for Radioactive Sources.

Sufficient data has been published to make some order of magnitude calculations on the source strenghts, specific activities, and total neutron flux necessary when using a mass spectrograph to aid in the assignment of mass numbers to the specific radioactive characteristics of various nuclei.

The requirements for identification are that enough atoms, N_o, of the radioactive isotope must be transferred to an isotope line on the receiving plate so that the position of the line can be determined by the use of β-ray sensitive plates or Geiger-Mueller counter methods.

$$N_o \left(1 - e^{-\frac{.693\ t}{T}}\right) = 10^5,$$

where t is the time of exposure during which the auto-radiograph plate is in contact with the collecting plate, and T is the period of half-life of the radioactive isotope.

A Geiger-Mueller counter is more sensitive than a photographic plate for radioactive substances of short half life values. Assuming a small volume, low background counter would require 8 disintegrations per minute at the source to give a definite reading above normal background, then a counter would be more sensitive than a plate for values of $T \leq 6$ days, and N_o could be reduced proportionately to T for values less than 6 days.

A source strength greater than 0.5 millicuries T_{sec}^{-1} is required for a single auto-radiograph using the Nier-Bleakney type spectrograph and a thermal ionization source. High specific source activity, 10^4 to 10^6 mc gm^{-1} T_{sec}^{-1}, is an aid in making short mass-spectrum exposures, but not enough data is available to calculate the actual specific activity in the experiments published to date.

The source strengths and specific activities used so far for the study of neutron induced activities could be attained in many cases for charged particle reaction products obtained from cyclotron bombardment, where the end product can be obtained essentially « carrier-free », from (d, n), (p, n), (d, α), (p, α), (α, p) or other reactions where Z is changed.

When the hot spark source is used the source strength must be greater than 10^5 T_{sec}^{-1} millicuries. The fact that one-half milligram of material at the source is used up to photograph ~ 5 mass-spectra indicates a figure of 10^6 T_{sec}^{-1} curies per gram for the specific activity of the source.

Analysis of Stable Products of Nuclear Reactions.

Another very interesting application of mass spectrometers has been made possible in many cases by the high neutron flux available from uranium reactors. The capture of a slow neutron by an atomic

nucleus results in an isotope of one unit greater mass number, which may be stable, or unstable by β radioactivity. In the case of a stable end product the ordinary methods of nuclear and chemical research cannot be appplied and identification of the neutron capturing nucleus has not hitherto been possible.

Gadolinium has a high capture cross-section σ, for thermal neutrons and so provides a favorable case for study. After an oxide sample had been irradiated for an unannounced period of time in the Clinton Laboratory pile, the relative abundances of the isotopes were compared with those from a normal sample. Gd^{155} had been decreased by about 12 percent of its normal amount and Gd^{156} had been increased, the charge amounting to — 2 % and + 2 % shifts relative to all the isotopes. Gd^{157} was decreased by 40 percent of its initial amount and Gd^{158} increased (L—2).

The number of product nuclei B formed in the irradiation

$$B = A_o (1 - e^{-\sigma n_o}) = 0.4\ A_o$$

where A_o is the number of Gd^{157} nuclei initially present. σ is the atomic cross-section for $Gd^{157} \simeq 185{,}000 \times 10^{-24}$ cm²/atom (S—4, L—2), and n_o represents the total number of neutrons which have traversed the thin sample per square centimeter. Solving for n_o a value of 3×10^{18} neutrons per cm² is obtained. The high value of n_o utilised in this and related experiments, Table III, shows that similar studies by this method of isotopic analysis would be very difficult if not impossible, if the neutrons had to be provided from cyclotrons or other high intensity neutron sources ordinarily available.

Samarium and cadmium, Figure 15, are other elements with large neutron capture cross sections which have been investigated by the hot spark and photographic recording technique to determine the isotopes responsible for the major share of the slow neutron capture (L—2, D—1).

The still greater sensitivity and higher accuracy of electrical measurements make possible the use of the Nier-Bleakney type spectrometer in cases less favorable for study by the hot spark method (I—4). Absolute isotope abundance measurements are generally good to ± 1 percent and comparative measurements attain ± 0.1 percent accuracy. With practice, a sample of material equivalent to 10^{-3} cm³ of vapor at atmospheric pressure can be analyzed with an accuracy of ± 1 per cent in favorable cases (T—1, N—1). The sample should

TABLE II

A. Fission chain end products :

Z	Symbol	Mass No.	Remarks	Reference
36	Kr	83	—	T-1
—	—	84	—	—
—	—	85	T = 10y, isomer of 4 h. Kr^{85}	—
—	—	86	—	—
54	Xe	131	—	T-1
—	—	132	—	—
—	—	134	—	—
—	—	136	—	—

B. U - Reactor neutron absorbing isotopes :

Z	Symbol	Mass No.	*Absorption cross-section* $\times 10^{24}$ cm^2	Reference
48	Cd	113	$\sim$ 20.000	M-1, D-1
		any other	$<$ 500	—
62	Sm	149	$\sim$ 52.000	L-1
64	Gd	155	$\sim$ 50.000	L-2
—	—	157	$\sim$ 185.000	—
80	Hg	196	3.100	I-4
—	—	198	small	—
—	—	199	2.500	—
—	—	200	$<$ 60	—
—	—	201	$<$ 60	—
—	—	202	$<$ 60	—
—	—	204	$<$ 60	—

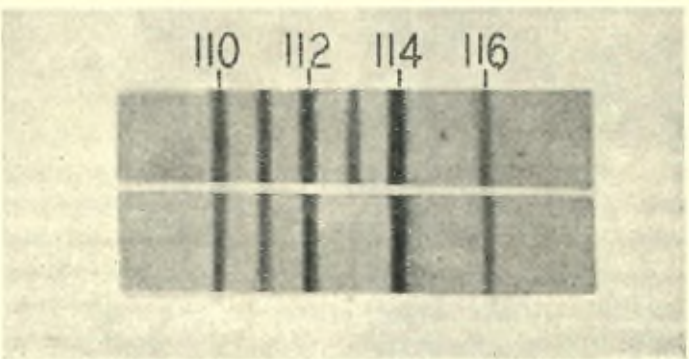


Fig. 15
Reference D-1. A. J. Dempster, *Physical Review.*
Normal Cadmium (above).
Isotopes Altered by Neutron Absorption (below).

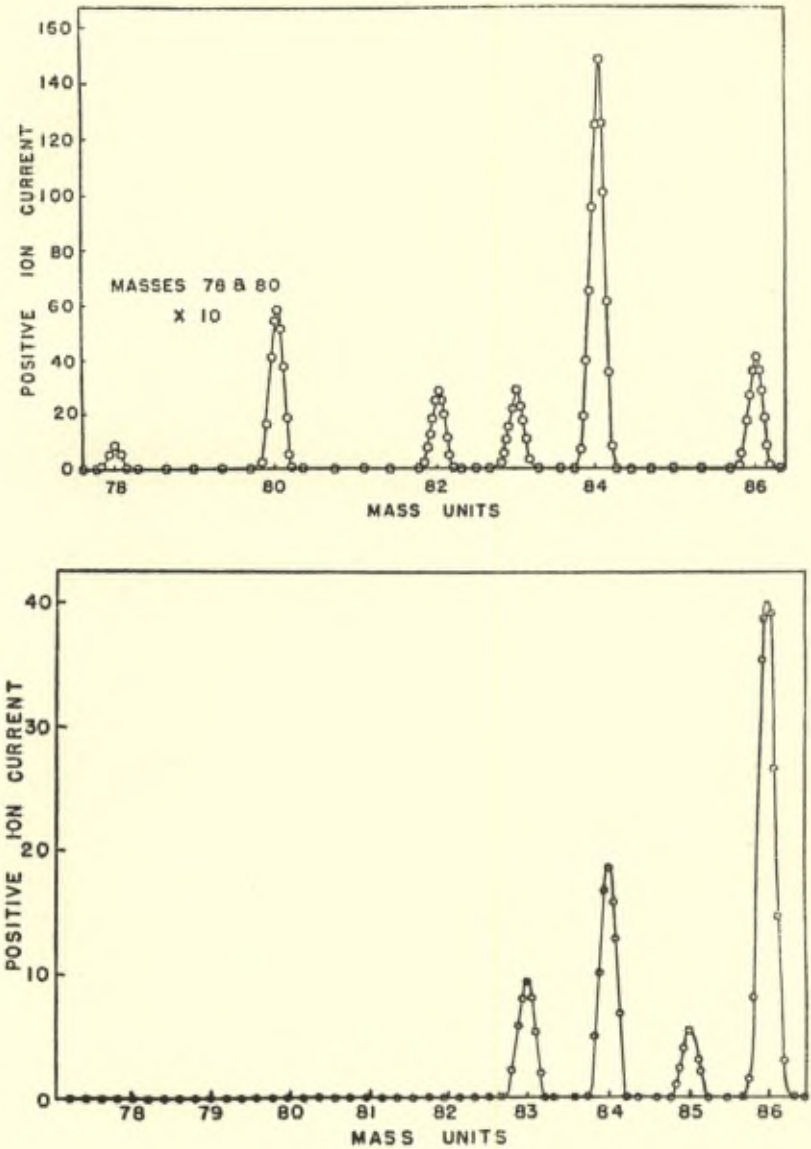


Fig. 16

Reference T 1. H. G. Thode, R. L. Graham, *Canadian Journal of Research.*
Normal Krypton (above).
Fission Product Krypton (below).

have a vapor pressure of 10^{-4} mm of mercury or more at temperatures below 300° C.

Thode has investigated stable isotopes of krypton and xenon which are in seven cases the stable end products of different fission chains, Figure 16. The study of all of the elements from about Z = 30 to Z = 65 by spectrometer methods will help to obtain the fission yield-mass number curve with greater accuracy than is attainable from activity measurement experiments. As yet there is no evidence for the *direct* formation of stable isotopes in the fission process, but the mass spectrometer approach is one way of attempting to discover fission products of this type.

It is natural to consider whether greater sensitivity could be obtained so that smaller samples could be analyzed. One should certainly try the use of secondary emission multiplier tubes which can respond to single positive ions which are amplified in the multiplier by a factor of 10^5 to 10^6 and the individual pulses can be still further amplified and recorded by the usual counting techniques (C—2). More time will be required for an analysis and there will be greater difficulties with residual gases, but this development has attractive features for aiding in the solution of some problems in chemistry, physics, and biology, as well as in the analysis of nuclear reactions where only very small test samples are available.

C-1 L. Cartan, *J. de Physique* **8,** 453 (1937).

C-2 A. A. Cohen, *Phys. Rev.* **63,** 219*A* (1943).

D-1 A. J. Dempster, *Phys. Rev.* **71,** 829*L* (1947).

H-1 G. Hevesy, M. Pahl, *Nature* **130,** 846 (1932); **131,** 434 (1933).

H-2 R. J. Hayden, L. G. Lewis, *Phys. Rev.* **70,** 11*A* (1946).

H-3 R. J. Hayden, M. G. Inghram, *Phys. Rev.* **70,** 89*L* (1946).

H-4 R. Herzog, *Z. Phys.* **89,** 447 (1934).

I-1 M. G. Inghram, R. J. Hayden, *Phys. Rev.* **71,** 130*L* (1947).

I-2 M. G. Inghram, R. J. Hayden, *Phys. Rev.* **71,** 144*A* (1947).

I-3 M. G. Inghram, R. J. Hayden, D. C. Hess Jr., *Phys. Rev.* **71,** 270*L* (1947).

I-4 M. G. Inghram, D. C. Hess Jr., R. J. Hayden, *Phys. Rev.* **71,** 561*L* (1947).

I-5 M. G. Inghram, R. J. Hayden, D.-C. Hess Jr., *Phys. Rev.* **71,** 643*L* (1947).

I-6 M. G. Inghram, D. C. Hess Jr., R. J. Hayden, G. W. Parker, *Phys. Rev.* **71,** 743*L* (1947).

L-1 L. G. Lewis, R. J. Hayden, *Phys. Rev.* **70,** 111*A* (1946).

L-2 R. E. Lapp, J. R. Van Horn, A. J. Dempster, *Phys. Rev.* **71,** 745 (1947); *Abstract* **70,** 104*A* (1947).

M-1 B. J. Moyer, B. Peters, F. H. Schmidt, *Phys. Rev.* **69,** 666 (1946).

N-1 A. O. Nier, *Rev. Sci. Inst.* **11,** 212 (1940).

N-2 A. O. Nier, *Rev. Sci. Inst.* **18,** 398 (1947).

P-1 T. J. Parmley, B. J. Moyer, *Bulletin of the Am. Phys. Soc.* **22,** (1947). No. 4, *Abstract E6.*

R-1 W. Rall, *Phys. Rev.* **70,** 112*A* (1946).

S-1 G. T. Seaborg, *Rev. Mod. Phys.* **16,** 1 (1944).

S-2 J. A. Swartout, G. E. Boyd, A. E. Cameron, C. P. Keim, C.-E. Larson, *Phys. Rev.* **70,** 232*L* (1946).

S-3 A. E. Shaw, W. Rall, *Phys. Rev.* **70,** 117*A* (1946); *Rev. Sci. Inst.* **18,** 278 (1947).

S-4 E. Segré, Isotope Chart (1946), in *The Science and Engineering of Nuclear Power* (1947), Addison-Wesley Press, Inc., Cambridge, Massachusetts.

S-5 W. M. Schwartz, M. L. Pool, *Phys. Rev.* **64,** 43*A* (1943).

T-1 H. G. Thode, R. L. Graham, *Canadian J. of Res.*, A, **25,** 1 (1947).

W-1 T. R. Wilkins, A. J. Dempster, *Phys. Rev.* **54,** 315 (1938).

Discussion du rapport de M. Bainbridge

M. Rittenberg. — Dr. Bainbridge states that for maximum accuracy in the sector spectrometer H should be kept constant and V varied. If in the analysis of hydrogen this is done an incorrect answer is obtained while if V is kept constant and H is varied a more nearly correct value is obtained.

M. Bainbridge. — I had said earlier that to obtain freedom from selective effects one must maintain identical trajectories throughout the spectrometer for ions of different m/e values. One must maintain the magnetic intensity of the main analysing field at a constant value if the auxiliary field (for collimating the electron beam at the source) is fixed. As is true in nearly all sector spectrometers, this has proved to be the best practice in the usual measurements of relative abundance where the percentage mass differences do not exceed, say 10%.

Hydrogen furnishes an unusual and more difficult case than the heavier elements. V is varied over a much greater range than 10%. Apparently the main accelerating field penetrates into the region where the ions are formed and has a large effect on the number of ions extracted. This effect is evidently obviated if the magnetic field is varied and the electric field is fixed at a constant value. The effect of small differences in the ion trajectories at the source is not prominent for the wide slits and relatively low resolving powers needed for hydrogen and the lighter elements. This is probably the case with your instrument. Another mass spectrometer might well behave differently.

Direct comparison of a sample of unknown composition with one or more samples of known composition will give the most accurate results. I believe that is the procedure in use at Prof. Rittenberg's laboratory and is certainly the most reliable one for measurements on Hydrogen.

M. Joliot. — Je demanderais à M. Bainbridge de nous indiquer quelques limites de sensibilité pour la détection d'ions d'éléments divers.

Pour certains éléments l'analyse et le dosage par la spectrographie de masse doit être plus aisée que par la spectrographie optique ou les Rayons X. Ceci a un intérêt pour l'examen des puretés extrêmes que doivent posséder, pour diverses raisons, des substances chimiques que l'on prépare au laboratoire ou industriellement.

M. Bainbridge. — The greatest ratio of currents measured in a single experiment with a magnetic spectrometer has been reported as 10^7 by Aldrich and Nier. This was the approximate ratio of He^4 to He^3 and so refers to a single element. HD ions were present to about the same intensity as He^3 and were resolved separately. So one in ten million is also about the best attained so far for the measurement of one element in the presence of another.

You mentioned general analysis and studies of purity where a number of elements and a wide range of masses may be present. In my opinion a satisfactory combination to accomplish this by mass-spectrographic means would be a Dempster hot-spark source combined with a Mattauch spectrograph. The Dempster source only requires a milligram or so of the material under study. The Mattauch spectrograph gives « double focusing » for ions over a wide range of masses so that a single mass spectrum may cover from say 10 to 200 mass units. One might achieve a sensitivity of detection of one in 100.000 in favorable cases. A small instrument of this type has been mentioned in the *Physical Review*. In general the sensitivity is inferior to optical spectroscopic methods for the detection of small amounts of an element in the presence of large concentrations of other elements.

Thermal ion sources have been used succesfully in the study of rare earths for which the intensities and characteristics of the ions produced differ somewhat from one element to another. I think that fluorescent X-ray analysis would be a much more satisfactory method for the investigation of the rare earths.

Finally I should say that for the general problems you mention, I do not believe that the mass-spectrographic method of analysis has yet attained the sensitivity of the older spectrographic methods which have reached a higher state of development and reliability in their longer period of use.

M. Paneth. — Professor Bainbridge has just mentioned the measurements made by Aldrich and Nier on the abundance of He^3 in He^4. It would interest me to learn whether Professor Bainbridge

considers the figures given in his report as final; according to them the abundance ratio of He^3 in atmospheric helium is about eight times as high as in helium from gas wells. If we take into account that helium is constantly diffusing through the terrestrial atmosphere into the void, we should rather expect an enrichment in the atmosphere of the heavier isotope of helium. If the opposite is true, an interesting question is put to the nuclear physicists and chemists as to the origin of the various species of helium in the interior of the earth and in the atmosphere.

M. Bainbridge. — I have confidence that the measurements of Aldrich and Nier are correct, but the samples may not be characteristic. Some fractionation may well have occured in the purification of the helium although it is difficult to envisage that the greater concentration of He^3 in atmospheric helium is entirely due to that effect. The very difficult experiment should be performed of examining helium samples truly characteristic of atmospheric helium, and of well helium from many sources.

As you know, Nier has been very keen on geological problems among others, and I believe he has plans to study the He^3 problem in much greater detail.

M. Timmermans. — Je voudrais demander à M. Bainbridge quelles sont les formalités à remplir pour obtenir les radioéléments préparés en Amérique.

M. Bainbridge.—I have with me a copy of the official announcement of the U. S. Atomic Energy Commission which lists the regulations. I am very glad to give you my copy which is more complete than the published press releases.

In discussions here and in England I found there have been some unfortunate misinterpretations of the regulations which I hope this pamphlet can correct. This was sent to me by Dr. Paul Aebersold in answer to my request for the complete official announcement which was distributed on September 4th. Unfortunately he failed to include the catalogue of isotopes, but that will be mailed on application, and Professor Paneth has a list from the U. S. Embassy in London.

May I add that the rules governing the purchase and distribution of isotopes outside the U. S. A. do not differ in any important regard

from those under which U. S. scientists operate except for two provisions :

1. A requirement has been added that a progress report must be submitted six months after the shipment of the material;

2. Isotopes will not be released for industrial use. The same prohibition is imposed in the United States but material is made available for industrial research purposes. I do not believe the announcement is unambiguous on the subject of industrial research. A direct inquiry would be necessary to settle the question.

M. Joliot. — En France nous avons eu d'abord connaissance d'une note rédigée par la Commission de l'Energie atomique des Etats-Unis, note destinée à la Presse mondiale.

C'est sans doute la forme abrégée de rédaction, en particulier celle concernant les conditions de publication des résultats obtenus à l'aide des radioéléments artificiels de provenance des U. S. A., qui a pu prêter à la mauvaise interprétation signalée par M. Bainbridge.

Isotopes in the Spectroscopy of Polyatomic Molecules with Special Reference to the Benzene Molecule

By M. C. K. INGOLD (London)

I.

GENERAL PRINCIPLES, AND A SUMMARY SHOWING SCOPE OF APPLICATIONS.

When undertaking an investigation in the field of band spectroscopy, one's usual object is to discover as much as possible about the geometry and mechanics of some molecule in its ordinary or « ground » state in the first instance, though at some stage the centre of interest may shift to an « excited » state. Such information has to be gathered from a study of the radiation which the molecule can be caused to to absorb or emit. From the distribution of frequencies and intensities in the radiation, one may seek to elucidate, first, the shape and dimensions of the molecule in its equilibrium configuration, then its vibration forms and their frequencies, and finally the system of forces within the molecule which maintains the configuration and controls the frequencies — in short, the statics and dynamics of the stereochemistry of the molecule.

In pursuing such objects, it is of enormous advantage to be able to work with a number of isotopically isomeric forms of the molecule under examination. There are several reasons for this : all depend on the circumstance that, in replacing an element by its isotope, we

alter no nuclear charges, nor therefore any electronic wave-functions, so that the whole internal force system remains unchanged. One result of this is that the sole effect of an isotopic substitution on the vibration frequencies of a molecule arises from known changes in certain atomic masses. There are accordingly calculable relationships, which are expressed by a general formula known as the Teller-Redlich product theorem, between the frequencies of corresponding fundamental vibrations of any pair of isotopically isomeric molecules. Such relationships are independent of the common, initially unknown, force system, but they will depend both on the configuration assumed for the molecule in equilibrium, and on the particular fundamental modes of vibration to which they apply. Accordingly, the experimental verification of a set of relationships for all the observed frequencies affords a means, not only of establishing the geometry of the equilibrium configuration, or « model », of the molecule, but also of identifying the frequencies observed in the spectra, *i. e.*, of assigning them to the various vibrational modes of which the model is capable.

A further strong reason for undertaking parallel investigations with isotopically isomeric forms of the molecule which is being studied arises in connexion with one's interest in its internal force-system. This has to be defined in terms of the elastic constants, which measure the resistance which the molecule displays to different types of deformation. But for all molecules of more than two atoms, the number of elastic constants, even of Hooke's-law constants, is greater than the number of fundamental vibration frequencies. For an n-atomic molecule without symmetry, the number of Hooke's-law constants is $(3n\text{-}6)(3n\text{-}5)/2$, whilst the number of fundamental frequencies is only $3n\text{-}6$. The presence of symmetry will reduce the former figure and may reduce the latter; but these reductions take place always in such a way that the number of elastic constants remains larger than the number of frequencies — until both become unity in the limit of diatomic molecules. Thus the water molecule has 6 Hooke's-Law constants but only 3 fundamental frequencies, the ethylene molecule 18 constants but only 12 fundamental frequencies, and the benzene molecule 34 constants but only 20 frequencies. It follows that even a complete knowledge of the vibration frequencies of a polyatomic molecule does not furnish enough data for the calculation of the internal force system. But the same force system occurs in all the isotopically isomeric forms of the molecule under consideration.

Hence the vibration frequencies of all those forms, when determined, become available to build up the needed body of data.

The work which has hitherto been carried out on these lines has related exclusively to hydrogen compounds, deuterium having been used for the purpose of producing the required isotopic isomerides. Most of the work has been concerned with molecules in their ground states; and the observational material has therefore consisted of the Raman and infra-red spectra of ordinary and deuterated forms of the substances. The following is a list of such investigations :

HCN — Raman and infra-red spectra of DCN investigated. Harmonic force-system determined :
Dadieu and Kopper, *Wien Anz.* 92, (1935);
Bartunek and Barker, *Phys. Rev.* **48**, 516 (1935).

H_2O — Raman and infra-red spectra of DHO and D_2O investigated. Full Hooke's-law force-system calculated :
Rank, Larsen and Bordner, *J. Chem. Phys.* **2**, 464 (1934);
Bartholomé and Clusius, *Z. Elektrochem.* **46**, 529 (1934);
Barker and Sleator, *J. Chem. Phys.* **3**, 660 (1935);
Darling and Dennison, *Phys. Rev.* **59**, 128 (1940).

H_2S — Raman and infra-red spectra of DHS and D_2S investigated. Harmonic force-system determined :
Bailey, Thompson and Hale, *J. Chem. Phys.* **4**, 625 (1936);
Sprague and Neilson, *J. Chem. Phys.* **5**, 85 (1937);
Neilson and Neilson, *J. Chem. Phys.* **5**, 277 (1937);
Murphy and Vance, *J. Chem. Phys.* **6**, 426 (1938).

H_2Se — Raman and infra-red spectra of DHSe and D_2Se investigated. Harmonic force-system determined :
Dadieu and Engler, *Wien Anz.*, 138 (1935);
Cameron, Sears and Neilson, *J. Chem. Phys.*, **7**, 994 (1939).

C_2H_2 — Raman and infra-red spectra of C_2HD and C_2D_2 investigated. Harmonic force-system determined :
Randall and Beck, *Phys. Rev.*, **45**, 124 (1934);
Colby, *Phys. Rev.*, **47**, 388 (1935);
Glockler and Morrell, *J. Chem. Phys.*, **4**, 15 (1936);
Neilson, *Phys. Rev.*, **57**, 346 (1940);
Stitt, *J. Chem. Phys.*, **8**, 56 (1940).

NH_3 — Raman and infra-red spectra of ND_3 investigated. Height of pyramid and energy of inversion determined :
Manning, *J. Chem. Phys.*, **3**, 136 (1935);
Migeotte and Barker, *Phys. Rev.*, **50**, 143 (1936);
Glockler and Wall, *J. Phys. Chem.*, **41**, 143 (1937);
Wall and Glockler, *J. Chem. Phys.*, **5**, 314 (1937);
Dennison, *Rev. Mod. Phys.*, **12**, 175 (1940).

PH_3 Raman and infra-red spectra of PD_3 investigated. Height of pyramid and energy of inversion determined :
De Hemptinne and Delfosse, *Bull. Sci. Acad. Belg.*, **21**, 793 (1935);
Sutherland, Lee and Wu, *T. Faraday Soc.*, **35**, 1366, *et seq.* (1939).

AsH_3 Infra-red spectrum of AsD_3 investigated. Height of pyramid and energy of inversion determined :
Sutherland, Lee and Wu, *loc. cit.*

H_2CO Infra-red spectrum of D_2CO investigated. Harmonic force-system determined :
Ebers and Neilson, *J. Chem. Phys.*, **5**, 822 (1937); **6**, 311 (1938).

CH_4 Raman and infra-red spectra of CH_3D, CH_2D_2, CHD_3 and CD_4 investigated. Full Hooke's-law force-system calculated :
Ginsberg and Barker, *J. Chem. Phys.*, **3**, 661 (1935);
MacWood and Urey, *J. Chem. Phys.*, **4**, 402 (1936);
Benedict, Morikawa, Barnes and Taylor, *J. Chem. Phys.*, **5**, 1 (1937);
Dennison, *Rev. Mod. Phys.*, **12**, 175 (1940).

CH_3Cl; CH_3Br Raman and infra-red spectra of CD_3Cl and CD_3Br investigated. Harmonic force-system determined :
Noether, *J. Chem. Phys.*, **10**, 81 (1942);
De Hemptinne and Doehaerd, *Bull. Acad. roy. Belg.*, 477 (1943).

$CHCl_3$ Raman spectrum of $CDCl_3$ investigated. Harmonic force-system determined :
Wood and Rank, *Phys. Rev.*, **48**, 63 (1935);
Redlich and Porders, *Monatsh.*, **67**, 328 (1936);
Voge and Rosenthal, *J. Chem. Phys.*, **4**, 137 (1936).

$CH_2 : CH_2$ Raman spectra of $CH_2 : CHD$, $CH_2 : CD_2$, $CHD : CHD$ (cis and trans), $CHD : CD_2$ and $CD_2 : CD_2$, and infra-red spectrum of $CD_2 : CD_2$ investigated. Full Hooke's-law force-system calculated :
Delfosse, *Ann. Soc. Sci. Brux.*, **B55**, 114 (1935);
Manneback and Verleysen, *Ann. Soc. Sci. Brux.*, **B56**, 349 (1936);
Chang, *Ann. Soc. Sci. Brux.*, **B58**, 87 (1938);
De Hemptinne, Jungers and Delfosse, *J. Chem. Phys.*, **6**, 319 (1938);
Bernard and Manneback, *Ann. Soc. Sci. Brux.*, **B 59**, 113 (1939);
Gallaway and Barker, *J. Chem. Phys.*, **10**, 48 (1942);
De Hemptinne and van Riet, *Bull. Acad. roy. Belg.*, 79 (1943).

C_6H_6 Raman and infra-red spectra of C_6H_5D, 1 : 4-$C_6H_4D_2$, 1 : 3 : 5-$C_6H_3D_3$, 1 : 2 : 4 : 5-$C_6H_2D_4$, and C_6D_6, and the Raman spectra of all other partly deuterated benzenes, investigated. Equilibrium symmetry established. Harmonic force-system determined. Data obtained for calculation of full Hooke's-law force-system :
Angus, Bailey, Hale, Ingold, Leckie, Thompson and Wilson, *J. Chem. Soc.*, 912 *et seq.* (1936);
Redlich and Stricks, *Monatsh*, **67**, 213 (1936);
Langseth and Lord, *Kgl. Danske Vidensk. Selsk.*, **16**, 6 (1938);
Bailey, Best, Carson, Gordon, Hale, Herzfeld, Hobden, Ingold, Leckie, Poole, Weldon and Wilson, *J. Chem. Soc.*, 222 *et seq.* (1946).

The Raman and infra-red spectra of deuterated forms of many other molecules have been studied, *e. g.*, CH_3OH, CH_3NH_2, $H.CO_2H$, $CH_3.CO_2H$, C_2H_6, $CH_3.CH_2Br$, $CH_2Br.CH_2Br$, CH_2:CHBr and $CH_2 : CBr_2$. These investigations, however, have not yet been pressed far enough to yield new geometrical or dynamical information about the molecules concerned. For instance, in spite of the close attention that has been given to the problem, it still seems uncertain whether ethane possesses the eclipsed (D_{3h}) or staggered (D_{3d}) equilibrium configurations although indirect considerations, mainly of a thermochemical nature, point rather decidedly to the staggered conflguration.

Mention must also be made of the fact that spectral appearances, due to the rarer isotopes of elements other than hydrogen, have sometimes been found a useful aid in the assignment of observed vibration frequencies to fundamental modes of vibration of a molecule. An example is furnished by the satellite bands, due to the heavier isotope of chlorine, in the infra-red spectrum of methyl chloride.

It is also possible to use isotopic substitution, according to the general principles described, for the study of the geometrical and dynamical characteristics of excited polyatomic molecules. The observational material will now be the absorption and fluorescence spectra of the isotopically isomeric molecules in that part of the ultraviolet range which is relevant for electronic transitions between the already studied ground state and the excited state under examination. This use of isotopes in spectroscopy is a more recent development. Indeed, only one example is at present available for report : it also uses deuterium as source of the required isotopically isomeric forms :

C_6H_6 The state (indicated by the asterisk) is the first excited singlet state. Transitions involving it occur near 2600 A^o. Absorption spectra of C_6H_5D, 1 : 4-$C_6H_4D_2$, 1 : 3 : 5-$C_6H_3D_3$, 1 : 2 : 4 : 5-$C_6H_2D_4$ and C_6D_6, fluorescence spectra of 1 : 4-$C_6H_4D_2$, 1 : 3 : 5-$C_6H_3D_3$, 1 : 2 : 4 : 5-$C_6H_2D_4$ and C_6D_6, and resonance-fluorescence spectra of 1 : 3 : 5-$C_6H_3D_3$ and C_6D_6 excited by Hg 2537 A^o have been investigated. Equilbrium symmetry of excited molecule established. Dimensions estimated. Harmonic force-system calculated. Data obtained for the calculation of an improved force-system :

Ingold and Wilson, *J. Chem. Soc.*, 941 *et seq.* (1936);
Sklar, *J. Chem. Phys.*, **5**, 669 (1937);
Goeppert-Mayer and Sklar, *J. Chem. Phys.*, **6**, 645 (1938);
Nordheim, Sponer, Sklar and Teller, *J. Chem. Phys.*, **7**, 247 (1939);
Sponer, *J. Chem. Phys.*, **8**, 705 (1940);
Beck and Sponer, *J. Chem. Phys.*, **10**, 575 (1942);
Garforth, Ingold and Poole, *J. Chem. Soc.*, 406 *et seq*, (1948).

Electronic transitions involving excited states of deuterated forms of other polyatomic molecules have been investigated, *e. g.*, NH_3, $CH_3.NH_2$, C_2H_4. These studies, however, have not yet been pursued far enough to furnish new dynamical information about the excited states involved.

We may now proceed directly to a more detailed consideration of the main examples by which it is proposed to illustrate the general principles stated. The examples are the ground state, and first excited state, of the benzene molecule.

II.

EQUILIBRIUM SYMMETRY AND VIBRATION FREQUENCIES OF THE GROUND STATE OF BENZENE.

Since one's object is to obtain information on the geometry and dynamics of the ground state of benzene through the observation of its vibration frequencies in the Raman and infra-red spectra, it is necessary first to point out that any molecule, such as benzene, which possesses considerable symmetry, may be unable to record the frequencies of all its vibrations in either type of spectrum. The intensity with which any vibration may appear in either spectrum is shown by the physical theory of the spectra to be either in principle zero, or in principle different from zero, according as a certain physical quantity, which is dependent on the vibration, a polarisability in the case of the Raman spectrum, and an electric moment in the case of the infra-red spectrum, vanishes or not as a result of the symmetry of the molecule. Such vanishings, due to symmetry, are expressed in what are called « selection rules ». They occur to quite a considerable extent with benzene, and, of course, to an equal extent with hexadeuterobenzene, which has exactly the same symmetry. The result is that a number of the fundamental vibrations of each of these molecules can leave no direct record of their frequencies in either the Raman or the infra-red spectrum. The precise number and kind of these vibrations

which are thus prevented from recording themselves will be dependent on the equilibrium symmetry of the molecule, which is the first of the questions to be investigated. It is not necessary here to go into all the theoretical possibilities : it will suffice to make the following statement.

Benzene, having 12 atoms, has 30 vibrational degrees of freedom. On the assumption that benzene has the equilibrium-symmetry which we now know it to possess, theory shows that these 30 vibrational degrees of freedom correspond to 20 frequencies, 10 of the vibrations being non-degenerated, with one degree of freedom each, whilst 10 are doubly degenerated, with two degrees of freedom each; and furthermore, that, of the 20 frequencies, 7 only are allowed in the Raman spectrum, whilst 4 only are allowed in the infra-red spectrum. The 7 Raman frequencies and the 4 infra-red frequencies are different, so that a total of 11 out of the 20 fundamental frequencies are in principle observable in one spectrum or the other. All this is true equally for hexadeuterobenzene.

The first stage of isotopically-aided spectroscopic work on the ground state of benzene was carried out with the two isotopic isomerides, benzene and hexadeuterobenzene. It was directed to the establishment of the equilibrium symmetry of the molecule. Up to 1935 this was in doubt. The theory of mesomerism required the symmetry of a plane, strictly regular hexagon. On the other hand, bands had been observed, in both the Raman and infra-red spectra of benzene, which should not have appeared if the molecule really had this high degree of symmetry. These bands were not inconsistent, however, with a lower equilibrium symmetry, such as might, perhap, correspond to the Kekulé structure for benzene.

It was necessary, first, to find the real reason for the appearance of the extra bands in the Raman and infra-red spectra of benzene. They were traced to the use, deliberately or accidently, of liquid benzene. In a liquid any individual molecule is under external forces, which, although they are always varying under thermal motion, are scarcely ever so balanced as to leave undisturbed the equilibrium symmetry about which the molecule is in vibration. Since it is the equilibrium symmetry which forbids the appearance of certain vibrations in the spectra, one can understand how the cohesive forces in a liquid may cause forbidden frequencies to become observable. No deception need be caused by this effect if one knows that one is using a liquid. The real difficulty was that some observers of the infra-red

absorption spectrum of benzene, who claimed to have worked with the vapour of that substance, must have allowed thin films of liquid benzene to condense on the end-plates of their absorption tubes. A very thin film of liquid will do a great deal of absorbing, and much of the absorption that these workers recorded must have been caused by the liquid films rather than by the vapours. Such films are easily got rid of by warming the end-plates. The recognition of this situation completely cut away the basis of the arguments that had led spectroscopists to reject the plane, regular hexagonal, model of benzene in favour of a less symmetrical model.

On the positive side, it was possible, in the same stage of investigation, to show, (*a*) that the selection rules for the plane regular hexagonal model are indeed precisely fulfilled by the Raman and infra-red spectra of benzene, and also by the corresponding spectra of hexadeuterobenzene; and (*b*) that the 11 spectrally active fundamental frequencies of benzene, and the 11 of hexadeuterobenzene, which this model possesses could all be recognised in the spectra, and could be assigned to their appropriate vibrations. The assignments were not only consistent with theoretical predictions concerning the polarisation of bands in the Raman spectrum, and the rotational contours of bands in the infra-red spectrum; they also gave quantitative agreement with all the relationships, which could be calculated from the model with the aid of the product theorem, between the two sets of frequencies.

Having thus established the equlibrium symmetry of benzene, and having observed and identified the 11 spectrally active vibration frequencies in both benzene and hexadeuterobenzene, the next task was to identify the 9 inactive vibration frequencies of each of these molecules. This was necessary in order to complete our knowledge of their vibration frequencies.

The plan for doing this follows. Vibrations, which are forbidden in a spectrum for reasons of symmetry, may become allowed, and in principle observable, if at least part of the symmetry is removed by dissymetric substitution; but then the vibrations appear with altered frequencies, which in general bear no simple relation to the original frequencies. If, however, the substitution is an isotopic one, there exist, as has been mentioned, certain relationships between the altered active and original inactive frequencies. In general these relationships are not of themselves sufficient to enable the inactive frequencies to be calculated; but they form the main part of the body of data

needed for this purpose. The rest one must hope to secure from supplementary investigations, of which by far the most fruitful consists in the detailed study of the overtones and combination tones of all these substances, particular attention being paid to active first overtones and binary combination tones involving inactive fundamental frequencies.

There was another reason for studying the vibrational spectra of the less symmetrically deuterated benzenes; for one of the objects of this work was to provide material for the determination of the forces within the benzene molecules. Now we have already noted that the number of fundamental frequencies of benzene is smaller than the number of its elastic constants. For example, 10 relations exist between the 20 fundamental frequencies of benzene and the 20 of hexadeuterobenzene, so that all these frequencies are collectively equivalent to 30 independent data; and even that is not enough to permit a complete determination of the 34 Hooke's-law constants of benzene. But just the same force-system is present in all the partly deuterated benzenes. A few of *their* frequencies are, then, what we need in order to provide an adequate basis for undertaking a full determination of the Hooke's-law forces. Actually it has been possible to indentify, not a few, but almost all the frequencies of the partly deuterated benzenes studied.

It was important, of course, not only to observe the frequencies of the partly deuterated benzenes, but also to identify the corresponding vibrations. Owing to the high symmetry of benzene and hexadeuterobenzene, the Raman and infra-red spectra of these molecules each contain relatively few fundamental frequencies : had it been otherwise one might not have been able to interpret the spectra. If we should at once proceed to a deuterated benzene so dissymmetrically substituted that all vibrations are spectroscopically « allowed », very complex spectra would be observed, the interpretation of which present great difficulties. Therefore the method followed is to remove symmetry from the benzene molecule in graded steps by properly oriented substitution, thus allowing previously forbidden vibrations to appear a few at a time. This procedure gives the best chance of arriving eventually at a completely correct interpretation even of the most complex spectra, because at each stage of complexity the added complication is limited, and a full understanding of the related simpler spectra is available to assist and control interpretation. As in the work on benzene and hexadeuterobenzene, considerable

use has been made of the frequency shifts produced by isotopic substitution for the purpose of assigning frequencies to their proper vibrations; and therefore when two isotopically isomeric benzenes exist, such as mono- and pentadeuterobenzene, which have identical symmetry and therefore the same active vibrations, both have been examined. These considerations determined the general scheme, which has been to work in order through the list, given in table I, of isotopically isomeric benzenes. Most of this work has now been done, though the spectroscopic study of pentadeuterobenzene is not yet complete.

TABLE I

Benzenes examined and their Symmetry Classification.

Symmetry	Formulæ	Some symmetry elements
D_{6h}	C_6H_6; C_6D_6	3-fold axis; centre.
D_{3h}	1 : 3 : 5-$C_6H_3D_3$	3-fold axis (no centre).
D_{2h} (= V_h)	1 : 4-$C_6H_4D_2$; 1 : 2 : 4 : 5-$C_6H_2D_4$	Centre (no 3-fold axis).
D_{1h} (= C_{2h})	C_6H_5D; C_6HD_5	(No centre; no 3-fold axis).

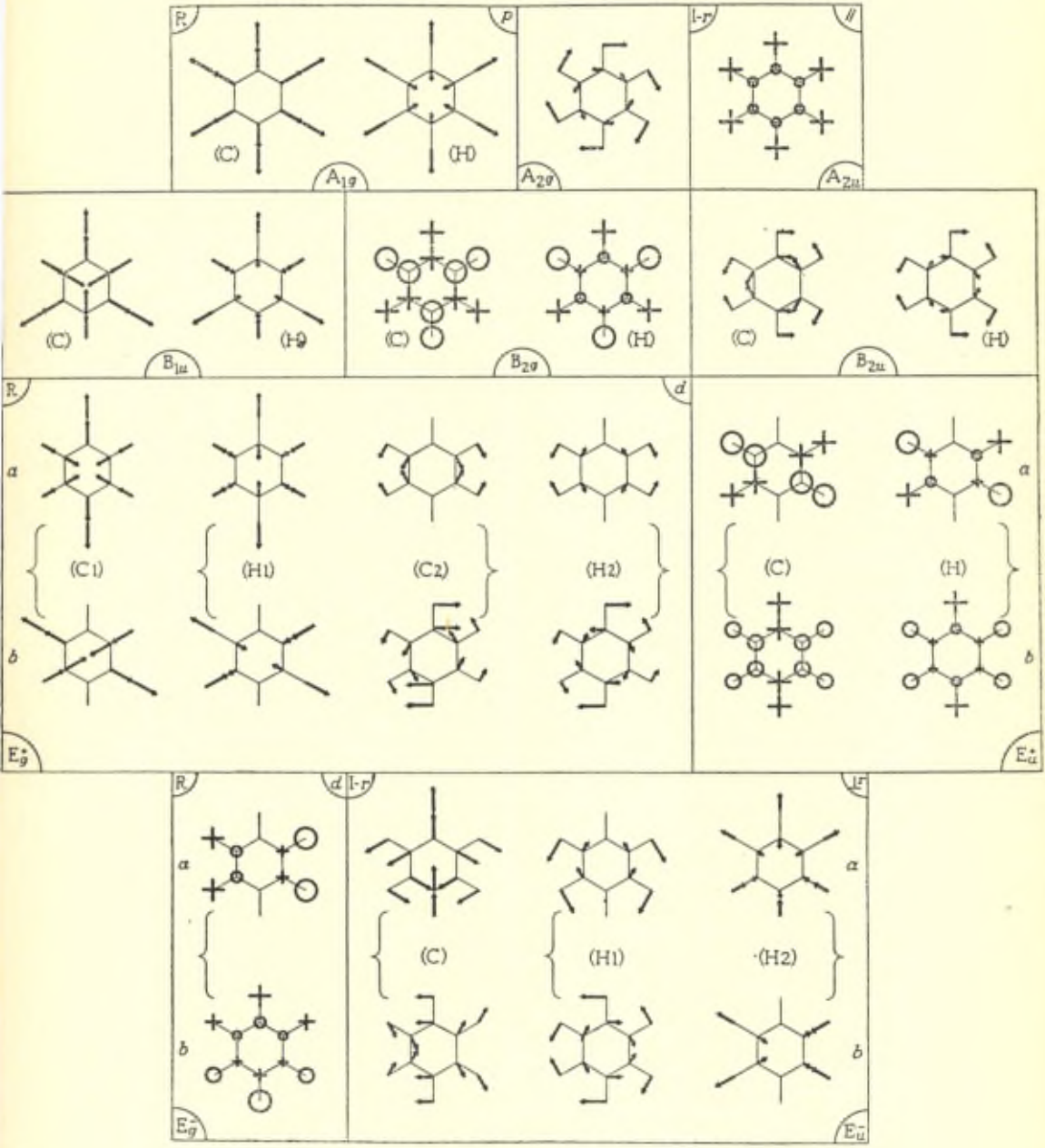
Each line in Table I represents a symmetry class. In all the molecules, the plane of the ring is a plane of symmetry. The numerical subscripts in the symmetry labels (col. 1) mean that the axis perpendicular to the ring plane is a six-fold, three-fold, two-fold, and one-fold symmetry axis, and also that the ring-plane contains six, three, two and one two-fold axes, in the four symmetry classes taken in order. Some other symmetry properties of the classes are explicitly given in the table. Clearly the four classes correspond to a progressive reduction of symmetry.

Vibrations are classified according to their symmetry. Some preserve all the equilibrium symmetry of the molecule (they are called totally symmetrical). Others, in varying degrees, preserve less than total symmetry (and are called non-totally symmetrical). When a molecule possesses a 3-fold axis of symmetry, as do benzene, hexadeuterobenzene and 1 : 3 : 5-trideuterobenzene, a vibration which does not preserve this symmetry is degenerate. This is because it can be represented in three ways which differ only in orientation; and since there is one relation between the three, *viz.*, that the vector sum of their atomic displacements is zero, they clearly correspond to two independent vibrations of the same frequency (fig. 17).

The symmetry classification of vibrations is illustrated in fig. 17 for the case of the D_{6h} benzenes, *viz.*, benzene and hexadeuterobenzene. The 30 diagrams, representing the 30 vibrational degrees of freedom,

Fig. 17.

Vibration forms of the D_{6h} model.



Notes. — (1) Vector perpendicular to the plane of the ring represented + O.

(2) For each point-set ↑ : ↑ = + : + = O : o = 2 : 1.

(3) R = Allowed in the Raman spectrum (p = polarised lines; d = depolarised lines).

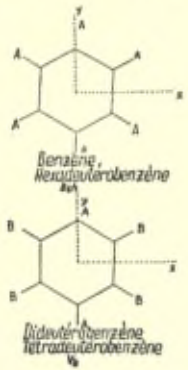
I-r = Allowed in the infra-red spectrum (// = parallel bands; ⊥r = perpendicular bands).

are placed in 10 rectangles, each corresponding to a different symmetry class. The 10 rectangles are arranged in 4 rows. The top row contains all the vibrations which have 6-fold symmetry with respect to the axis of symmetry perpendicular to the ring (they are all labeled A, with various subscripts). The second row of rectangles contains the vibrations which preserve 3-fold symmetry with respect to the same axis (they are labeled B). All the vibrations of the first two rows are non-degenerate. All the remainder are degenerate; and their two degrees of freedom are represented, in each case, by a braced pair of diagrams, corresponding to independent vibrations of identical frequency. The third row of rectangles contains the diagrams of all the vibrations for which the axis perpendicular to the ring is a 2-fold axis of symmetry (they are labeled E^+, with subscripts). The fourth row contains all those for which the axis perpendicular to the ring is only a 1-fold axis (they are labeled E^-).

The distribution of the diagrams into different rectangles within each row has reference to the behaviour of the vibrations with respect to other features of the symmetry of the molecule in equilibrium. Thus the diagrams in the first rectangle preserve symmetry with respect to a 2-fold axis through para-atoms (as is indicated by the subscript 1), and also maintain symmetry with respect to the centre of symmetry (as is indicated by the subscript g). These vibrations, indeed, are totally symmetrical. The single vibration in the second rectangle fails to preserve symmetry with respect to the 2-fold para-axis (as is denoted by the subscript 2), though it does maintain symmetry with respect to the centre (subscript g). The vibration in the third rectangle fails to preserve symmetry with respect to the 2-fold para-axis (subscript 2), and also with respect to the centre (as is denoted by the subscript u). The trigonal vibrations of the second row are similarly sub-divided. Those of the first rectangle preserve the 2-fold para-axis, but not the centre of symmetry. Those of the second rectangle fail to preserve the 2-fold axis, but do maintain the centre. Those of the third rectangle maintain neither of these symmetry elements. Of the degenerate vibrations in the third row of rectangles those in the left-hand rectangle preserve the centre of symmetry, whilst those in the right-hand rectangle do not. The same distinction applies to the left and right-hand members of the fourth row of rectangles. It will be noted that the representations of the degenerate pairs have been so made that the upper member of each pair preserves the 2-fold para axis whilst the lower member does not.

For the purpose of distinguishing between the vibrations of those symmetry classes which contain more than one, it is convenient to rely on more approximate descriptions. In the A_{1g} class, for example, it is possible to rigorously prove (*a*) that the motions of the atoms of each CH-group will be parallel in one vibration and anti-parallel in the other; and (*b*) that the ratio of the carbon amplitudes in the two vibrations will bear to the ratio of the hydrogen amplitudes the same proportions as the mass of a hydrogen atom bears to a mass of a carbon atom. But for classificatory purposes it is sufficient to reduce this to the rough statement that in one vibration each carbon atom carries its hydrogen atom with it, whilst in the other most of the moving is done by the hydrogen atoms. We call the former vibration a carbon vibration, and the latter a hydrogen vibration, using the specific symbols A_{1g} (C) and A_{1g} (H). The A_{2g} and A_{2u} vibrations, which are alone in their symmetry classes, are obviously hydrogen vibrations; so also is the degenerate vibration E_g^-. Each of the *B* symmetry classes contains two vibrations, one of which is a carbon, and the other a hydrogen, vibration; the same is the case with the degenerate symmetry class E_u^+. On the other hand, the symmetry class E_g^+ contains two carbon and two hydrogen vibrations, whilst the symmetry class E_u^- contains one carbon and two hydrogen vibrations, so that, in these classes, we have to add a number to the symbolic description of a vibration, using such labels as E_g^+ (C2), E_u^- (H1), etc., as indicated beside the relevant diagrams in fig. 17.

In order to be able to represent the symmetry classification of the vibrations of all the partly deuterated benzenes, it is convenient to take rectangular co-ordinate axes, $j = x, y, z$, as shown in the following diagrams, in each of which either A represents protium and B deuterium, or *vice versa* :



We furthermore, allow C_p^j to denote an axis of p-fold symmetry parallel to the co-ordinate axis j, σ_j, to represent a plane of symmetry perpendicular to the axis j, and J to signify a centre of symmetry. Then the labels of the symmetry classes of the vibrations of the various isotopically isomeric benzenes are as shown in table II.

TABLE II

Symmetry Class Symbols of Vibrations of D_{6h}, D_{3h}, V_h, C_{2v} molecules.

Group	Class symbol	Class symbol
D_{6h}	A = sym. to C_3^z and to C_2^z	1 = sym. to C_2^y
	B = sym. to C_3^z, not to C_2^z	2 = antisym. to C_2^y
	E^+ = degen., sym. to C_2^z	g = sym. to J.
	E^- = degen., antisym. to C_2^z	u = antisym. to J
D_{3h}	A = sym. to C_3^z	1 = sym. to C_2^y 2 = antisym. to C_2^y
	E = degenerate	' = sym. to σ_z '' = antisym. to σ_z
V_h	A = sym. to C_2^z and to C_2^y	
	B_1 = sym. to C_2^z, not to C_2^y	g = sym. to J
	B_2 = sym. to C_2^y, not to C_2^z	u = antisym. to J
	B_3 = antisym. to C_2^z and to C_2^y	
C_{2v}	A = sym. to C_2^y	1 = sym. to σ_z
	B = antisym. to C_2^y	2 = antisym. to σ_z

When the symmetry, D_{6h} of benzene or hexadeuterobenzene becomes degraded to the symmetry, D_{3h}, of 1 : 3 5 -trideuterobenzene, the 6-fold axis, as distinct from the coincident 3-fold axis, disappears, and also the centre of symmetry disappears. It thus becomes impossible for a vibration to have any particular properties, such as symmetry or antisymmetry, with respect to these lost elements of symmetry. Reference to fig. 1 shows that the D_{6h} vibrational symmetry classes, A_{1g} and B_{1u}, differ with respect to just those properties which cease to be available in the vibrations of a molecule of equilibrium symmetry D_{3h}. It follows that in the D_{3h} molecule these two symmetry classes will coalesce into a single symmetry class, called A_1' (according

to table II). Similarly, the D_{6h} classes, A_{2g} and B_{2u}, will merge to form the D_{3h} class A_2', whilst the D_{6h} classes A_{2u} and B_{2g}, will become fused to give the D_{3h} class A_2''. The D_{6h} symmetry classes E_g^+ and E_u^- both preserve the plane of the molecule as a plane of symmetry, but differ in their properties with respect to the centre of symmetry. In the D_{3h} molecule the centre of symmetry disappears, and so these two symmetry classes join together to form the class E'. Similarly the D_{6h} classes E_u^+ and E_g^- join together to form the D_{3h} class E''. The ways in which the vibrational symmetry classes coalesce when the equlibrium symmetry D_{6h} is degraded by partial deuteration to V_h or C_{2v} can be similarly discussed. The general pattern of the relationships which these arguments disclose is shown in table III.

TABLE III

Relations between Symmetry Classes of Benzenes of different Symmetry.

D_{6h}	A_{1g},	B_{1u}	E_g^+,	E_u^-	A_{1u},	B_{1g}	E_u^+,	E_g^-	A_{2g},	B_{2u}	E_g^+,	E_u^-	A_{2u},	B_{2g}	E_u^+,	E_g^-
D_{3h}	A'_1		E'		A''_1		E''		A'_2		E'		A''_2		E''	
C_{2v}	A_1				A_2				B_1				B_2			
V_h	A_g		B_{2u}		A_u		B_{2g}		B_{1g}		B_{3u}		B_{1u}		B_{3g}	
D_{6h}	A_{1g},	E_g^+	B_{1u},	E_u^-	A_{1u},	E_u^+	B_{1g},	E_g^-	A_{2g},	E_g^+	B_{2u},	E_u^-	A_{2u},	E_u^+	B_{2g},	E_g^-

A consideration of the physical mechanism of the production of a Raman spectrum, and of an infra-red absorption spectrum, shows that a vibration must change the polarisability of the molecule if it is to appear in the Raman spectrum, and must alter the electric moment if it is to leave its record in the infra-red spectrum. The A_{1g} vibrations of fig. 17 must obviously change the polarisability of the benzene molecule, though they will not disturb its (zero) electric moment. These vibrations therefore appear as fundamentals in the Raman spectrum only, and, it may be added (though the reason must be omitted), they give polarised lines. The only other vibrations which appear in the Raman spectrum are those of the E_g^+ and E_g^- symmetry classes; and they give depolarised lines. The vibration A_{2u} clearly produces a variation of electric moment in a direction normal to the plane of the ring. It will therefore record itself as a fundamental

band in the infra-red spectrum, the band having a characteristic rotational contour (and here again, the reason must be omitted) of what is called the «parallel» type. The only other vibrations which appear in the infra-red spectrum are those of the E_u^- class : they cause a variation of electric moment in the plane of the molecule, and produce bands with a rotational contour of the « perpendicular » type.

Now we have seen that when the equilibrium symmetry of the molecule is degraded from that of benzene or hexadeuterobenzene to that of 1 : 3 : 5-trideuterobenzene, all distinctions of symmetry between the D_{6h} vibration classes A_{1g} and B_{1u} disappear. It follows that in benzene and hexadeuterobenzene the B_{1u} vibrations did not appear in the Raman spectrum because, and only because, the variation of polarisability was cancelled by just those symmetry properties which a D_{3h} molecule cannot possess. Such cancellation will, then, not occur in 1 : 3 : 5-trideuterobenzene; and thus all the four vibrations of its symmetry classe A_1' will be active in the Raman spectrum. This illustrates the general principle that when, on account of a reduction of the equilibrium symmetry of the molecule, two or more symmetry classes coalesce, any spectral activity previously present in one of the combining classes becomes possessed by all vibrations of the combination class. In like manner, because the single A_{2u} vibration of benzene and hexadeuterobenzene is active in the infra-red spectrum, all three of the A_2'' vibrations of 1: 3: 5-trideuterobeuzene (which arise by combining the A_{2u} and B_{2g} symmetry classes) will be active in the infra-red spectrum. All three of the E'' vibrations (which arise by combining the E_g^- and E_u^+ symmetry classes) will be active in the Raman spectrum. Finally, all seven of the E' vibrations of 1 : 3 : 5-trideuterobenzene will be active both in the Raman and infra-red spectra, because the E_g^+ vibrations of benzene and hexadeuterobenzene appeared in the Raman spectrum and the E_u^- vibrations in the infra-red spectrum. Thus, in 1 : 3 : 5-trideuterobenzene, 17, instead of 11, of the 20 fundamental frequencies are in principle capable of direct observation : and they have all been, not only observed, but also identified.

In 1 : 4-dideuterobenzene, and 1 : 2 : 4 : 5-tetradeuterobenzene we have no longer a 3-fold axis of symmetry and therefore no degenerate vibration : each of the vibrations which was degenerate in the benzenes of higher symmetry now splits into two vibrations having different frequencies. Furthermore, now that the plane of

the molecule possesses a unique direction, they have different symmetries. Thus the four E_g^+ vibrations shown in the top row of the appropriate rectangle of fig. 17 mix with the two A_{1g} vibrations to form a symmetry class, called A_g, of six vibrations which, naturally, will be active in the Raman spectra of 1 : 4-di- and 1 : 2 : 4 : 5-tetra-deuterobenzene. On the other hand, the four E_g^+ vibrations shown in the bottom row will mix with the A_{2g} vibration to form a symmetry class B_{1g}, of five vibrations, all of which will also be active in the Raman spectra of these two benzenes, even though the A_{2g} vibration is inactive in benzene and hexadeuterobenzene. The other relations of this nature are all indicated in table III, and the over-all result is that, of the 30 distinct vibrations, 15 are active in the Raman spectrum and 13 others are active in the infra-red spectrum, so that 28 of the 30 frequencies are directly observable. For 1 : 4-di- and for 1 : 2 : 4 : 5-tetra-deuterobenzene, all the 28 frequencies have been both observed and identified.

The situation with respect to the vibrations of mono- and penta-deuterobenzene is that, of their 30 fundamental frequencies, 27 can appear in the infra-red spectrum, whilst all these and one other can appear in the Raman spectrum. All the 28 spectrally active frequencies of monodeuterobenzene have been observed and identified. However the work on pentadeuterobenzene is as yet incomplete, only its Raman spectrum having so far been studied.

It is not necessary to go into the details of the recognition and identification of all these active frequencies, since the principles employed are essentially the same as those previously used for benzene and hexadeuterobenzene.

By using the observed frequencies of the vibrations of the partly deuterated benzenes, in conjunction with the theoretical relations summarised in the Teller-Redlich product theorem, it is possible at once to proceed a considerable way in the determination of the frequencies of the nine spectrally inactive vibrations of benzene and hexadeuterobenzene. One of the inactive vibrations is alone in its symmetry class : the frequencies of this vibration, for benzene and for hexadeuterobenzene, can be directly calculated. The other eight vibrations occur as pairs in four symmetry classes : here we can calculate, for benzene or for hexadeuterobenzene, the product of each pair of frequencies. For the determination of the individual frequencies of each of these pairs it is therefore necessary to have another datum.

In almost every case it has been found possible to obtain, not

TABLE IV

Correlation of the Fundamental Frequencies (cm.$^{-1}$) of the Ground States of Benzene, Hexadeuterobenzene, 1 : 3 : 5-Trideuterobenzene and Monodeuterobenzene.

DESCRIPTION OF VIBRATION	D_{6h}			D_{3h}		C_{2v}	
	Class	C_6H_6	C_6D_6	Class	$C_6H_3D_3$	Class	C_6H_5D
C-stretching	A_{1g}	991.6	943.2	A'_1	956.2	A_1	980.0
H-stretching		3061.9	2292.6		3052.7		3054.0
C-bending	B_{1u}	**1010**	**963**		1003.6		1006.8
H-stretching		**3060**	**2290**		2281.9		2269.0
C-bending	E_g^+	605.6	577.4	E'	593		601.8
H-stretching		3046.8	2264.9		2292		3060
C-stretching		1596	1551.5		1573.1		1591.1
H-bending		1178.0	867.3		1101.8		1175.6
C-deformation	E_u^-	1485	1333		1407		1480
H-bending		1037	813		833.5		1031.0
H-stretching		3080	2294		3084		3066
Out-of-plane	E_u^+	**405**	**352**	E''	372.7	A_2	**405**
Out-of-plane		**970**	**793**		947		**970**
Out-of-plane	E_g^-	848.9	661.7		710.2		849.9

DESCRIPTION OF VIBRATION	D_{6h}			D_{3h}		C_{2v}	
	Class	C_6H_6	C_6D_6	Class	$C_6H_3D_3$	Class	C_6H_5D
H-bending	A_{2g}	**1326**	**1037**	A'_2	**1230**	B_1	1292
C-stretching	B_{2u}	**1648**	**1571**		**1600**		1624
H-bending		**1110**	**825**		**920**		858
C-bending	E_g^+	605.6	577.4	E'	593		601.8
H-stretching		3046.8	2264.9		2292		3041
C-stretching		1596	1551.5		1573.1		1574.3
H-bending		1178.0	867.3		1101.8		1158.2
C-deformation	E_u^-	1485	1333		1407		1450
H-bending		1037	813		833.5		1076
H-stretching		3080	2294		3084		3079
Out-of-plane	A_{2u}	671	496.5	A_2	533	B_2	608
Out-of-plane	B_{2g}	**703**	**601**		691		698
Out-of-plane		**985**	**827**		915		922
Out-of-plane	E_u^+	**405**	**352**	E''	372.7		380
		970	**793**		947		995
Out-of-plane	E_g^-	848.9	661.7		710.2		778

TABLE V

Correlation of the Fundamental Frequencies (cm.$^{-1}$) of the Ground States of Benzene, Hexadeuterobenzene, 1 : 4-Dideuterobenzene, 1 : 2 : 4 : 5-Tetra-deuterobenzene and Monodeuterobenzene.

DESCRIPTION OF VIBRATION	D_{6h}			V_h			C_{2v}	
	Class	C_6H_6	C_6D_6	Class	$C_6H_4D_2$	$C_6H_2D_4$	Class	C_6H_5D
C-stretching	A_{1g}	991.6	943.2		978.0	960.9		980.0
H-stretching		3061.9	2292.6		3055.0	2285.0		3054.0
C-bending	E_g^+	605.6	577.4	A_g	596.0	589.0		601.8
H-stretching		3046.8	2264.9		2280.0	3045.0		3060
C-stretching		1596	1551.5		1587.0	1572.1		1591.1
H-bending		1178.0	867.3		1173.4	862.2	A_1	1175.6
C-bending	B_{1u}	**1010**	**963**		992	977		1006.8
H-stretching		**3060**	**2290**		2275	3078		2269.0
C-deformation	E_u^-	1485	1333	B_{2u}	1469	1353		1480
H-bending		1037	813		1033	819		1031.0
H-stretching		3080	2294		3060	2280		3066
Out-of-plane	E_u^+	**405**	**352**	A_u	**405**	**352**		**405**
Out-of-plane		**970**	**793**		**970**	**793**	A_2	**970**
Out-of-plane	E_g^-	848.9	661.7	B_{2g}	849.5	663.6		849.9

DESCRIPTION OF VIBRATION	D_{6h}			V_h			C_{2v}	
	Class	C_6H_6	C_6D_6	Class	$C_6H_4D_2$	$C_6H_2D_4$	Class	C_6H_5D
H-bending	A_{2g}	**1326**	**1037**		1309.0	954		1292
C-bending	E_g^+	605.6	577.4	B_{1g}	600.9	585.8	B_1	601.8
H-stretching		3046.8	2264.9		3042	2272		3041
C-stretching		1596	1551.5		1569.3	1564.0		1574.3
H-bending		1178.0	867.3		908.5	1255.3		1158.2
C-stretching	B_{2u}	**1648**	**1571**	B_{3u}	1603	1585		1624
H-bending		**1110**	**825**		814	1057		858
C-deformation	E_u^-	1485	1333		1413	1439		1450
H-bending		1037	813		1106	812		1076
H-stretching		3080	2294		3079	2280		3079
Out-of-plane	A_{2u}	671	496.5		597	548		608
Out-of-plane	E_u^+	**405**	**352**	B_{1u}	367	383	B_2	380
Out-of-plane		**970**	**793**		876	925		995
Out-of-plane	B_{2g}	**703**	**601**	B_{3g}	634.1	615.1		698
Out-of-plane		**985**	**827**		967	929.7		922
Out-of-plane	E_g^-	848.9	661.7		736	767.1		778

only the one necessary datum, but also a great deal of valuable confirmation of the determined fundamental frequencies, through the study of the higher harmonics, which can be found in the Raman and infra-red spectra of benzene and hexadeuterobenzene. They are almost all first overtones or binary summation tones. The fact that they can be interpreted, and applied to the problem of the determination of the inactive fundamental frequencies, is entirely due to the severely restricting effect of the high symmetry of the molecule on the number and type of higher harmonics which may appear in the Raman or the infra-red spectrum.

From a knowledge of the inactive frequencies of benzene and hexadeuterobenzene, it is possible to proceed by similar methods to a determination of the inactive frequencies of the partly deuterated benzenes. The two inactive frequencies of mono- and 1 : 4-di-deuterobenzene can be shown to be individually identical with certain frequencies of benzene, whilst the two inactive frequencies of 1 : 2 : 4 : 5-tetra- and penta-deuterobenzene are individually identical with the corresponding frequencies of hexadeuterobenzene. The three inactive frequencies of 1 : 3 : 5-trideuterobenzene present more difficulty, since they all belong to a single symmetry class : we can find the product of the frequencies and then have to seek for two further relations, involving these individual frequencies, amongst those higher harmonics which can be found in the Raman and infra-red spectra of 1 : 3 : 5-trideuterobenzene.

The result of the work up to this stage is that a list can be given of all the fundamental frequencies of the vibrations of those isotopically isomeric benzenes whose Raman and infra-red spectra have both been examined, *i. e.*, all the isomerides mentioned above except pentadeuterobenzene. These frequencies should suffice for the determination of all the Hooke's-law elastic constants of benzene.

The vibration frequencies are assembled in Tables IV and V. Two tables are necessary to show the correlation of the frequencies of one isomeride with those of another. This is because the symmetry of benzene and hexadeuterobenzene can be degraded to that of monodeuterobenzene by either of two parallel routes, *i. e.*, either through the intermediate symmetry of 1 : 3 : 5-trideuterobenzene, or through that of 1 : 4-di- and 1 : 2 : 4: 5-tetra-deuterobenzene; and the vibrations group themselves in different ways according to the route taken. Frequencies directly observed as fundamentals in the Raman spectrum or the infra-red spectrum or both are printed

in the Roman type. Those frequencies which, as fundamentals, are inactive in both spectra, and have therefore been obtained partly by calculation from observed frequencies of other isotopic forms, and partly by the observation of higher harmonic frequencies, are given in bold type. The figures given for the B_{2u}(C) frequency of benzene, and its analogues for the deuterated benzenes are still regarded as rather rough and provisional estimates. In the partial description given of the vibrations, the terms stretching and bending refer to planar motion according to a valency picture; and the term deformation is used when stretching and bending are simultaneously present. The out-of-plane vibrations are not separated in these tables into carbon and hydrogen types, because in certain cases the distinction becomes somewhat artificial.

III.

THE EQUILIBRIUM SYMMETRY AND VIBRATION FREQUENCIES OF THE FIRST EXCITED SINGLET STATE OF BENZENE.

The described study of the geometry and vibrations of the electronic ground state of benzene has permitted the commencement of an investigation with similar objects into those electronically excited states of benzene which are sufficiently long-lived to exhibit sharply defined vibration frequencies. One cannot, of course, hold the benzene molecule in an electronically excited state, as one holds it in the electronic ground state, for the purpose of a direct excitation of its vibrations. Evidence about the vibrations of an excited state has to be gathered from a study of electronic transitions between the ground state and the excited state in question. The spectral record of such electronic transitions will display a band-structure due to accompanying changes of vibration frequencies, with or without changes of vibrational quantum number; and, if one is sufficiently well-informed about the vibrations of the lower electronic state to recognise the exact part which they play in the production of the observed band structure, one may be able to sort out the

contribution made by the vibrations of the upper electronic state, and thus to reach conclusions concerning their frequencies.

An investigation on these lines has a significant place in the contemporary developement of spectroscopy. At the present time, the principles underlying the analysis of rotation and vibration spectra of diatomic, and of polyatomic, molecules are fully known; and the same is true of the electronic spectra of atoms, and of diatomic molecules. But the fifth and last remaining branch of optical spectroscopy; the electronic spectroscopy of polyatomic molecules, is still in its rudimentary stages; and benzene, with its high symmetry and very practical possibilities of isotopic modification, is an almost ideal example with which to open up the study of this branch of spectroscopy.

In the investigation to be reported, considerable use is made of both the mentioned properties of the benzene molecule. The experimental material consists in the ultra-violet absorption and fluorescence spectra of the various isotopically isomeric benzenes in the spectral region relevant to the required electronic transition. The benzenes must be used as vapour in order to secure adequate resolution, and the dispersion must be sufficient to show the details of the band structure.

The analysis depends largely on comparisons between the spectra of isotopically modified benzenes. It proceeds by an application to the upper state of two principles neither of which has been applied before to the vibrations of an excited polyatomic molecule. The first depends on the circumstance that, just as in the electronic ground state, so also in an excited state, isotopic substitution does not change the force-field. From that alone, and without a knowledge of the force-field, a number of calculable relationships, which are summarised in the Teller-Redlich formula, arise between the upper-state frequencies of isotopically isomeric molecules. The precise relationship depends on the forms of the vibrations, and thus guides the assignment of frequencies to vibrations. The second depends on the use of simplified force-systems. Just as has so often been done in the study of the vibrations of molecules in their ground states, so for an excited molecule it is possible to utilise the first few definitely identified vibration frequencies in order to compute a rough force-system (the same for all the isotopically isomeric molecules), from which one may calculate approximate values of other vibration frequencies, thus securing a guide with the aid of which they may be definitely identified by reference back to the spectra.

The symmetry of benzene has been represented by the symbol D_{6h}. When it is intended to refer to the symmetry of the nuclear charges, but not of the nuclear masses, this will be written $\mathbf{D_{6h}}$. This is because the nuclear charges control the electronic motion, the forms of which will be symbolised by means of heavy type-italics being reserved, as heretofore, for the representation of nuclear motion. Just as for nuclear motion, so also for electronic motion, the possible forms can be classified according to what symmetry they preserve. The twelve classes, 8 non degenerate (**A**, **B**) and 4 degenerate (**E**), are $\mathbf{A_{1g}}$, $\mathbf{A_{1u}}$, $\mathbf{A_{2g}}$, $\mathbf{A_{2u}}$, $\mathbf{B_{1g}}$, $\mathbf{B_{1u}}$, $\mathbf{B_{2g}}$, $\mathbf{B_{2u}}$, $\mathbf{E_g^+}$, $\mathbf{E_u^+}$, $\mathbf{E_g^-}$ and $\mathbf{E_u^-}$. These symbols refer to the total electronic orbital wave function, just as the corresponding italic symbols relate to the total nuclear vibrational wave function (as well as to the normal co-ordinate in the special case of fundamental vibrations). It is usual further to classify electronic states according to the total spin of the electrons; and triplet as well as singlet forms of benzene could be produced by suitable forms of excitation. However, the electronic ground state of benzene is a singlet state, and therefore only singlet excited states will participate in the stronger transitions involving the ground state. The investigation to be reported is concerned only with singlet states, and it is therefore unnecessary here to introduce a symbol for the electronic spin.

The above 12 symmetry classes do not all contain electronic states, just as not all of the corresponding nuclear symmetry classes contain vibrations. It can be shown that non-ionised benzene, because of the even number of its electrons, cannot have electronic states which are antisymmetric with respect to the molecular plane. This reduces the symmetry classes which are available for occupation by electronic states to the six following : $\mathbf{A_{1g}}$, $\mathbf{A_{2g}}$, $\mathbf{B_{1u}}$, $\mathbf{B_{2u}}$, $\mathbf{E_g^+}$, $\mathbf{E_u^-}$.

Benzene has several ultra- violet absorption systems. The first runs out from about 2600 $A°$, and it is the vibrational structure of this transition which has been studied in detail. A stronger transition starts at about 2000 $A°$, and this is overlapped by a still stronger one at about 1850 $A°$. After that two Rydberg series appear which run out to a common limit at 1350 $A°$.

E. Hückel first discussed the lower electronic states of benzene as molecular states of the six unsaturation electrons; and his work has been brilliantly developed by Sklar. The six atomic orbitals $2p\pi$ of these electrons combine to produce the following molecular orbitals in order of increasing energy : $\mathbf{a_{2u}}$, $\mathbf{e_g^-}$, $\mathbf{e_u^+}$, $\mathbf{b_{2g}}$. Here the

symmetry symbols have their usual meaning except that, by custom, lower case letters are used for single-electron wave functions. Pauli's principle allows the $\mathbf{a}_{2u}$ and $\mathbf{b}_{2g}$ shells each to accommodate two, and the degenerate $\mathbf{e}_g^-$ and $\mathbf{e}_u^+$ shells four, electrons. The electronic ground state, formed by filling the $\mathbf{a}_{2u}$ and $\mathbf{e}_g^-$ orbitals, has the symmetry $\mathbf{A}_{1g}$. Here the electronic motion preserves all the symmetry of the$\mathbf{D}_{6h}$ molecular model.

The lowest excited states are obtained by promoting an electron from an $\mathbf{e}_g^-$ orbital to an $\mathbf{e}_u^+$ orbital. Because of the degeneracy of these orbitals, two non-degenerate and one degenerate states may thus arise, which have the symmetries $\mathbf{B}_{2u}$, $\mathbf{B}_{1u}$ and $\mathbf{E}_u^-$. According to Sklar's calculations of the energy relationships, the $\mathbf{B}_{2u}$ state lies the lowest of these three, and is therefore the first singlet excited state of benzene. The molecular model still has the symmetry $\mathbf{D}_{6h}$, but now the electronic wave-function changes sign on rotation about any 2-fold axis through para-atoms. Goeppert-Mayer and Sklar's further calculations place the $\mathbf{B}_{1u}$ state higher on the energy scale and the $\mathbf{E}_u^-$ state higher still. Neither the $\mathbf{B}_{2u}$ nor the $\mathbf{B}_{1u}$ state has the symmetry of a component of the electric moment, and therefore transitions between the ground stage and each of these states is forbidden. On the other hand, the $\mathbf{E}_u^-$ state has the symmetry of an electric moment in the plane of the ring, and hence transitions between the ground state and this state are allowed. Sklar first assigned as a $\mathbf{B}_{2u}$ state the upper state of the transition starting near 2600 A°. Though the transition is forbidden under the equilibrium symmetry of the nuclear system, it would be able to occur when, in either of the interacting states, the molecular symmetry became suitably reduced by an appropriate nuclear vibration. Goeppert-Mayer and Sklar assigned the absorption system starting around 2000 A° as a forbidden transition to a $\mathbf{B}_{1u}$ state, and the strong absorption system beyond 1850 A° as an allowed transition to an $\mathbf{E}_u^-$ state.

A hypothesis concerning the symmetry of the electronic motion in the upper electronic state enables selection rules to be formulated which should regulate the appearance of vibrational changes in association with the electronic transition; and a verification that these selection rules are obeyed in detail will constitute a proof of the correctness of the identification of the electronic state. In the present case, Sklar's hypothesis concerning the symmetry of the electronic wave function of the upper state of the near-ultra-violet transition of benzene is amply confirmed in the spectra. It follows

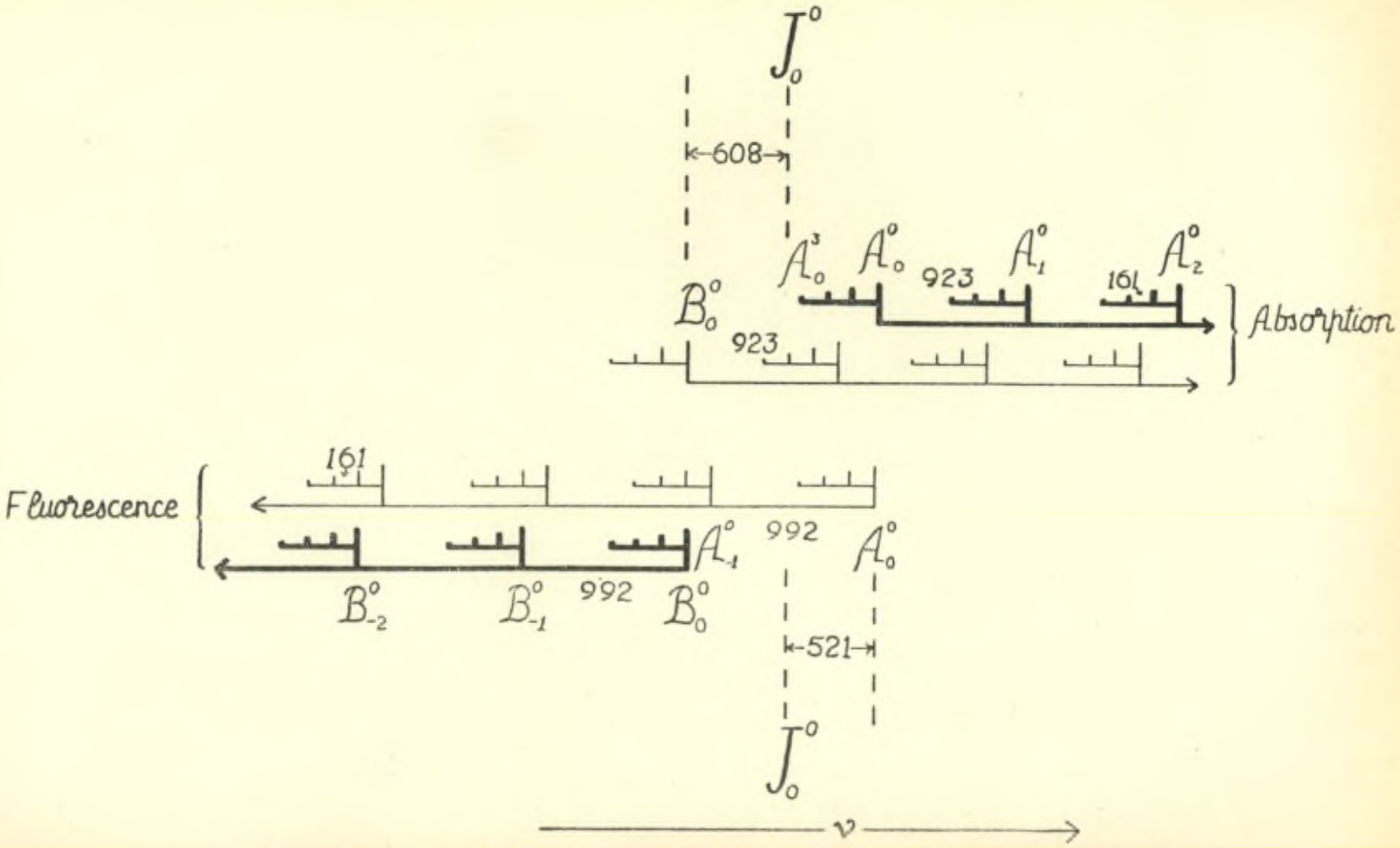
that the excited molecule, like the normal molecule, of benzene has the full symmetry of a plane regular hexagon.

The selection rules for an allowed electronic transition require that the quantum numbers of the totally symmetrical vibrations should be the principal ones to change. Thus one expects, accompanying the 0-0 band, strong progressions 0-1, 0-2..., in these vibrations. One must also expect that sequences involving transitions 1-1, 2-2..., in all the non-totally symmetrical vibrations will be prominent, except in so far as these bands are weakened by the Boltzmann factors of their initial vibrational levels. As weak bands, one might observe such as depend on changes of 2 in the quantum numbers of non-totally symmetrical vibrations, *i. e.*, transitions 0-2, 1-3..., or symmetrically equivalent combinations, such as combined 0-1, 0-1 changes of pairs of non-totally symmetrical vibrations of the same symmetry class.

In a forbidden transition the 0-0 band cannot appear. Associated with the electronic change there must be a vibrational transition of such a type as will produce the perturbation which makes the electronic transition possible. The symmetry which the vibration must possess is readily calculated. The simplest form of vibrational perturbation would be a one-quantum change, such as 0-1, 1-2, 1-0 or 2-1 transition, of some vibration of the right symmetry. Upon each vibrational transition fulfilling the required conditions, all the vibrational changes which may accompany an allowed electronic transition may be superposed.

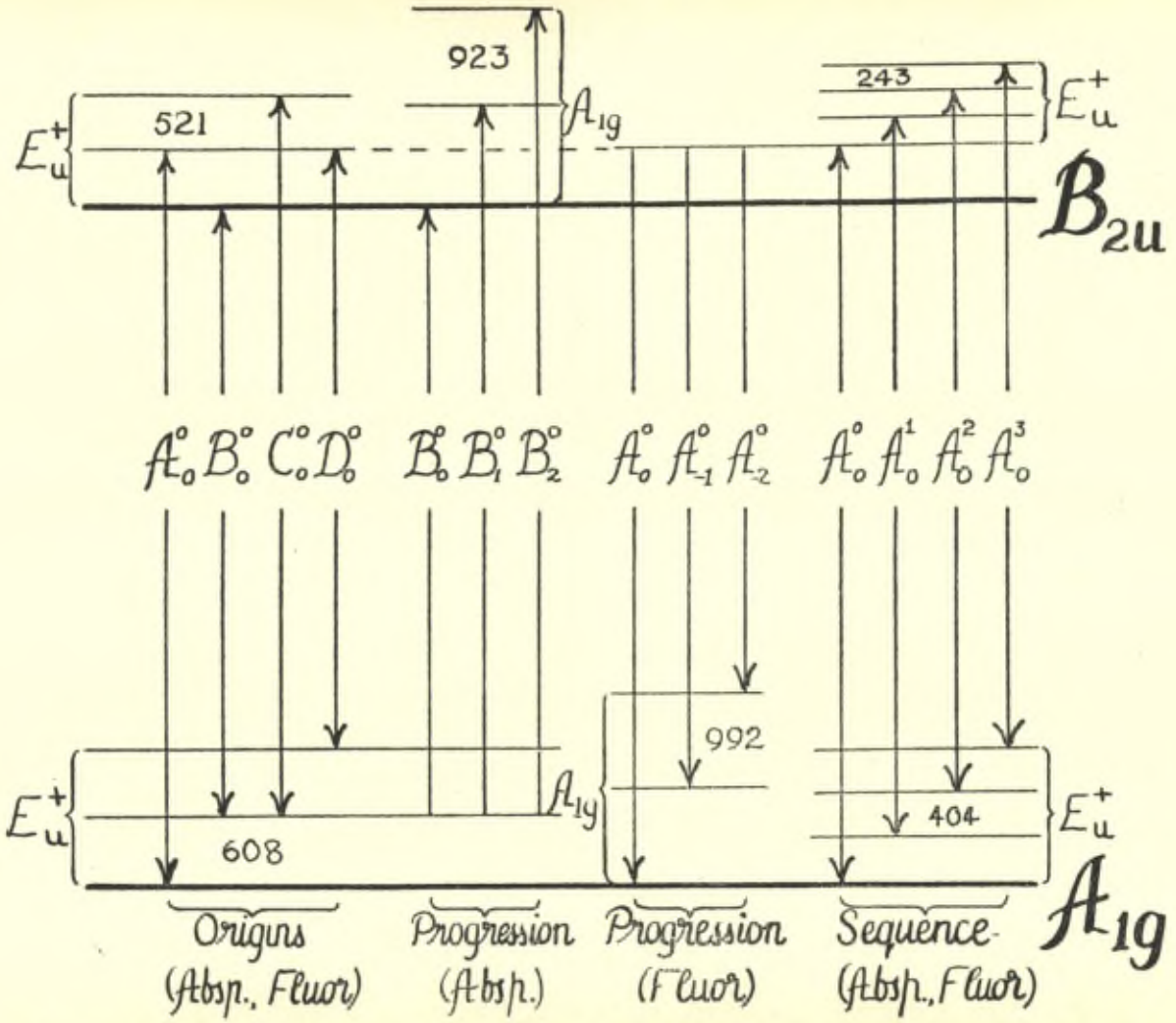
Sklar pointed out that, in the case of the $\mathbf{A_{1g}}$-$\mathbf{B_{2u}}$ electronic transition of benzene, a vibration of E_g^+ symmetry can provide the necessary perturbation, and furthermore that, amongst fundamental vibrations, it is the only kind that can so act. He assumed that a one-quantum change in one of the four E_g^+ fundamental vibrations of benzene does indeed allow the $\mathbf{A_{1g}}$-$\mathbf{B_{2u}}$ electronic transition to produce the band-system near 2600 A°. Nordheim, Sponer, Teller and he then showed that this theory accounts very well for the salient features of the structure of the relevant absorption and fluorescence spectra. The main perturbing vibration is the planar carbon-bending vibration E_g^+ (C1), whose ground-state frequency, as given by the Raman spectrum of liquid benzene, is 606 cm.$^{-1}$. The absorption and fluorescence spectra themselves give the frequencies 608 cm.$^{-1}$ in the ground state and 521 cm.$^{-1}$ in the excited state.

Fig. 18



General pattern of stronger bands in the first ultraviolet absorption and fluorescence band-systems of benzene vapour.

Fig. 19



Interpretation of the pattern of stronger bands in the first ultraviolet absorption and fluorescence systems of benzene.

The general pattern of the two related spectra is shown in a simplified form in fig. 18. In absorption, two « active origins », A_0^0 and B_0^0, 1129 cm.$^{-1}$ apart, are the starting points of progressions in the interval 923 cm.$^{-1}$ which run to higher frequencies, the progression starting from the higher frequencies being much the stronger. In fluorescence, the same two origins start progressions in the interval 992 cm.$^{-1}$ which run to lower frequencies, the progression starting from the lower frequency being much the stronger. From each band in all these progressions, sequences start out : in both absorption and fluorescence they run to lower frequencies, and the interval, 161 cm.$^{-1}$, is common to them all. Then (though this is omitted from fig. 18 for simplicity) the whole of the described pattern is repeated much more weakly with a shift of 87 cm.$^{-1}$ towards lower frequencies. Many bands are present which do not conform to this description, which nevertheless represents the main framework of the two spectra.

The interpretation will be clear from Fig. 19. In absorption the origins A_0^0 and B_0^0 involve upward transitions, 0-1 and 1-0 respectively, of the perturbing E_g^+ (C1) vibration. The second is weakened by its Boltzmann factor. Their separation is 608 + 521 = 1129 cm.$^{-1}$. The weaker origins C_0^0 and D_0^0, which appear 87 cm.$^{-1}$ on the low-frequency side of A_0^0 and B_0^0 respectively, involve upward transitions, 1-2 and 2-1 respectively, of the same vibration. Both are weakened, the second very much so, by their Boltzmann factors. One understands why they appear 608 — 521 = 87 cm.$^{-1}$ from the stronger origins. In fluorescence, A_0^0 and B_0^0 involve downward transitions, 1-0 and 0-1 respectively, of the E_g^+ (C1) vibration. Since we are concerned with fluorescence at pressures permitting a thermal distribution of vibrational energy in the excited state, the former of these transitions will now be weakened by the Boltzmann factor. The origins C_0^0 and D_0^0 correspond in fluorescence to downward transitions, 2-1 and 1-2 respectively, and therefore both will be weakened by their Boltzmann factors, the former more than the latter.

The main progressions depend on quantum changes in the ring-swelling vibration A_{1g} (C). Its frequency, as given in the Raman spectrum, is 992 cm.$^{-1}$. Its frequencies, as given by the ultraviolet spectra, are 992 cm.$^{-1}$ in the ground state and 923 cm.$^{-1}$ in the excited state. In absorption, progressions in the upper-state frequency, involving upward transitions 0-1, 0-2, ..., start from each active origin and run to higher frequencies. In fluorescence, progressions in the

lower-state frequency, involving downward transitions 0-1, 0-2, ..., run to lower frequencies.

The sequences in the interval 161 cm.$^{-1}$ arise from 1-1, 2-2, ..., transitions of the degenerate out-of-plane vibration E_u^+ (C). The frequency of this vibration, as given by the Raman spectrum of liquid benzene, is 405 cm.$^{-1}$. The ultraviolet spectra give 404 cm.$^{-1}$ for the frequency in the ground state and 243 cm.$^{-1}$ for the frequency in the excited state. The difference $404 - 243 = 161$ cm.$^{-1}$ is the sequence interval, common to the absorption and fluorescence spectra. In principle, all vibrations can produce such sequences. But the outstanding persistence of the sequences of this vibration is evidently due very largely to the fact that its frequencies are so much lower than those of any other vibration, and that therefore its Boltzmann factors are not a serious obstacle to its appearance in *n-n* transitions up to several quanta.

The whole of this description applies equally well to the absorption and fluorescence spectra of hexadeuterobenzene, except, of course, that the vibration frequencies are different for both the ground and excited states.

The D_{3h} benzene, 1 : 3 : 5-trideuterobenzene, still has the symmetry $\mathbf{D_{6h}}$ with respect to its nuclear charges. Thus the electronic transition is still forbidden for itself, though it becomes allowed in the presence of a perturbing vibration of the right symmetry. It is easily shown that this is the symmetry of the E' class. In fact it is found that the absorption and fluorescence spectra of 1 : 3 : 5-trideuterobenzene depend overwhelmingly on the perturbing effect of that E' vibration, called E' (C1), which most closely resembles the E_g^+ (C1) vibration of a D_{6h} benzene. This produces four active origins, A, B, C and D, similar to those of the D_{6h} benzenes.

As before the main progressions must depend on quantum changes, unrestricted by symmetry, in totally symmetrical vibrations. But now there are two totally symmetrical carbon vibrations, called A_1' (C) and A_1' (C'), which are similarly related to the D_{6h} ring-swelling vibration, A_{1g} (C) : in one most of the ring-swelling movement is in the CD-groups, whilst in the other most of it is in the CH-groups. It can therefore be understood why, in place of a simple progression of a D_{6h} benzene, one now finds two progressions, with slightly different intervals, as well as a mixtures of the two.

The principal sequences depend on *n-n* transitions of a vibration, called E'' (C), which is a close analogue of the D_{6h} vibration E_u^+ (C);

and the sequences are closely similar to the D_{6h} sequences. As with benzene and hexadeuterobenzene, the ground-state frequencies, as determined from the ultraviolet spectra, can all be checked against the values given by Raman spectra.

In the two investigated V_h benzenes, 1 : 4-dideuterobenzene and 1 : 2 : 4 : 5-tetradeuterobenzene, the electronic transition is still forbidden, but now two principal vibrations allow it to appear. They are the vibrations A_g (C1) and B_{1g} (C1) into which the E_g^+ (C1) vibration of a D_{6h} benzene, or the E' (C1) of a D_{3h} benzene, splits when the degeneracy is removed by the destruction of the hexagonal or trigonal axis of symmetry. The frequencies of these two vibrations lie quite close together both in the ground state and in the excited state, and hence the active origins appear in the spectra as close doublets.

Except that they must inherit the doublet character of their origins, the main progressions are simple, since there is only one vibration, A_g(C), which closely corresponds to the A_{1g}(C) vibration of a D_{6h} benzene. However, new complications arise in the sequences, because there are two vibrations which derive directly from the E_u^+(C) vibration of a D_{6h} benzene, *viz.*, the two vibrations, A_u(C) and B_{1u}(C), into which the degenerate vibration splits when degeneracy is removed by the non-trigonal arrangement of deuterium atoms. Thus one observes duplicated and mixed sequences in place of the simple sequences of the D_{6h} and D_{3h} benzenes.

The situation with respect to the C_{2v} molecule, monodeuterobenzene, may be summarised by saying that it combines the complications of the D_{3h} and V_h benzenes. In all cases frequencies of the ground state, as determined from the ultraviolet spectra agree well with the values already found by the study of Raman and infra-red spectra.

It was necessary for the purpose in view to carry the analyses of all these spectra far enough to include large numbers of bands which were by no means amongst the strongest in their respective spectra. But this it was possible to do, knowing the selection rules, and all the ground-state fundamental frequencies, and having as working tools the Teller-Redlich product theorem, and the method of force-field calculation, both applied to the excited state in the knowledge that isotopic substitution could not change the internal force-system of that state. The same two methods, and the same basic principle, allowed a number of upper-state frequencies to be estimated which could not be obtained by direct analysis of the spectral observations.

The determined fundamental frequencies of the upper electronic states of the various isotopically isomeric benzenes studied are given in tables VI and VII. As in the case of the ground state frequencies, two tables are necessary in order to furnish a scheme of correlation between the frequencies of molecules having the different types of symmetry of those employed. The figures in Roman type represent fundamental frequencies which are directly obtained from observed and assigned spectral bands, whilst the frequencies given in bold type are estimated in various ways. Those which are given in bold type without added brackets are either calculated from directly determined fundamental frequencies by means of the product rule, or derived from an exact, or very close, identity with determined frequencies which is revealed by considerations relating to the involved normal co-ordinates. These frequencies should be correct to 1 % or better. The figures which are given in bold type and in brackets are obtained from other frequencies with the help of force-field calculations, supplemented by various, partly empirical adjustments and controls. These data are less accurate than the others.

There is a simple form of comparison between the fundamental frequencies of the ground and excited states of isotopically modified benzenes which can be made at once, although a more extensive comparison of such frequencies is implicit in the discussion, given in the next Section, concerning the effect of the electronic excitation on the elastic constants of the benzene molecule. It follows from the product theorem that an electronic excitation should alter the frequency of any vibration which is alone in its symmetry class by approximately the same factor in isotopically different molecules. The factor would be exactly the same except for an effect, due to the small change in the moment of inertia of the molecule on excitation, which the product theorem takes into account, and except also for a small effect due to the anharmonicity of the vibrations, which the product theorem neglects. Confining attention, because of the greater precision of the figures, to those vibrations whose upper-state frequencies are given by observed bands, one finds, in the two available cases, that the factors by which the fundamental frequencies are changed by the electronic excitation are the same for benzene and hexadeuterobenzene to 0.6 % and 0.3 %. For vibrations which are not alone in their symmetry classes the product theorem requires only that the product of the frequencies of a given symmetry class will change by almost the same factor in two isotopically different

TABLE VI

Correlation of Fundamental Frequencies (cm^{-1}) of the Upper Electronic State (B_{2u}) of Benzenes of the Symmetry Types D_{6h}, D_{3h} and C_{2v}.

DESCRIPTION OF VIBRATION	D_{6h}			D_{3h}		C_{2v}	
	Class	C_6H_6	C_6D_6	Class	$C_6H_3D_3$	Class	C_6H_5D
C-stretching	A_{1g}	923	879	A'_1	893	A_1	920
H-stretching		3130	**2340**		3135		3129
C-bending	B_{1u}	**985**	**940**		988		990
H-stretching		**3130**	**2340**		2300		3081
C-bending	E_g^+	521	499	E'	513		517
H-stretching		3080	2320		3085		2348
C-stretching		1470	1403		1428		1472
H-bending		**1130**	830		824		—
C-deformation	E_u^-	**[1470]**	**[1320]**		—		—
H-bending		**[940]**	**[740]**		—		—
H-stretching		**[3130]**	**[2300]**		2330		—
Out-of-plane	E_u^+	243	208	E''	223	A_2	243
Out-of-plane		706	590		680		**706**
Out-of-plane	E_g^-	585	454		495		585

DESCRIPTION OF VIBRATION	D_{6h}			D_{3h}		C_{2v}	
	Class	C_6H_6	C_6D_6	Class	$C_6H_3D_3$	Class	C_6H_5D
H-bending	A_{2g}	**[1210]**	**[940]**	A'_2	—	B_1	—
C-stretching H-bending	B_{2u}	**[1510]** **[1010]**	**[1440]** **[750]**		— —		— —
C-bending H-stretching C-stretching H-bending	E_g^+	521 3080 1470 **1130**	499 2320 1403 830	E'	513 3085 1428 824		517 3072 1446 —
C-deformation H-bending H-stretching	E_u^-	**[1470]** **[940]** **[3130]**	**[1320]** **[740]** **[2300]**		— — 2330		— — —
Out-of-plane	A_{2u}	513	382	A''_2	411	B_2	476
Out-of-plane Out-of-plane	B_{2g}	365 775	306 663		360 715		360 720
Out-of-plane Out-of-plane	E_u^+	243 706	208 590	E''	223 680		230 690
Out-of-plane	E_g^-	585	454		495		552

TABLE VII

Correlation of Fundamental Frequencies (cm^{-1}) of the Upper Electronic State (B_{2u}) of Benzenes of the Symmetry Types D_{6h}, V_h and C_{2v}.

DESCRIPTION OF VIBRATION	D_{6h}			V_h			C_{2v}	
	Class	C_6H_6	C_6D_6	Class	$C_6H_4D_2$	$C_6H_2D_4$	Class	C_6H_5D
C-stretching	A_{1g}	923	879	A_g	909	895	A_1	920
H-stretching		3130	**2340**		3132	2355		3129
C-bending	E_g^+	521	499		511	509.5		517
H-stretching		3080	2320		2355	3133		2348
C-stretching		1470	1403		1489	1414		1472
H-bending		**1130**	830		**1075**	**820**		—
C-bending	B_{1u}	**985**	**940**	B_{2u}	—	—		990
H-stretching		**3130**	**2340**		—	—		3081
C-deformation	E_u^-	[1470]	[1320]		—	—		—
H-bending		[940]	[740]		—	—		—
H-stretching		[3130]	[2300]		—	—		—
Out-of-plane	E_u^+	243	208	A_u	243	208	A_2	243
Out-of-plane		706	590		706	591		**706**
Out-of-plane	E_g^-	585	454	B_{2g}	585	452		585

DESCRIPTION OF VIBRATION	D_{6h}			V_h			C_{2v}	
	Class	C_6H_6	C_6D_6	Class	$C_6H_4D_2$	$C_6H_2D_4$	Class	C_6H_5D
H-bending	A_{2g}	**[1210]**	**[940]**		—	—		—
C-bending	E_g^+	521	499	B_{1g}	516.5	505		517
H-stretching		3080	2320		3075	2333		3072
C-stretching		1470	1403		1469	1414		1446
H-bending		**1130**	830		—	—		—
C-stretching	B_{2u}	**[1510]**	**[1440]**		—	—	B_1	—
H-bending		**[1010]**	**[750]**		—	—		—
C-deformation	E_u^-	**[1470]**	**[1320]**	B_{3u}	787	—		—
H-bending		**[940]**	**[740]**		—	—		—
H-stretching		**[3130]**	**[2300]**		—	—		—
Out-of-plane	A_{2u}	513	382		435	419		476
Out-of-plane	E_u^+	243	208	B_{1u}	222	233	B_2	230
Out-of-plane		706	590		655	665		690
Out-of-plane	B_{2g}	365	306	B_{3g}	357	351		360
Out-of-plane		775	663		775	649		720
Out-of-plane	E_g^-	585	454		457	535		552

molecules. It is found, however, that the factors are nearly the same, not only for the products, but also for the individual frequencies, the largest deviation being 1.7 %. This result, which is illustrated in Table VIII, is clearly due to the valency forces, which separate the vibrations rather completely into bond stretching and bending types, without much of the mixing which would cause marked changes in the vibration forms when the molecule is isotopically substituted.

TABLE VIII

Percentage Reduction undergone by some Fundamental Frequencies (cm.$^{-1}$) of Benzene and Hexadeuterobenzene on Electronic Excitation.

	A_{1g}		E_g^+			
C_6H_6 (ground state) . .	992	3062	606	3047	1596	1178
C_6H_6 (excited state). . .	923	3130	521	3080	1470	1130
% Drop (C_6H_6)	7.0	—2.2	14.0	—1.1	7.9	4.9
C_6D_6 (ground state). . .	943	2293	577	2265	1551	867
C_6D_6 (excited state). . .	879	2340	499	2320	1403	830
% Drop (C_6D_6)	7.3	—2.2	15.7	—2.5	9.5	4.3

	A_{2u}	B_{2g}		E_u^+		E_g^-
C_6H_6 (ground state) . . .	671	703	985	405	970	849
C_6H_6 (excited state) . . .	513	365	775	243	706	585
% Drop (C_6H_6)	23.6	48.0	21.3	40.0	27.2	31.1
C_6D_6 (ground state) . . .	496	601	827	352	793	662
C_6D_6 (excited state) . . .	382	306	663	208	590	454
% Drop (C_6D_6)	23.0	49.0	19.8	40.9	25.6	31.4

IV.

EFFECT OF AN ELECTRONIC EXCITATION ON THE GEOMETRY, ELASTIC PROPERTIES AND ZERO-POINT ENERGY OF THE BENZENE MOLECULE.

The simplest normal-co-ordinate treatment which has been applied to the benzene molecule is that of Wilson, who adopted a valency-force potential energy function containing six constants, and from

this derived a complete set of equations for the twenty fundamental frequencies in terms of the six constants. Wilson's function did not deal satisfactorily with the out-of-plane vibrations, but Bell has shown how it may be modified, without the introduction of any further constants, in order to correct for this shortcoming. The Wilson-Bell function works at least as well for the excited (B_{2u}) state of benzene as for the ground (A_{1g}) state, and therefore provides a good basis for the assessment of the salient features of the effect of the A_{1g}-B_{2u} electronic transition on the elastic properties of the benzene molecule.

The potential energy function may be written thus :

$$2V = F\Sigma r^2 + f\Sigma s^2 + \Delta\Sigma\rho^2 + \delta\Sigma\sigma^2 + \Gamma\Sigma\Phi^2 + \gamma\Sigma\mu^2$$

Here V is the potential energy of deformation of the molecule, and the quantities under the summation signs are the geometrical variables in terms of which the deformation is expressed; whilst the quantities outside the summation signs are elastic constants. The geometrical variables are defined as follows : r is the deviation of the length of a C-C bond, and s that of a C-H bond, from their respective equilibrium lenghts; ρ is the angular deformation of an internal ring-angle, whilst σ is the angular deviation, in the plane of the ring, of a C-H bond from the bisector of the associated external ring angle : $\Phi = \varphi_C + \varphi_H$, where φ_C and φ_H are the respectieve angles through which a given C-C bond is twisted by the out-of-plane displacements of the two attached carbon atoms, and two attached hydrogen atoms; and μ is the angle by which the bond of a hydrogen atom deviates from the plane of the nearest three carbon atoms. The elastic constants correspond. The quantities F and f are force-constants : they express the restoring force per unit change of length of a C-C bond, and of a C-H bond, respectively. The quantities Δ, δ, Γ, and γ are moment-constants : they measure the restoring moment generated per unit angular deformation. For Δ, the deformation is that of an internal ring-angle; for δ it is the angular deviation, in the plane of the molecule, of a C-H bond from the bisector of the external ring angle; for Γ, it is the angle through which a C-C bond is twisted by the out-of-plate displacements of adjacent atoms; and for γ, it is the angle by which the bond of a hydrogen atom deviates from the plane of the nearest three carbon atoms. Each of the summations in the potential energy function extends over six similar terms, one for each of six like atoms or six like bonds.

The values of the above elastic constants, for both the ground and excited states of benzene, have been determined by an application of the Wilson-Bell function to the frequency data of Tables IV-VII. The results are in Table IX. They express directly the dynamical properties of the molecule in its ground and excited states : and they also lead to conclusions about the dimensions of the molecule, and the differences which they exhibit in the two electronic states.

TABLE IX

Elastic Constants of the Benzene Molecule in its Normal and Excited States.

	Force-constants (dynes per cm.)		Moment-constants (dyne-cm. per radian.)			
	$10^{-5}F$	$10^{-5}f$	$10^{12}\Delta$	$10^{12}\delta$	$10^{12}\Gamma$	$10^{12}\gamma$
Ground state	7.61	5.06	13.7	8.0	1.02	2.62
Excited state	6.53	5.35	10.5	6.6	0.32	1.43
% Drop	14	-6	23	17	68	45

As noted in Section III, the electronic excitation $A_{1g} \longrightarrow B_{2u}$ involves the promotion of one of the six unsaturation electrons from a molecular orbital of e_g^- symmetry to one of e_u^+ symmetry. On an energy scale, the electron can be considered to have been raised about half-way towards complete ionisation (ionisation potential 9.19 electron-volts). From a stereo-electronic standpoint, the excited orbital has one additional nodal surface intersecting the geometrical bands, and therefore must have increased anti-bonding properties. From both points of view, one would expect the excitation to diminish both the strength of the C-C bonds and their resistance to stretching.

Consistently with its average content of three electrons, a C-C bond of normal benzene exhibits a degree of resistance to stretching which is intermediate between the degrees of resistance shown by the C-C bonds of normal ethane and ethylene :

Force-constants *(F)* of the normal molecules..	Ethane	Benzene	Ethylene
$10^{-5}F$ (with F in dynes/cm.)	4.5	7.6	9.8

It is the excess of resistance of the benzene bond, over that shown by the ethane bond, that one would expect to find considerably reduced as a result of the electronic excitation : and thus it seems very reasonable that $10^{-5}F$ for excited benzene should have a value 6.5, which is well-bracketed between 4.5 and 7.6.

Badger's relation between the force-constants and the lengths of bonds can be made the basis of a procedure for estimating the expansion which the carbon ring undergoes as a result of the electronic excitation. The relation takes the form :

$$X = k\ (x - K_{ij})^{-3}$$

where X is a force-constant in dynes/cm., x is the equilibrium bond-length in Angstrom units, k is a universal constant, the best value of which, for general purposes, is 1.85×10^5, and K_{ij} is a constant which depends on the principal quantum numbers of the bonding electrons, and for a carbon-carbon bond has the value 0.68. By making the plausible assumption that, for the bond of a benzene ring, the best value of k, though not exactly equal to the general average value, is, to a close approximation, the same for both electronic states, and using the determined values of F, one may write the following expression for the percentage increase which the carbon bond-length undergoes as a result of the electronic excitation :

$$100\ (1.39 - 0.68)\ \{\ (7.61/6.53)^{1/3} - 1\ \}\ /1.39 = 2.7\ \%$$

Douglas Clark's relationship,

$$X = Cx^{-6}$$

gives a similar result. Taking the constant C to be the same in both states, the percentage enlargement becomes :

$$100\ \{\ (7.61/6.53)^{1/6} - 1\ \} = 2.6\ \%$$

Thus the C-C bond-length in the excited molecule must be close to 1.43 A°.

In 1941 Kynch and Penney reported a quantum mechanical calculation using the electron-pair method, of some of the properties of the excited state of benzene. For the length of the C-C bond, they obtained 1.44 $A°$, in good agreement with the value given above. For the ring-swelling frequency they found 918 cm.$^{-1}$, and remarked that « this agrees moderately well with the experimental measurement of 940

cm.$^{-1}$». It agrees better with the true experimental value 923 cm.$^{-1}$.

It is of considerable interest that the electronic excitation *increases* the stretching frequency and force-constant of the C-H bonds. If one could completely remove an unsaturation electron from a single carbon atom, the effect might well be to shorten the C-H bond considerably, and increase its resistance to stretching, both because of the increased effective nuclear charge of the carbon atom, and because of the incompleteness of its valency shell. A rough estimate, based on known bond-lengths, gives 0.1 A° as the probable order of magnitude of the contraction in this case. Actually, the excitation partly removes, from the near locality of each carbon nucleus, one-sixth of an unsaturation electron : and so one may plausibly suppose that the resultant contraction is likely to be only of the order of 0.01 $A°$. This, however, is the correct order of magnitude for correlation with the determined increase of 6 % in the force-constant f. Alternatively, one can use known force-constants in order to estimate directly the probable order of magnitude of the increase in force-constant, and discover in this way that an increase of the order of 5 % would be reasonable.

In an application of Badger's relation to a C-H bond, the constant K_{ij} takes the value 0.34. Using this relation as before, together with the determined values of the force-constant f, one may calculate the percentage shortening of the bond thus :

$$100\ (1.08 - 0.34)\ \{\ 1 - (5.06/5.35)^{1/3}\ \}\ /1.08 = 1.3\ \%$$

Alternatively, employing Douglas Clark's relations as before, one finds for the contraction :

$$100\ \{\ 1 - (5.06/5.35)^{1/6}\ \} = 0.9\ \%$$

Thus the C-H bond-length in excited benzene must be close to 1.07 $A°$.

Sklar's theory of the electronic transition, and its ample verification in the analysis of the spectra, establishes the plane-hexagonal (D_{6h}) symmetry of the equilibrium nuclear configuration of the excited benzene molecule. The above estimates of the equilibrium C-C and C-H bond-lengths in the excited state therefore complete the determination of the geometry of the model of the excited molecule.

Since the planar variations of the three valency angles of any one carbon atom involve the relation that their sum is zero, the individual variations are expressible in terms of two parameters. The constant Δ

measures the restoring moment which is produced when the C.C.C. angle changes in one direction by a certain amount, and each C.C.H. angle simultaneously changes in the other direction by half that amount. The constant δ measures the moment which is called into operation when the two C.C.H. angles simultaneously change in opposite directions by equal amounts, the C.C.C. angle remaining unaltered.

Independently of the unsaturation electrons, the trigonal constitution of the carbon orbitals of the three saturated electron-pair bonds tends to place the latter at angles of 120° to one another; and the repulsive forces of the three electron-pairs on one another tend to keep them close to these relative positions. However, the superposed effect of the unsaturation electrons in helping to maintain the structure is shown by the observed weakening of the restoring moments, as measured by the moment-constants Δ and δ, when the unsaturation shell becomes attenuated as a result of the electronic excitation. It should be noted that the unsaturation electrons will exert their effect in at least two ways, — partly through the repulsive forces which they themselves exert on the different saturated electron-pairs, and partly by shortening the C-C bonds, and thus enabling the three saturated electron-pairs of any one carbon atom to exert stronger repulsive forces on one another than they would otherwise. No precise statement can be made about the anticipated magnitude of the weakening which will be suffered, as a result of the electronic excitation, by the whole of this contribution of the unsaturation electrons to the angular rigidity of the system, though one might suppose the percentage reduction of the moment-constant, Δ and δ, to be of the same order of magnitude as that of the stretching force-constant F, in so far as the changes in Δ and δ result from the effects considered. Actually, these should be the only major effects on the constant δ : and, as Table VI shows, the percentage change which δ undergoes is, in fact, not very different from that of the force-constant F.

However, the above effects are expected to be reinforced by another in the case of the moment-constant Δ. This further effect arises from the repulsive forces between the different spin-coupled electron-pairs of the unsaturation shell itself. These pairs will tend to keep apart, and their repulsion will accordingly tend to open the internal ring-angles. The effect of this will be to steepen, on the side of small angles, the potential hollow which restricts the variation of a ring-

angle. But even such a one-sided steepening of the potential hollow must make it narrower, and must therefore increase the angular vibration frequency, and the corresponding moment-constant. The decrease of this contribution to the moment-constant Δ, as the unsaturation shell becomes attenuated by excitation, must also be included amongst the causes of the observed reduction of this constant. Table VI shows that the percentage reduction of Δ is, actually, appreciably larger than is that of the other planar moment-constant δ.

The two out-of-plane constants, Γ and γ, suffer much more drastic reductions, as a result of the electronic excitation, than do any of the planar elastic constants. This is not difficult to understand.

The constant Γ measures the resistance of a C-C bond to such torsion as arises from unequal out-of-plane displacements of the atoms attached on each side of it. Now according to our theories of chemical binding, the whole of such resistance to torsion must come from the unsaturation electrons : for the saturated electron-pair belonging to any bond is supposed to have full cylindrical symmetry about the line of the bond. It follows that any change in the state of the unsaturation shell must profoundly affect the constant Γ, and that, in particular, a replacement of bonding by anti-bonding character within the shell, such as occurs during the electronic excitation, must strongly reduce Γ. A second reason for the reduction of Γ consists in the diminution of the non-bonding repulsive forces exerted by the unsaturation shell on the neighbouring saturated electron pairs.

The second out-of-plane constant, γ, measures resistance to the bending of a hydrogen atom out of the plane of the nearest three carbon atoms. Since this type of hydrogen motion is tangential to the contours of electron-density of the saturated electron-pairs belonging to the C-C bonds, these electron-pairs will contribute relatively little to the harmonic restoring moment (1). But since the hydrogen motion lies across the common nodal plane of the unsaturation electrons, and is directed as nearly as any bending motion can be towards their density gradients, these electrons will contribute largely to the restoring moment. Thus the harmonic restoring moment will owe its existence largely to the unsaturation electrons. It should

(1) That the saturated electron-pairs can in principle make a finite, if small, contribution to the harmonic restoring moment is not intuitively obvious, but can be established by a short calculation.

be noted that they exert their effect in this case solely by their repulsive action on the electrons of the C-H bond, and not indirectly through shortening the C-C bonds. It follows that any change of state of the unsaturation shell must strongly influence the moment-constant γ; and that, in particular, the attenuation of the shell, as a result of the electronic excitation, must very considerably reduce that constant.

Reference is made here to the attenuation of the unsaturation shell during excitation, and not to the increase of antibonding properties within it, because the latter effect is clearly less important for interactions with outside electron-pairs than when the bonded character of the unsaturation shell itself is in question,— as it is in the problem of the torsion constant Γ. That may be partly why, although the out-of-plane bending constant γ is, indeed, much reduced, it is not, as Table VI shows, so greatly reduced as is the torsion constant Γ.

The remaining subject for discussion relates to the zero-point energies of the two electronic states of the benzene molecule. They are accessible by calculation in those cases in which one has a sufficiently complete knowledge of the fundamental vibration frequencies. The difference between them directly affects the position of the electronic origin of the transition. The frequency of the origin is accurately known from spectral observation. Furthermore, since isotopic substitution necessarily affects zero-points energy, an isotope effect on the position of the electronic origin is to be expected. Such an isotope effect is in fact observed.

Although the $\mathbf{A_{1g}}$-$\mathbf{B_{2u}}$ transition is forbidden, so that no 0-0 band appears in any of the spectra to mark the electronic origin of the band-system, the analyses of these absorption and fluorescence spectra are so consistent and complete that the position of the origin in any of them can be fixed with as much certainty as if it were marked by a visible band. The origin frequencies of the transitions of all those isotopically different forms of benzene, the ultraviolet spectra of which have been studied, are assembled in Table X.

Since all these figures are accurate to within 2 cm.$^{-1}$, it will be evident that the different benzenes have not all the same electronic origin : that of hexadeuterobenzene lies 200 cm.$^{-1}$ further out towards the far-ultraviolet than that of benzene. Furthermore, the shift with isotopic substitution is an approximately linear function of the number of deuterium atoms present in the molecule, although small,

but apparently systematic, departures from linearity can be discerned.

TABLE X

Frequencies (cm.$^{-1}$) of the Electronic Origins of the First Ultraviolet Band-systems of the Isotopically Isomeric Benzenes, as observed in either Fluorescence or Absorption.

	Origin Frequency	Difference	Deviation from linearity
Benzene	38090	34	± 0
Monodeuterobenzene	38124	30	+ 1
1 : 4-Dideuterobenzene	38154	30	− 3
1 : 3 : 5-Trideuterobenzene	38184	35	− 6
1 : 2 : 4 : 5-Tetradeuterobenzene	38219		− 4
Pentadeuterobenzene	—	2 × 35	—
Hexadeuterobenzene	38290		± 0

The energy of an electronic transition is not simply the difference between the electronic energies of the two combining states : it is this, *plus* the difference between their nuclear zero-point energies. To a very close approximation the electronic energy of each state remains the same in an isotopic substitution, and therefore the difference between the electronic energies of the two states remains the same. However, the vibrational zero-point energy of each state will be changed by isotopic substitution, and, if the changes are different, then the contribution of the zero-point energy to the transition energy will be different for the isotopically isomeric molecules.

For simplicity, let us first consider a vibration of benzene which is alone in its symmetry class, *e. g.*, the vibration of E_g^-(H). We can then discuss it independently of the other vibrations. Since it is doubly degenerate, its contribution to the zero-point energy ($h\nu/2$ in each of 2 degrees of freedom), if expressed in frequency units, is equal to its fundamental frequency. For the electronic ground state, this is 849 cm.$^{-1}$. In the electronic excitation the forces are loosened and the frequency drops, so that the contribution of this vibration to the zero-point energy of the upper electronic state is only 585 cm.$^{-1}$. The difference between these two quantities, — 264 cm.$^{-1}$, is the contribution of the zero-point energy of the vibration to the energy of a transition from one « vibrationless » electronic state to the other. Its sign is opposite to that of the electronic energy difference,

so that its effect is to shift the electronic origin to a lower frequency than that which the origin would have if there were no zero-point energy. Starting again in the ground state, let us make the isotopic substitution which converts benzene into hexadeuterobenzene, thus reducing the fundamental frequency, and therefore the zero-point energy contribution, of the vibration by a factor which is given by the product rule. The energy is, in fact, reduced to 662 cm.$^{-1}$. In the upper electronic state also, the zero-point energy contribution becomes reduced by a factor, which is likewise given by the product rule, and is very nearly the same as before. This energy accordingly becomes 454 cm.$^{-1}$. The difference between these two quantities, — 208 cm.$^{-1}$, could obviously be calculated by reducing the former difference, — 264 cm.$^{-1}$, by almost the same factor. (The various factors could be considered identical, if one would neglect anharmonicity and the small difference in the size of the molecule in its two electronic states). Each of the differences, — 264 cm.$^{-1}$ for benzene, and — 208 cm.$^{-1}$ for hexadeuterobenzene, is a negative contribution to the energy of electronic excitation of the relevant molecule : each shifts the origin towards lower frequencies but the reduction of frequency is greater for benzene than for hexadeuterobenzene. Thus the difference between the differences, + 56 cm.$^{-1}$, represents the contribution of the zero-point energy of this vibration to the upward displacement of the excitation energy of hexadeuterobenzene relatively ot that of benzene.

Only three vibrations of benzene are alone in their symmetry classes, and it is not theoretically rigorous to discuss the other seventeen on similar lines. But to quite a reasonable degree of approximation one may do so. For it has been shown empirically that the electronic excitation not only reduces, as the product theorem necessitates, the products of frequencies of the same symmetry class by substantially the same factor for benzene and hexadeuterobenzene; it also reduces each individual frequency by approximately the same factor for both compounds (cf. Section III, especially Table VIII). This fact is sufficient to ensure that any vibration whose frequency is considerably reduced by the conversion of benzene into hexadeuterobenzene, and is also considerably reduced by the electronic excitation, will act like the E_g^-(H) vibration considered above : it will supply to the energy of the electronic excitation of each compound a contribution, consisting of a difference of zero-point energies, which displaces the electronic origin towards lower frequencies, but does so in such a way as to

leave the electronic origin of hexadeuterobenzene at a higher frequency than that of benzene.

The great majority of the vibrations of benzene have their frequencies reduced both by complete deuteration of the molecule and by the electronic excitation, although in some cases the changes are not very large. All these, if they have any decided effect on the relative positions of the electronic origins, will act in the way described. Thus it is not unnatural that the total contribution of the zero-point energy of all the vibrations to the energy of the electronic excitation should be of the kind illustrated; and that, accordingly, the electronic origin of the hexadeuterobenzene transition should be found at a higher frequency than that of the benzene transition.

One group of vibrations is anomalous, *viz.*, the hydrogen stretching vibrations. These have their frequencies reduced (obviously) by deuteration, but increased quite appreciably by the electronic excitation. It will be evident from the foregoing discussion that the zero-point energy contribution of such vibrations will be in the direction of raising the energy of the electronic excitation; and furthermore, that the magnitude of the contribution will be greater for benzene than for hexadeuterobenzene. It follows that, if this difference were the dominating one, the electronic origin of the transition of benzene would lie higher than that of the transition of hexadeuterobenzene. However, the effect of the hydrogen-stretching vibrations is, in fact, not powerful enough to prevail against the contrary action of all the other vibrations.

We may now consider the origin shifts of the partly deuterated benzenes. One might have expected that their electronic origins would space themselves out according to the number of deuterium atoms; and that, in rough approximation, the spacing would correspond to a linear interpolation between the electronic origins of benzene and hexadeuterobenzene. It is true that, as symmetry is reduced by the isotopic loading of the partly deuterated benzenes, with the consequence that certain vibrational symmetry classes become fused together, many of the vibrations which are characteristic of benzene and hexadeuterobenzene become mixed. But when two vibrations thus mix, even when they do so rather completely, as do, *e. g.*, the ring-swelling, and plane-trigonal ring-bending, vibrations of benzene or hexadeuterobenzene in forming the two totally symmetrical carbon vibrations of 1 : 3 : 5-trideuterobenzene, the effect on the frequencies is merely to push them apart in such a way that

their sum, and therefore the sum of their zero-point energies, is not very different from what it would have been if the isotopic loading of the molecule had been so levelled out as to stop the mixing, *i. e.*, if, in this example, the molecule had been supplied with six hydrogen atoms of atomic weight 1.5. In short, the sum of the frequencies will not differ greatly from a linearly interpolated sum. Many of the mixings which occur when successive deuterium atoms are introduced into benzene are more complicated than this, and in particular, involve more than two vibrations, but such mixings may be analysed into successive two-component mixings, and thus the rough principle of a linear shift of summed frequencies may still be expected to hold. Furthermore, the deviations shown by particular groups of mixed vibrations, due to the dissymmetric pushing apart of frequencies, are likely to occur about equally often in either direction, so that for the complete sum of all the vibration frequencies, and therefore for the total vibrational zero-point energy, the law of linear interpolation might be expected to hold rather well. Now all this is true not only for the ground state, but also for the excited state : linear interpolation should hold for each state separately, and therefore also, for the difference between the zero-point energies of the ground and excited states. In short, it should hold for the contribution of zero-point energy of excitation, and for the effect of zero-point energy on the position of the electronic origin.

Before proceeding to make quantitative comparisons between the observed isotope shifts of the electronic origin and the already given fundamental frequencies of the two combining states, it is necessary to remark that a second cause of isotope shifts exists in principle. It consists in the occurrence in the molecular case of the effect, with which we are familiar in atoms, arising from the altered reduced masses of the electrons. Although one cannot calculate this effect in molecules, it is easy to show that it must be too small to have any relevance to the observations under discussion. The Rydberg constants for a protium atom and a deuterium atom differ by the factor $1/(2 \times 1835) = 1/3670$. Therefore, if the benzene transition, amounting as it does to 38090 cm.$^{-1}$, consisted in the excitation of an electron which belonged entirely to one hydrogen atom, or entirely to all the hydrogen atoms, and not to any of the carbon atoms, we should expect the electronic energy of the transition to be altered in hexadeuterobenzene, on account of the altered reduced mass of the electron, by a quantity of the order of $38090/3670 = 10$ cm.$^{-1}$. However,

all the spectral evidence points to the conclusion that the optical electron has actually very little to do with the hydrogen atoms, and that it belongs essentially to the carbon atoms. For this reason one must not expect the isotope shift in the purely electronic part of the excitation energy to be of a greater order of magnitude than 1 cm.$^{-1}$, — which is negligible in relation to the effect under discussion.

From this it follows that, in seeking to account quantitatively for the origin shifts, we may quite properly confine attention to the zero-point energy contribution to the transition energy. A quantitative test is possible for benzene and hexadeuterobenzene, because for these molecules complete sets of vibration frequencies have been given for both the combining electronic states. The cases of the partly deuterated benzenes must for the present be allowed to rest on the discussed rule of linear interpolation.

The results of the test, applied to benzene and hexadeuterobenzene, are shown in Table XI; in which, for convenience, energies are expressed in wave-numbers. The calculated isotopic shift of the electronic origin is 208 cm.$^{-1}$, whilst the observed shift is 200 cm.$^{-1}$. The agreement is closer than one had any right to expect, having regard to the errors to which some of the frequencies are liable.

TABLE XI

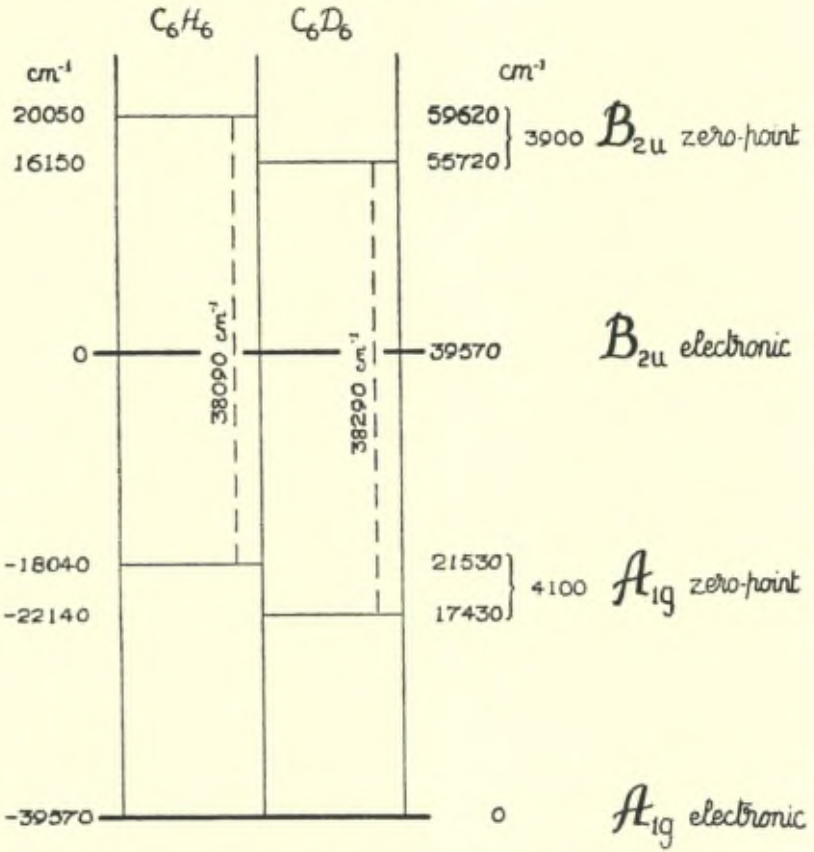
Zero-point Energies (cm.$^{-1}$) of the States and Transitions of Benzene and Hexadeuterobenzene, interpreting the Isotope Shift of the Electronic Origin of their Band Systems.

	C_6H_6	C_6D_6	Isotope Shift
Excited state (B_{2u})	20050	16154	— 3896
Ground State (A_{1g})	21535	17431	— 4104
Transitions ($A_{1g} - B_{2u}$)	— 1485	— 1277	+ 208

Attention may be directed to the considerable magnitude of the zero-point energy of benzene. It appears that one would have to heat that substance to more than 1300°C in order to add as much energy, again, or to more than 1500°C to add as much vibrational energy as the molecule already possesses at the absolute zero of temperature. A notable fact is that the zero-point energy in either state is of the order of one-half of the energy of the electronic transition.

This is shown in fig. 20, which is a graphical expression of the data for benzene and hexadeuterobenzene, contained in Tables VII and VIII, the units of wave-numbers having been rounded off in order to secure consistency. The figure shows that the electronic transitions actually take place rather far from those positions in the energy diagram in which one usually thinks of them.

Fig. 20



Electronic and zero-point energy levels of normal and excited benzene and hexadeuterobenzene (energies in cm.$^{-1}$).

Finally, a remark may be made concerning the purely electronic part of the transition energy. Having assessed the contribution of nuclear zero-point energy to the transition energy, the purely

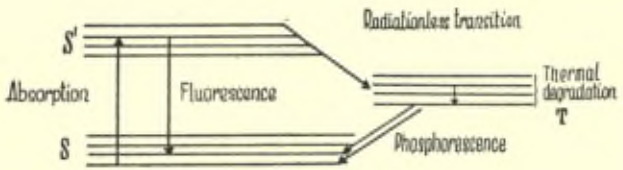
electronic energy contribution can be given in a corrected form. It will, of course, be the same (to within ± 1 cm.$^{-1}$) for all isotopically isomeric benzenes. One sees from Fig. 21 that the electronic part of the transition energy is :

$$39570 \text{ cm.}^{-1} = 4.88 \text{ electron-volts.}$$

Sir William Ramsay and Ralph Forster Laboratories, University College, London, W. C. 1.

Discussion du rapport de M. INGOLD

M. Calvin. — I would like to call the attention of the conference to a third method of examining the vibrational pattern of the ground states of polyatomic molecules. This method results from the observations of G. N. Lewis on the phosphorescence of polyatomic molecules. Under the proper conditions it is possible to get an appreciable fraction of the molecules into a metastable state by excitation with light in its absorption region. The molecules remaining in the phosphorescent state for an appreciable length of time, lose all of their vibrational excitation by thermal degradation so that when they do pass by emission to the ground electronic state only vibrational levels of the ground state are involved. This is illustrated in the following scheme :



Thus a study of the structure of the phosphorescent emission (T ⟶ S) can give information concerning vibrational frequencies of the ground states of polyatomic molecules which may not be available by Raman or infra-red methods. Furthermore these phosphorescence spectra are observed in the visible, near ultraviolet and near infra-red regions, and generally at very low temperatures where the bands are very sharp.

M. Ingold. — I agree that phosphorescence spectra may prove most valuable for confirming by direct observation some frequencies of the ground state which are otherwise known only in some indirect way, *e. g.*, through Raman or infra-red combination tones, or by calculation from observed frequencies of isotopically modified forms.

Dr Calvin has indicated that a considerable simplification may be expected to arise from the circumstance that phosphorescence transitions usually start from a vibrationless upper state, with the result that the only fundamental vibration frequencies which are involved in the production of the phosphorescence band-system are those of the ground state. But the identification of any *new* fundamental frequency, *i. e.*, one which cannot otherwise be found at all, would be made difficult by the absence of vibrational selection rules for such essentially forbidden transitions.

M. de Hemptinne. — Faisant usage de la fonction potentielle de la molécule du benzène, fonction qui permet de calculer les fréquences des divers composés isotopes, ne serait-il pas utile de calculer de façon plus générale l'effet d'une variation de masse plus grande que celle qui correspond au double de la masse de l'hydrogène. Des calculs de ce genre ont été faits par Manneback et nos élèves à propos des vibrations gauches de l'éthylène. Ils ont pu donner des indications précieuses sur l'effet de l'augmentation de la masse d'un des vibrateurs sur l'ensemble des vibrations gauches de la molécule. Ceci facilite l'interprétation des spectres des dérivés substitués dans lesquels l'action d'une variation de la masse s'ajoute à l'action de la variation de la force.

M. Backer. — I have heard with great interest Prof. Ingold's report of his important work. I should like to put two practical questions.

How have the pure deuterium derivatives of benzene been prepared? What is the influence of the introduction of deuterium on the melting point of benzene?

M. Ingold. — Monodeuterobenzene was prepared by the Grignard reaction using bromobenzene and deuterium oxide.

$$C_6H_5Br \rightarrow C_6H_5.MgBr \xrightarrow[D_2O]{} C_6H_5D + MgBr(OD)$$

1 : 4 — Dideuterobenzene was prepared from *p*-dibromobenzene by the Grignard reaction, but it was found necessary to introduce the deuterium atoms one at a time, in order to obtain an isotopically pure product :

$$Br.C_6H_4Br \rightarrow Br.C_6H_4.MgBr \xrightarrow[D_2O]{} Br.C_6H_4.D + MgBr(OD)$$

$$D.C_6H_4Br \rightarrow D.C_6H_4.MgBr \xrightarrow[D_2O]{} D.C_6H_4.D + MgBr(OD)$$

It is possible to produce the dimagnesium compound $BrMg.C_6H_4$-MgBr, but the benzene obtained by decomposition of this with pure heavy water in dry ether contains a few units % of light hydrogen in the positions which should be occupied by deuterium. The light hydrogen presumably comes from the ether, which the dimagnesium compound decomposes to a small extent, probably through the production of intermediate free radicals. Professor Langseth's report refers to this matter. The trouble does not arise if one does not allow more than one magnesium atom at a time to enter into the aromatic molecule.

Pure 1 : 3 : 5-trideuterobenzene was obtained by using the rule that direct deuteration of the benzene nucleus by means of heavy acids (in Brönsted's sense, *i. e.*, deuteron-donors), such as D_3O^+, D_2SO_4, etc., follows the same orientation laws as do ordinary aromatic substitutions. Thus aniline was deuterated by D_3O^+ exclusively in the 2 : 4 : 6-position. From the product the amino-group was eliminated by the use of the diazo-reaction.

1 : 2 : 4 : 5 — Tetradeuterobenzene was also prepared by direct deuteration, in this case of p-dibromobenzene by means of D_2SO_4. From this product the two bromine atoms were eliminated by decomposing the Grignard derivative with light water.

Hexadeuterobenzene was prepared by the direct deuteration of benzene with D_2SO_4; and pentadeuterobenzene was obtained by brominating hexadeuterobenzene, and then getting rid of the bromine atom, as before, by the use of the Grignard reaction.

The melting point of benzene is about 5.5°, and the melting point of the deuterated benzenes are higher than this by 0.2° for every deuterium atom, up to the limit of hexadeuterobenzene, the melting point of which is 6.8°.

M. Timmermans signale que la très faible influence exercée par le remplacement d'atomes d'hydrogène par le deuterium sur la température de fusion du benzène n'a rien d'inattendu. En effet, le remplacement d'un hydrogène par un halogène diminue la symétrie de la molécule et abaisse notablement le point de fusion. Mais lorsqu'on remplace un ou plusieurs hydrogènes par du deutérium cela n'a aucun effet sur la symétrie de la molécule et seul se fait sentir l'influence de la masse 2 du deuterium remplaçant la masse 1 de l'hydrogène. Dans les autres cas de substitution d'un

élément par un de ses isotopes, un effet sur la température de fusion n'a jamais pu être observé; il n'y a d'exception que lorsqu'on remplace l'hydrogène par le deutérium, où une faible différence de la température de fusion est observable tant pour l'élément lui-même que pour ses combinaisons courantes (ex. : $H_2O = D_2O \longrightarrow = + 1.1.$); ce qui donc est intéressant dans le cas présent c'est de pouvoir séparer l'influence sur le point de fusion exercée par 1) la symétrie

2) la masse.

M. Berthelot. — J'aimerais demander à M. Calvin si les raisons qui déterminent la longue durée de vie du niveau métastable du benzène sont connues.

M. de Hemptinne. — Je signale que les points de fusion et d'ébullition de la famille d'isotopes du $CH_2Br - CH_2$ Br ont été mesurés par J. C. Jungers et ses collaborateurs; ils ont trouvé :

	Pt Fusion	Pt Ebullition
CHH Br — CHH Br	10.0 — 10.1	131.87
CHH Br — CHD Br	10.1 — 10.2	131.55
CHD Br — CHD Br	10.2 — 10.3	131.20
CHH Br — CDD Br	10.2 — 10.3	131.25
CHD Br — CDD Br	10.3 — 10.4	130.77
CDD Br — CDD Br	10.4 — 10.5	130.46

M. Calvin. — The long life of the phosphorescent state has been attributed by Lewis to the prohibition of the triplet-singlet transition, *i. e.*, the phosphorescent state is a triplet state, while the ground state is a singlet state. This has been confirmed by a direct measurement of the magnetic susceptibility of at least one phosphorescent molecule, fluorescein, for which the paramagnetic susceptibility was found to be only slightly less than that of molecular oxygen. The transition S' — T is not subject to the selection rule since it is a radiationless transition.

M. Briner. — Dans quelle mesure l'état de polarisation (mesuré par le facteur de dépolarisation) est-il modifié dans le benzène par les différentes substitutions d'un hydrogène par le deuterium?

M. Ingold. — Most of the Raman fundamentals of benzene, hexadeuterobenzene and the partly deuterated benzenes are essentially

depolarized, *i. e.*, their depolarisation factors a closely approximate to the theoretical maximum of 6/7. As to the rest, the depolarisation factors of polarised Raman lines of corresponding fundamental vibrations of isotopically isomeric benzenes of the same symmetry seem to be identical to within the rather considerable error of measurement. The theory of such effects is complicated, and exact conclusions are not easily reached by means of theory alone.

M. Langseth. — I want to express my admiration for the very great work carried through by Prof. Ingold and his co-workers concerning the molecular spectra of the benzene molecule. It is of extremely great interest to test the theory experimentally as is done in this work. There are certain minor points in the proposed analysis which perhaps still may be open to discussion. Here I only want to ask Dr Ingold one question. The frequency of the B_{2u} Carbon frequency is given to 1648 cm^{-1} in $C_6 H_6$ and 1571 $cm.^{-1}$ in $C_6 D_6$. Are these figures a result of computation (on the basis of the Teller-Redlich product rule, or on the basis of a solution of the vibrational problem) or are these frequencies observed in any of the deuterium derivatives of benzene ?

With regard to the interpretations of the nature of the forces within the benzene molecule given in the last part of Dr Ingold's report I want to say that, according to my experience, one has to be extremely careful about attributing a definite physical meaning to the arbitrary constants appearing in an approximate potential function like the Wilson-Bell function used. It is very probably approximately right, but it might on the other hand very well be pretty much wrong. I think one has to solve the vibrational problem on the basis of a general potential function and *afterwards* try to find out about the physical meaning of the arbitrary constants.

M. Ingold. — The determination of the B_{2u}-carbon frequency has been a source of difficulty. The basis of the solution proposed for this problem is essentially experimental, but it is somewhat indirect and there is an evident need for confirmation. We are hoping later to obtain confirmation through the study of the infra-red spectra of 1 : 2-di- and 1 : 2 : 3 : 4-tetra-deuterobenzene. The evidence at present available runs along the following lines.

The vibration is inactive in most of the spectra which have been

studied in connection with the vibrations of the ground state of benzene; but its fundamental frequency should appear, though probably with low intensity, in the infra-red spectrum of mono-, 1 : 4-di-, and 1 : 2 : 4 : 5-tetra-deuterobenzene. Unfortunately, allowed combination tones in the relevant region are numerous for these spectra, and there is a real possibility mistaking the required fundamental frequency, even if it would be observed, for a combination tone. There are no less than four sets of weak frequencies which can be selected from these three infra-red spectra, and considered for assignment to the B_{2u}-like carbon vibrations, on the ground that the frequency shifts from one isotopic benzene to another are approximately what is to be expected for this vibration. In every case, however, alternative interpretations, as combination tones, are possible; and therefore the position at this stage of the argument is simply that these four sets of frequencies are indicated for consideration.

One of the four sets, however, offers certain advantages of interpretation in quite a different direction, and it is for that reason that we point, tentatively, to this set as providing a probable solution to this problem. It has been noticed empirically that combination tones which appear with outstanding strength in the Raman or infra-red spectra of the different isotopic forms of benzene, and whose strength cannot be attributed to intensity borrowed from a neighbouring active fundamental by the mechanism of Fermi resonance, almost always belong to analogous combinations of vibrations. Now in the Raman spectra of benzene, monodeuterobenzene and 1 : 4-dideuterobenzene there are combination tones of quite outstanding intensity at 2618 cm.,$^{-1}$, 2590 cm.$^{-1}$ and 2583 cm.$^{-1}$, respectively. One would like to give them analogous explanations. It is also fairly clear that the benzene frequency 2618 cm.$^{-1}$ is that of a combination of which the B_{2u} frequency is one component : no other simple interpretation presents itself. Now if we adopt one particular set of the above-mentioned infra-red frequencies, *viz.*, 1624 cm.$^{-1}$ in monodeuterobenzene, 1603 cm.$^{-1}$ in 1 : 4-dideuterobenzene, and 1585 cm.$^{-1}$ in 1 : 2 : 4 : 5-tetradeuterobenzene, as representing the fundamental frequencies of the active analogues, in these deuterated compounds, of the B_{2u}-carbon vibration of benzene, we are able to explain the Raman combination tones in completely corresponding ways. They would represent combinations of the B_{2u}-like carbon frequencies with an out-of-plane frequency, 970 cm.$^{-1}$, of a vibration, labeled E_u^+-hydrogen, which is absolutely identical in benzene,

monodeuterobenzene and 1 : 4-dideuterobenzene (but no other deuterated benzene). The vibration is identical in these three compounds because the atoms on that para-axis, which in the deuterated compounds contains the deuterium atoms, do not move. Mono- and 1 : 4-di-deuterobenzene are thus the only deuterated benzenes for which this vibration is exactly the same as in benzene. And benzene, monodeuterobenzene and 1 : 4-di-deuterobenzene are the only benzenes amongst those which we have studied, which possess this outstanding Raman combination tone near 2600 cm.$^{-1}$. The attractive consistency of this interpretation, a consistency which it has not been found possible to obtain in any other way, is our main reason for selecting the infra-red frequencies mentioned for the B_{2u}-like carbon vibration of the partly deuterated benzenes. These values lead by way of the Teller-Redlich product theorem, to the values 1648 cm.$^{-1}$ and 1571 cm.$^{-1}$ for the corresponding frequencies of benzene and hexadeuterobenzene.

Theoretical calculations do not give much help with this problem. At the extremes of amplitude of this vibration, the C-C bonds become alternately long and short, as if they were approaching the condition of being the single and double bonds of the Kekulé formula. This suggests that it is less justifiable for this vibration than for the most others to treat the electronic energy as constant and separable from the energy of nuclear motion. The effect of supposing that, toward the turning points of a vibration, the electronic wave function becomes more Kekulé-like would be to reduce the expected value of the frequency. We do not know how powerful this effect is, and for the present may, on grounds of theory, expect the B_{2u}-carbon frequency to lie anywhere between wide limits, say 1300-1900 cm.$^{-1}$.

I agree with Dr Langseth that a more extended potential energy function would allow a more exact interpretation of the involved elastic constants. Our approximate physical interpretation of the constants of the simple function used must be judged by its internal consistency.

Les isotopes comme moyen d'investigation des spectres de bandes

par M. DE HEMPTINNE

Les composés du deutérium dans la série grasse

I.

INTRODUCTION

L'examen spectroscopique d'une molécule polyatomique comporte l'étude de son spectre I. R. lointain et proche, de son spectre Raman et si possible l'étude de son spectre électronique situé le plus souvent dans l'ultra-violet lointain, plus rarement, tout au moins pour les corps de la série grasse, dans l'ultra-violet proche et la région visible.

Faisant usage des raies d'absorption et éventuellement des raies d'émission obtenues, le spectroscopiste cherchera à déterminer la valeur énergétique des différents états de la molécule et en tout premier lieu les fréquences propres de la molécule dans son état normal. Il pourra alors chercher à déterminer pour cette molécule une fonction potentielle qui relie l'énergie potentielle de la molécule à la variation des entre-distances entre atomes, aux masses des atomes et aux forces de rappel s'opposant aux déformations.

La fonction permettra de retrouver par le calcul les données de l'expérience et éventuellement de déterminer certaines grandeurs que pour une raison ou une autre l'expérience est impuissante à donner; elle permettra également de contrôler les hypothèses de base se rapportant à la géométrie de la molécule envisagée.

Le spectroscopiste cherchera à déduire de l'examen des harmoniques des fréquences fondamentales le degré d'anharmonicité des vibrations dans le but de déterminer les états vibratoires élevés et de pouvoir estimer les énergies de dissociation.

L'étude de la molécule dans l'état électronique normal devrait être suivie de celle de la molécule dans les états électroniques supérieurs, en faisant usage à la fois des données obtenues pour la molécule à l'état normal et des conclusions que l'on peut tirer de l'analyse des spectres d'absorption ou d'émission ultra-violet.

Le premier travail du spectroscopiste est donc de déterminer les fréquences propres de la molécule c'est-à-dire d'identifier les fréquences expérimentales.

Les moyens employés se trouvent décrits dans une série d'excellents articles et d'ouvrages (38, 44, 45, 69).

On peut les résumer comme suit :

1. Détermination de l'état de polarisation des raies Raman.

2. Détermination du profil des raies et, si possible, de la structure fine des bandes d'absorption I. R. Détermination de la variation du coefficient d'absorption avec la polarisation de la lumière incidente.

3. Confrontation avec l'expérience des règles résultant de la symétrie supposée de la molécule, règles qui déterminent les activités en Raman et I. R.

La confrontation se fait par la détermination des raies observées uniquement dans le spectre Raman, de celles observées uniquement dans le spectre I. R. et de celles observées à la fois dans les deux spectres, de leur état de polarisation et de leur nature.

4. Examen des analogies que présentent les spectres de molécules de structures semblables; l'observation du déplacement des raies et de la variation du spectre consécutifs à la substitution d'un atome par un autre (45, 49).

5. Confrontation entre la valeur des raies expérimentales et les prévisions théoriques, lorsque l'établissement d'une fonction potentielle est possible.

Bien que, en principe, l'utilisation de ces méthodes dût permettre d'identifier avec certitude les fréquences fondamentales, l'impossibilité pratique où l'on se trouve d'obtenir un ensemble complet de données expérimentales ne permet pas d'atteindre l'objectif désiré.

En effet, un certain nombre de vibrations, théoriquement actives soit en Raman soit en I. R., ne donnent pas de raies pratiquement observables du fait de leur trop faible intensité; par ailleurs, certaines raies pourraient être interprétées aussi bien comme raies de combinaison que comme vibrations fondamentales; des coïncidences

arithmétiques peuvent induire en erreur, d'autant plus facilement que l'anharmonicité des vibrations introduit une correction dont la grandeur est le plus souvent inconnue.

Lorsque la molécule contient des atomes d'hydrogène, leur substitution par le deutérium peut rendre d'appréciables services.

La substitution d'un atome par son isotope de masse double modifiera profondément les fréquences dans lesquelles cet atome se trouve principalement impliqué.

Le déplacement plus ou moins grand des fréquences facilitera donc l'identification de celles-ci.

De plus la substitution d'un hydrogène par le deutérium peut affecter la symétrie de la molécule; on assistera alors à une modification du degré de dégénérescence de certaines raies.

Les règles d'activité et de polarisation, corollaires à la symétrie de la molécule, se trouveront modifiées, elles évolueront de façon prévisible comme l'exigent les modifications apportées de la symétrie.

En outre, les homologues des raies interprétées comme raies de combinaison et comme harmoniques des fréquences propres doivent, pour les molécules substituées, se retrouver à des endroits déterminés, rendant les coïncidences arithmétiques moins probables.

On aura ainsi non seulement un moyen puissant pour identifier les raies, mais encore un moyen de contrôler les hypothèses faites, concernant la symétrie même de la molécule.

Enfin la géométrie de la molécule et les forces entre atomes isotopes restant inchangées, aucun paramètre nouveau ne s'introduit dans la fonction potentielle de la molécule à l'occasion de la substitution d'un atome par son isotope; toutes les données expérimentales nouvelles obtenues pourront donc servir de valeurs de départ pour la détermination de la fonction, ou serviront de contrôles aux calculs théoriques.

L'attribution des raies se trouve aussi facilitée par l'application de la loi du rapport des produits des fréquences dénommée parfois loi Teller Redlich ou encore « Rapport Isotopique ». Elle assure que, pour une molécule déterminée, le produit des fréquences appartenant à un type spectral déterminé divisé par le produit analogue pour la même molécule, dont les atomes sont remplacés par leur isotope, est indépendant des constantes des forces et dépend seulement des masses et de la géométrie de la molécule.

On peut étendre la règle à des molécules isotopes dont la symétrie est moindre que celle de la molécule non substituée. Il faut alors

appliquer la règle aux fréquences du type de symétrie de la molécule la moins symétrique.

L'extension de la règle est aisée lorsqu'il n'y a pas de fréquences dégénérées, car les corrélations des fréquences sont presque évidentes; elle demande un peu plus d'attention lorsqu'il y a des fréquences dégénérées (voir Herzberg, p. 231). Noether (59) a formulé une règle liant entre elles les fréquences de molécules différentes mais de structures semblables en proposant pour les halogénures de méthyle la relation empirique suivante justifiée théoriquement par la suite :

$$\frac{\nu_{(CD_3Cl)}}{\nu_{(CH_3Cl)}} = \frac{\nu_{(CD_3Br)}}{\nu_{(CH_3Br)}}$$

Il faut bien reconnaître que, en pratique, on ne peut appliquer ces règles qu'à des molécules relativement simples.

Pour pouvoir s'attaquer à des molécules plus compliquées, E. B. Wilson (80, 81) a développé une méthode permettant, tout en limitant les erreurs à 1 %, d'appliquer la règle de Teller-Redlich, séparément aux hautes et aux basses fréquences.

Enfin tout dernièrement dans une suite de travaux ayant pour but de justifier la règle empirique de Noether, W. F. Edgell (18) montre comment et dans quelle mesure la règle peut s'appliquer à chaque fréquence isolément.

La connaissance des harmoniques des familles de deutéro-substitués, jointe à l'écart entre le rapport théorique et expérimental des produits des fréquences, écart dû à l'anharmonicité et jointe éventuellement aussi à la mesure de la structure de rotation des raies, permet d'estimer de façon précise l'anharmonicité de la molécule et d'en déduire une fonction potentielle plus conforme à la réalité.

La modification apportée à la symétrie des molécules permet en outre dans quelques cas spéciaux de formuler des conclusions certaines au sujet de la structure et de la rotation libre de certaines molécules.

Passons en revue quelques catégories importantes de molécules de la série grasse pour lesquelles des substitués lourds ont été préparés et étudiés. Laissant aux ouvrages spécialisés le soin de rapporter la discussion complète des spectres, nous nous bornerons en général à mettre en évidence les résultats acquis, grâce au deutérium, et à souligner les problèmes que la méthode de substitution de l'hydrogène par le deutérium peut résoudre.

II.

MOLÉCULES LINÉAIRES

L'acétylène et l'acide cyanhydrique (HCN) sont deux molécules linéaires pour lesquelles la substitution de l'hydrogène par le deutérium a pu fournir nombre de renseignements. Nous nous bornerons à développer ici quelques considérations se rapportant à la première de ces molécules.

ACÉTYLÈNE

La facilité de préparation des deutéro-acétylènes et la simplicité de la géométrie de ces produits font que ces molécules furent parmi les premiers composés deutérés étudiés.

L'examen des spectres Raman et I. R. de la molécule C_2H_2 montre de façon indiscutable que cette molécule présente la symétrie $D_{\infty h}$; il en sera donc de même pour C_2D_2, tandis que la molécule C_2HD, moins symétrique présente la symétrie $C_{\infty v}$.

Le tableau I emprunté au livre de Kohlrausch (45) donne les règles de sélection pour les vibrations Raman et I. R. de molécules ayant les symétries indiquées. Les indications *s* et *as* expriment que la vibration en question est symétrique ou antisymétrique par rapport à l'élément de symétrie se trouvant au haut de la colonne; *e* se rapporte à des fréquences dégénérées; les indications *p*, *dp*, *v* se rapportent aux raies Raman et indiquent qu'elles sont polarisées, dépolarisées ou interdites; enfin M_z, $M_\perp$, *ia* se rapportent aux bandes I.R. et indiquent si elles sont du type parallèle, normal ou si elles sont interdites.

TABLEAU I

Symétrie $D_{\infty h}$				Symétrie $C_{\infty v}$		
Type	C_∞	i	Activité	Type	C_∞	Activité
Σ_g^+ ou A_g	s	s	p ia	—	—	—
Σ_u^+ ou A_u	s	as	v M_z	Σ^+ ou A	s	p M_z
Π_g ou E_g	e	s	dp ia	—	—	—
Π_u ou E_u	e	as	v $M_\perp$	Π ou E	e	dp $M_\perp$

Il s'ensuit que, le spectre Raman des C_2H_2 ne peut donner que les fréquences Σ_g^+ et Π_g. Le spectre I. R. peut donner les autres fréquences fondamentales : Σ_u^+ et Π_u.

Pour la molécule C_2HD, au contraire, toutes les fréquences propres sont théoriquement actives tant en Raman qu'en I. R.

Les fréquences fondamentales des acétylènes sont données dans le tableau suivant :

TABLEAU II

Symétrie :	C_2H_2 $D_{\infty h}$	C_2D_2 $D_{\infty h}$	C_2HD $C_{\infty v}$	
ν_4 (Π_g)	611,8	(505)	518,8	ν_4 (II)
ν_5 (Π_u)	729,1	539,1	683	ν_5 (II)
ν_2 $\Sigma_g^+)$	1973,8	1762,4	1851,2	ν_2 (Σ^+)
ν_3^{CH} (Σ_u^+)	3287	2427	2584	ν_3 (Σ^+)
ν_1^{CH} (Σ_g^+)	3373,7	2700,5	3334,8	ν_1 (Σ^+)

Les attributions sont confirmées par la règle de Teller Redlich qui est d'application particulièrement aisée dans le cas d'une molécule linéaire simple comme celle dont nous nous occupons ici.

Les rapports théoriques sont :

$$\frac{(\omega_1\,\omega_2)_{C_2H_2}}{(\omega_1\,\omega_2)_{C_2D_2}} = \sqrt{\frac{m_D}{m_H}} = 1{,}413; \qquad \frac{(\omega_3)_{C_2H_2}}{(\omega_3)_{C_2D_2}} = \sqrt{\frac{m_D\,(m_C + m_H)}{m_H\,(m_C + m_D)}} = 1{,}361$$

$$\frac{(\omega_4)_{C_2H_2}}{(\omega_4)_{C_2D_2}} = \sqrt{\frac{m_D\,I_{C_2H_2}}{m_H\,I_{C_2D_2}}} = 1{,}20; \qquad \frac{(\omega_5)_{C_2H_2}}{(\omega_5)_{C_2D_2}} = \sqrt{\frac{m_D m_C + m_H}{m_H m_C + m_D}} = 1{,}335$$

Les valeurs expérimentales correspondantes sont :

$$\frac{(\nu_1\,\nu_2)_{C_2H_2}}{(\nu_1\,\nu_2)_{C_2D_2}} = 1{,}399 \qquad \frac{(\nu_3)_{C_2H_2}}{(\nu_3)_{C_2D_2}} = 1{,}354$$

$$\frac{(\nu_4)_{C_2H_2}}{(\nu_4)_{C_2D_2}} = 1{,}211 \qquad \frac{(\nu_5)_{C_2H_2}}{(\nu_5)_{C_2D_2}} = 1{,}352$$

Le spectre Raman de C_2H_2 et les spectres d'absorption dans les différentes régions I. R. fournissent de nombreuses raies supplémentaires à attribuer à des harmoniques ou à des raies de combinaison.

Les divers auteurs interprètent ces raies de façon différente et l'on trouvera dans la littérature plusieurs propositions également satisfaisantes mais portant toutes le flanc à certaines objections.

L'interprétation retenue par Herzberg (p. 290 et suiv.) offre l'avantage de pouvoir s'adapter aussi bien aux résultats expérimentaux concernant l'acétylène léger qu'à ceux se rapportant aux acétylènes deutérés. Toutefois, comme le reconnaît du reste l'auteur, elle peut prêter à discussion.

Aussi serait-il fort utile d'étendre les mesures faites sur C_2D_2 aux fréquences supérieures à 5120 Å, afin de pouvoir établir pour cette molécule un tableau aussi complet que celui donné pour C_2H_2 et pouvoir ainsi confirmer les attributions des bandes I. R. de cette dernière molécule.

Il est en effet étrange de constater que, alors que C_2H_2 présente dans l'infra-rouge photographique de belles structures de rotation parfaitement étudiées, l'étude de cette intéressante région a été seulement amorcée pour HDC_2 et que l'étude de la même région, pour la molécule C_2D_2, se fait encore attendre. Voilà une lacune qui devrait être comblée.

L'étude de la structure fine de rotation des molécules isotopes offre grand intérêt. En effet, l'observation et l'analyse des raies de rotation d'une molécule linéaire permettent de déterminer avec précision le moment d'inertie de cette molécule.

Dans le cas d'une molécule triatomique, les distances entre atomes peuvent en être déduites. Il n'en est pas de même pour l'acétylène car la molécule étant formée de 4 atomes, on ne dispose que d'une relation pour déterminer deux inconnues. Cependant l'analyse conjointe de la structure de rotation de la molécule isotope permet de faire cette détermination.

En effet, si « a » est la distance d'un atome « C » au centre de symétrie et « b » la distance de H à ce même centre, on a pour C_2H_2 :

$$I_{(C_2H_2)} = 2m_C a^2 + 2m_H b^2 \qquad (1)$$

et pour C_2D_2 :

$$I_{(C_2D_2)} = 2m_C a^2 + 2m_D b^2. \qquad (2)$$

Par ailleurs, le centre de symétrie de C_2HD ne coïncide plus avec le centre de masse; soit « d » la distance entre ces deux centres; on aura:

$$I(C_2HD) = 2m_C a^2 + (m_H + m_D)\, b^2 - Md^2 \quad (3)$$

et :

$$d = \frac{mD - mH}{M}\, b \quad (4)$$

où M représente la masse totale de la molécule C_2HD.

Ces quatre relations permettent non seulement de déterminer « a » et « g » à partir des valeurs expérimentales des I déduites de l'analyse de la structure de rotation, mais encore de contrôler cette détermination.

Les mesures faites sur C_2H_2 et C_2HD donnent finalement (38) :

$$r_e\ (C \equiv C) = 1{,}202 \quad 10^{-8}\text{cm}$$
$$r_e\ (C - H) = 1{,}059 \quad 10^{-8}\text{cm}$$

Enfin l'acétylène léger, tout comme l'H_2, est de par sa symétrie un mélange de variétés ortho et para; sa structure fine de rotation présente donc une alternance d'intensité dans le rapport de 1 à 3 à l'avantage des J impairs; l'alternance doit disparaître dans la structure fine de rotation de HDC_2, ce qui est confirmé par l'expérience.

Ces faits sont une preuve indubitable de la structure linéaire et symétrique de C_2H_2 et sont en accord avec la valeur 1/2 attribuée au spin du noyau H et avec la statistique de Fermi à laquelle obéit ce noyau. Comme le noyau de D a un spin 1 et que, d'autre part, il obéit à la statistique de Bose, la structure de rotation devrait présenter une alternance d'intensité dans le rapport de 2 à 1 en faveur des raies de J pairs.

La chose n'a pu encore être constatée expérimentalement, les mesures à grande dispersion, permettant l'analyse de la structure fine de rotation, faisant défaut pour C_2D_2.

III.

MÉTHANE ET QUELQUES DÉRIVÉS DU MÉTHANE

A. Fréquences fondamentales du méthane.

On trouvera un exposé assez complet de la question dans un article de Dennison (14) paru en 1940 ainsi que dans l'ouvrage de Herzberg (38) paru en 1945.

Ces auteurs se réfèrent aux travaux expérimentaux de Mac Wood et Urey, Ginsburg et Barker, Benedict, Morikawa, Barnes et Taylor, Childs et Jahn, Nielsen ainsi qu'aux travaux théoriques de Dennison, de J. Rosenthal et de Dennison et Johnston.

Pour le méthane léger, on n'a pu mesurer directement que trois fréquences fondamentales, deux d'entre elles apparaissent dans le spectre Raman, la troisième est donnée par le spectre I. R. La quatrième et dernière fréquence fondamentale n'a pas été mesurée directement mais peut se déduire de la mesure de certaines raies de combinaison.

Les données expérimentales sont donc très pauvres; aussi était-il des plus important de pouvoir les multiplier grâce à la substitution du deutérium à l'hydrogène.

L'allure des spectres Raman et I. R. du méthane et de ses substitués lourds confirme la structure tétraédrique de la molécule. Les spectres se modifient exactement comme l'exigent les règles d'activité et de dégénérescence en corrélation avec la symétrie envisagée.

L'attribution des raies et bandes expérimentales aux diverses fréquences propres est aisée.

Wagner (77, 78, 79) a montré dans une série de travaux comment les vibrations se modifiaient suite à la substitution d'un atome plus lourd à un atome plus léger. Cet auteur publie des graphiques donnant pour chaque vibration la variation des pourcentages d'influence des différentes liaisons de la molécule en fonction des variations des masses des atomes substitués.

Le remplacement successif des atomes d'hydrogène par des atomes de deutérium transforme la symétrie T_d de la molécule de méthane

en une symétrie C_{3v} pour H_3CD_v elle devient ensuite C_{2v} pour H_2CD_2 pour être à nouveau C_{3v}, suite à la substitution de trois atomes de deutérium à trois atomes d'hydrogène dans le méthane léger et revenir enfin à la symétrie T_d pour CD_4 (fig. 21).

Les règles de sélection ressortent du tableau suivant :

TABLEAU II

Groupe T_d						
Type	C_2^z	C_y	C_3	S_4		
A_1	s	s	s	s	$\rho = o$	ia
A_2	s	s	s	as	v	ia
E	s	s	e	e	dp	ia
F_1	s	e	e	e	v	ia
F_2	e	e	e	e	dp	M

Groupe C_{3v}				
Type	C_3^z	σ_x		
A_1	s	s	p	M_z
A_2	s	as	v	ia
E	e	e	dp	$M_\perp$

Groupe C_{2v}					
Type	C_2^z	σ_x	σ_y		
A_1	s	s	s	p	M_z
A_2	s	as	as	dp	ia
B_1	as	as	s	dp	M_x
B_2	as	s	as	dp	M_y

L'application de la règle de Teller Redlich aux fréquences non dégénérées de CH_4 et CD_4 permet d'estimer les corrections dues à l'anharmonicité de ces vibrations. On peut écrire en effet :

$$\frac{(\omega_1)_{CH_4}}{(\omega_1)_{CD4}} = \sqrt{\frac{m_D}{m_H}} \qquad (1)$$

$$\nu_{1\,(CH_4)} = \omega_{1\,(CH_4)}\,[1 - \alpha_1] \qquad (2)$$

$$\nu_{1\,(CD_4)} = \omega_{1\,(CD_4)}\left[1 - \frac{\omega_{1\,(CH_4)}}{\omega_{1\,(CD_4)}}\alpha_1\right] \qquad (3)$$

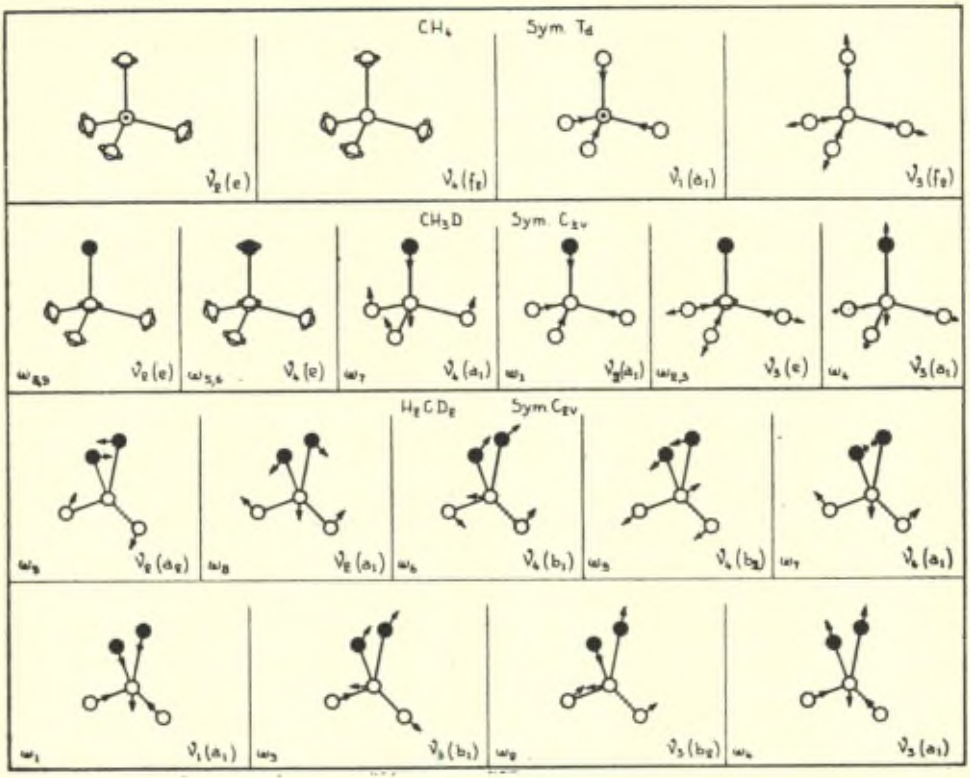


Fig. 21

Les ν sont les fréquences expérimentales entachées d'anharmonicité, les ω étant ces mêmes fréquences corrigées de l'anharmonicité, α étant la correction d'anharmonicité. Ces relations permettent de déterminer α à partir des données expérimentales.

La règle de Teller Redlich, jointe à la mesure de l'espacement des raies de rotation, qui est fonction de la constante d'anharmonicité α, permet de déterminer l'anharmonicité des deux fréquences dégénérées triples. Dennison donne les valeurs suivantes pour les différents α:

$\alpha_1 = 0{,}0397$ pour la vibration symétrique de valence CH.

$\alpha_2 = -0{,}0474$ pour la vibration de déformation double symétrique CH.

Dennison explique le signe moins, assez surprenant à première vue, par un effet stérique.

Il donne aussi $\alpha_3 = 0{,}0459$ et $\alpha_4 = 0{,}0393$ pour les 2 vibrations triples de valence et de déformation CH.

La connaissance de ces constantes permet de trouver les fréquences harmoniques à partir des données expérimentales ou mieux de calculer à partir des fréquences harmoniques théoriques ω les fréquences théoriques anharmoniques α que l'on compare avec les résultats expérimentaux.

Nous signalerons, à propos de l'éthylène, qu'il est également possible de calculer les fréquences anharmoniques en introduisant la correction sous forme d'une modification fictive des masses des vibrateurs.

Le tableau IV donne l'ensemble des résultats obtenus à ce jour. Les valeurs théoriques empruntées au travail de Dennisson sont écrites entre parenthèses.

Les fréquences calculées, compte tenu des corrections d'anharmonicité, sont écrites entre parenthèses au dessus des valeurs qu'auraient les fréquences si les vibrations étaient harmoniques. Les fréquences expérimentales sont reproduites en dessous des valeurs théoriques, elles sont suivies d'un R si elles sont données par le spectre Raman, d'un I si elles apparaissent dans le spectre I. R. L'astérisque indique, tout comme chez Herzberg, que la raie n'est pas obtenue directement, mais que sa valeur se déduit à partir de raies de combinaison.

TABLEAU IV

CH_4 (T_d)	$\nu_1(a_1)$	$\nu_2(e)$	$\nu_3(f_2)$	$\nu_4(f_2)$
	(3029,8) (2914,2) 2914,2 R	(1590,2) (1533,4) 1526 I* R*	(3156,5) (3018,4) 3020,3 I R	(1357,4) (1306,2) 1306,2 I
CH_2D (C_{2v})	$\nu_1(a_1)$	$\nu_2(e)$	$\nu_3(e)$ $\nu_3(a_1)$	$\nu_4(a_1)$ $\nu_4(e)$
	(3067) // (2948,5) 2982,2 I//	(1404,7) ⊥ — 1476,7 I⊥ R*	(3156,6)⊥ (3018) 3030,2 I (2283,6) // (2210,1) 2204,6 I	(1352) // (1174,5) ⊥ (1300,8) (1144,7) 1306,4 I// 1156 I⊥
CH_2D_2 (C_{2v})	$\nu_1(a_1)$	$\nu_2(a_1)$ $\nu_2(a_1)$	$\nu_3(b_2)$ $\nu_3(a_1)$ $\nu_3(b_1)$	$\nu_4(a_1)$ $\nu_4(b_2)$ $\nu_4(b_1)$
	(2234,2) (2170,7) 2139 R	(1241,2) (1393,1) — — 1285,6 R 1450 I	(2336,1) (3039,7) (3156,3) (2260,1) (2966) (3017,2) 2255 I 2974,2 R 3020 I	(1277,2) (1028,9) (1126,3) (1237,6) (994,4) (1090,7) 1235,2 I 1034,4 I R 1096,2 I
CD_3H (C_{3v})	$\nu_1(a_1)$	$\nu_2(e)$	$\nu_3(e)$ $\nu_3(a_1)$	$\nu_4(a_1)$ $\nu_4(e)$
	(2188,2) // (2122,2) 2141,1 R	(1259,7) ⊥ — 1239,2 R	(2336,9) ⊥ (3128,1) // (2260,3) (2992) 2268,6 R 2992 I//	(1034,2) // (1012,5) ⊥ (1004,2) (987,4) 1046 R 982 R
CD_4 (T_d)	$\nu_1(a_1)$	$\nu_2(e)$	$\nu_3(f_2)$	$\nu_4(f_2)$
	(2142,2) (2024,7) 2034,7 R	(983,4) (1036,4) 1054 R*	(2336,9) (2259,3) 2258,2 I R	(1026,4) (996,5) 995,6 I

B. Halogénures de Méthyle.

Fréquences fondamentales. — L'identification des fréquences fondamentales des halogénures de méthyle léger n'offre pas de difficulté majeure.

Faisant usage des données expérimentales, connues à l'époque, Wagner (77) et plus tard Linnett (51) purent établir une fonction potentielle permettant de prévoir théoriquement les fréquences des halogénures légers et lourds; toutefois comme le fait remarquer Dennisson (14), les rares vérifications possibles étaient illusoires tant que l'on ne disposait que des seules mesures de fréquences se se rapportant aux halogénures légers. Aussi était-il hautement souhaitable de pouvoir disposer également de données obtenues concernant les halogénures lourd et mixte. Dans le courant des dernières années, Noether (59) a fait paraître une série de travaux consacrés au spectre I. R. des CD_3Cl et CD_3Br; les mesures sont faites au moyen d'un réseau et peuvent donc prétendre à une grande précision; indépendamment, de Hemptinne (27, 28, 30) Doehaerd (16) et Courtoy (111*bis*), ont étudié les spectres Raman et I. R. des chlorures, bromures et iodures deutéro substitués. Les spectres I. R. mesurés par Courtoy sont obtenus au moyen d'un spectrographe à prisme; les résultats devront donc être considérés comme moins précis que ceux donnés par Noether; c'est pourquoi dans les tableaux qui suivent nous prendrons les résultats de Noether de préférence à ceux de Courtoy, lorsqu'un choix entre deux valeurs s'impose.

Doehaerd a repris les calculs de Linnett et les a étendus au calcul des fréquences propres des bisubstitués. Ces dernières molécules ne présentent plus qu'un plan de symétrie ce qui lève la dégénérescence des 3 fréquences doubles et rend par ailleurs le calcul malaisé. Une méthode d'approximation permet toutefois d'obtenir des données numériques suffisamment précises. Il y a 6 fréquences symétriques et 3 antisymétriques, toutes actives en Raman et I. R. Le dédoublement des fréquences doubles s'observe fort bien.

Les spectres Raman et I. R. se complètent admirablement; seules quelques rares lacunes subsistent encore dans les tableaux d'ensemble.

Les tableaux V, VI et VII suivants résument les résultats expérimentaux et théoriques obtenus.

Les raies Raman ont été obtenues en utilisant les produits à l'état liquide, alors que les spectres I. R. sont ceux des mêmes corps à

TABLEAU V

Chlorure de Méthyle

CH_3Cl (C_{3v})		CH_2DCl (C_s)		CD_2HCl (C_s)	CD_3Cl (C_{3v})
ω_1	(2911) 2924 R * 2967,0 I		(2960) 2985 R 2989 I	(2140) 2186 R 2188 I	(2087) 2115 R * 2160 I
ω_4	(708) 709 R * 733 I		(696) 696 R 717 I	(688) 689 R 700 I	(684) 683 R * 701 I
ω_1	(1333) 1370 R 1355,3 I		(1248) — 1265 I	(1239) — 1265	(994) 1018 R * 1030 I
$\omega_{2,3}$	(3043) 3086 R 3047,2 I	ω_2	(2206) 2220 R —	(3006) 3011 R —	(2270) 2283 R * 2300 I (réseau) * 2268 I (prisme)
		ω_3	(3042) — —	(2272) — —	
$\omega_{5,6}$	(1018) 1016 R * 1019,9 I	ω_5	(818) 824 R 824 I	(861) 864 R 872 I	(766) 769 R * 775 I
		ω_6	(983) — —	(771) — 775 I	
$\omega_{8,9}$	(1459) 1446 R * 1459,6 I	ω_8	(1438) — 1439 I	(1029) — 1055 I	(1051) 1051 R 1081 I
		ω_9	(1273) 1305 R —	(1292) 1305 R —	

TABLEAU VI

Bromure de Méthyle

CH_3Br		CH_2DBr		CD_2HBr	CD_3Br
ω_1	(2923) 2937 R * 2973 I		(2972) 2988 R 3006 I	(2149) 2185 R 2188 I	(2095) 2124 R * 2134 I
ω_4	(594) 595 R * 610 I		(580) 583 R —	(570) 573 R —	(562) 563 R * 577 I
ω_7	(1291) 1297 R * 1305,5 I		(1210) 1216 R 1230 I	(1202) 1201 R 1222 I	(968) 980 R * 987 I
$\omega_{2,3}$	(3055) 3046 R * 3061 I	ω_2	(2214) 2235 R 2188 I	(3018) 3019 R —	(2278) 2289 R * 2294 I
		ω_3	(3054) — —	(2280) — —	
$\omega_{5,6}$	(955) 942 R * 956,9 I	ω_5	(762) 765 R 770 I	(804) 811 R 816 I	(712) —
		ω_6	(926) — 920 I	(716) — 709 I	* 717 I
$\omega_{8,9}$	(1446) 1428 R * 1450,5 I	ω_8	(1425) — 1423 I	(1018) 1029 R 1036 I	(1043) 1048 R
		ω_9	(1254) — —	(1283) — —	* 1053 I 1048 I

TABLEAU VII

Iodure de Méthyle

CH_3I		CH_2DI		CD_2HI	CD_3I
ω_1	(2932) 2941 R * 2975 I		(2981) 2982 R 2980 I	(2154) 2178 R 2174 I	(2101) 2130 R 2151 I
ω_4	(524) 523 R * 532.9 I		(511) 511 R —	(501) 501 R —	(493) 493 R —
ω_7	(1243) 1241 R * 1251,8 I		(1164) 1161 R 1177 I	(1169) 1161 R 1177 I	(932) 938 R 948 I
$\omega_{2,3}$	(3063) 3049 R * 3061,4 I	ω_2	(2219) 2227 R 2174 I	(3025) 3013 R 3017 I	(2284) 2285 R —
		ω_3	(3063) — 3091 I	(2285) — —	
$\omega_{5,6}$	(886) 891 R * 881,6 I	ω_5	(708) 706 R 723 I	(753) 751 R 757 I	(655) 644 R 665 I
		ω	(864) — 876 I	(660) — —	
$\omega_{8,9}$	(1441) 1425 R * 1440,0 I	ω_8	(1419) 1408 R 1418 I	(997) 1006 R 1009 I	(1042) 1046 R 1050 I
		ω_9	(1244) — —	(1282) 1272 R —	

l'état vapeur. Ceci explique la différence entre les résultats obtenus en Raman et I. R.

Nous donnons, entre parenthèses, les fréquences théoriques obtenues, en utilisant une fonction potentielle très légèrement différente de celle proposée par Linnett.

Les lettres R et I indiquent que les raies ont été observées respectivement en Raman ou en Infra Rouge.

Les valeurs entre parenthèses sont les valeurs théoriques.

Les fréquences précédées d'un astérisque sont celles données par Noether.

C. Alcool méthylique.

L'alcool méthylique a donné lieu à de nombreuses études ayant pour objet la détermination et l'analyse de son spectre Raman et I. R.

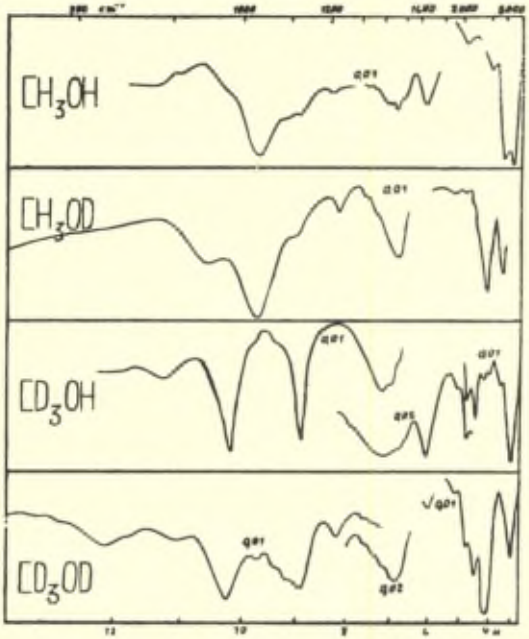
Les interprétations des différents auteurs coïncident en bien des points mais diffèrent sur bien d'autres. Une des causes des discordances est la différence qui existe entre le spectre du gaz et celui du liquide, différence due au phénomène d'association moléculaire caractéristique du radical OH.

La différence entre les spectres des vapeurs et des liquides n'est sensible que pour les raies dépendant principalement du groupe OH.

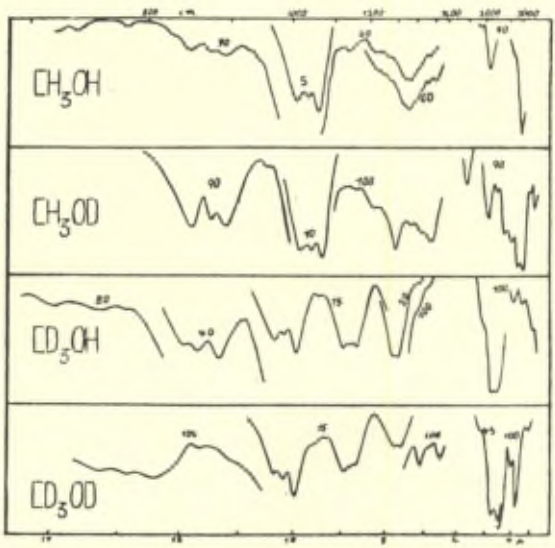
De façon générale, l'ensemble des raies apparaît large et sans structure de rotation dans le liquide. La fig. 22, représentant la région s'étendant de 12 à 4μ, montre la différence entre le spectre I. R. du liquide et de la vapeur.

Lorsque l'interprétation d'un spectre s'avère malaisée, il est extrêmement utile de pouvoir disposer des spectres des homologues deutérés, afin de pouvoir, par comparaison, confirmer ou infirmer les hypothèses faites et trouver ainsi les identifications conformes. Il est également utile de pouvoir confronter les valeurs expérimentales avec les prévisions théoriques. Tel est le cas de l'alcool méthylique.

Les travaux de Barker et Bosschieter (2), se rapportant à la molécule H_2DCOD, montrent à l'évidence qu'il n'y a pas rotation libre de OD autour de l'axe CO, car ils mettent en évidence pour cette molécule 2 isomères provenant de l'orientation différente de OD par rapport au D de la pyramide.



liquide



vapeur

Fig. 22

Ces vues sont confirmées par Lawson et Randall qui mettent la fréquence de torsion à 270 cm^{-1} pour l'alcool léger.

Puisqu'il n'y a pas de rotation libre il faut retrouver 3 fréquences OH, une fréquence de valence, une fréquence de torsion et une fréquence de déformation COH.

La première de ces fréquences a pu être particulièrement bien étudiée grâce aux spectres des molécules lourdes.

Le tableau suivant donne les interprétations de ces raies pour la molécule à l'état vapeur et à l'état liquide :

TABLEAU VIII

	CH_3OH	CH_3OD	CD_3OH	CD_3OD	Nature de la raie
Vapeur	—	—	—	—	torsion
	—	871	858	776	déformation plane. OH / OD
	3682	2719,7	3675	2720	valence ..... OH / OD
Liquide	(1) 965	941	886	825	
	(2) 1639	1218	1639	1214	
	(3) 3300	2405	3300	2420	

L'alcool à l'état liquide donne lieu au phénomène d'association moléculaire.

Les fréquences (2) et (3) des alcools liquides données dans le tableau sont certainement à attribuer au groupe OH ou OD; elles sont à mettre en parallèle avec les fréquences 1656 et 3440 observées dans H_2O liquide et 1208 et 2515 observées pour D_2O et attribuées respectivement à une déformation et une vibration de valence symétrique.

La comparaison des spectres des alcools deutéro substitués permet de déterminer avec certitude les harmoniques des fréquences de valence OH et OD et permet ensuite de déterminer les constantes d'anharmonicité et d'établir une fonction de Morse adéquate.

Le tableau IX résume les résultats obtenus (II).

G(v) et Go(v), suivant les notations de Herzberg, désignent les termes comptés respectivement à partir du minimum de la courbe potentielle ou à partir de l'état au zéro absolu.

$$G(v) = \omega_e (\upsilon + \tfrac{1}{2}) - \omega_e x_e (\upsilon + \tfrac{1}{2})^2.$$

TABLEAU IX

	CH_3OH	CH_3OD		CH_3OH	CH_3OD
x_e	0,02212	0,01614	ω_e	3851	2810
G (0)	1904	1394	Go (1) = G (1) — G (0)	3681 (3682)	2719 (2719,7)
G (1)	5585	4113	Go (2) = G (2) — G (0)	7191 (7150±40)	5348 (5335±15)
G (2)	9095	6742	Go (3) = G (3) — G (0)	10531 (10531)	7875 —
G (3)	12435	9279	Go (4) = G (4) — G (0)	13700 (13700,7)	10332 —
G (4)	15604	11726	Go (5) = G (5) — G (0)	16699 —	12689 —
G (5)	18603	14083		—	—

$D_e = 43515\ cm^{-1}$ $\qquad U(r - r_e) = D_e(1 - e^{-\beta(r - r_e)})^2$

$\beta = 2{,}198.\ 10^8$

Les fréquences expérimentales à comparer avec les valeurs théoriques sont données entre parenthèse au-dessous de celles-ci.

Les autres fréquences propres pourront s'identifier par comparaison entre résultats expérimentaux et prévisions théoriques; les calculs sont faits pour le radical H_3CO-, sans se préoccuper de l'H du groupe OH.

Le tableau suivant donne la comparaison entre expérience et théorie.

De nombreuses raies supplémentaires peuvent s'expliquer comme harmoniques ou combinaisons des fréquences fondamentales reprises dans le tableau précédent.

TABLEAU X

	CH_3O		CH_2DO		CD_2HO		CD_3O	
	obs.	calc.	obs.	calc.	obs.	calc.	obs.	calc.
ω_1	2834	2850	2882	2898	—	2100	2071	2048
ω_4	1034	1034	1037	1041	—	987	978	978
ω_7	1451	1453	1342	1345	—	1346	1128	1121
$\omega_{2,3}$ ω_2	2990	2978	2176	2161	—	2942	2249	225
$\omega_{2,3}$ ω_3			2980	2978	—	2227		
$\omega_{5,6}$ ω_5	1162	1167	907	929	—	967	903	900
$\omega_{5,6}$ ω_6			—	1093	—	909		
$\omega_{8,9}$ ω_8	1472	1475	1470	1467	—	1105	1066	1055
$\omega_{8,9}$ ω_9			1301	1328	—	—		

IV.

ÉTHYLÈNE ET DÉRIVÉS HALOGÉNÉS DE L'ÉTHYLÈNE

A. Ethylène.

La molécule d'éthylène est une de celles pour lesquelles l'étude du composé léger seul ne permet pas d'arriver à des conclusions certaines. Même si on a recours à des méthodes extra-spectroscopiques, telles que la mesure des chaleurs spécifiques par exemple, pour suppléer au manque de données expérimentales, les renseignements obtenus, tout en donnant des indications extrêmement précieuses, ne permettent pas de lever les doutes qui planent sur la détermination des fréquences propres de la molécule.

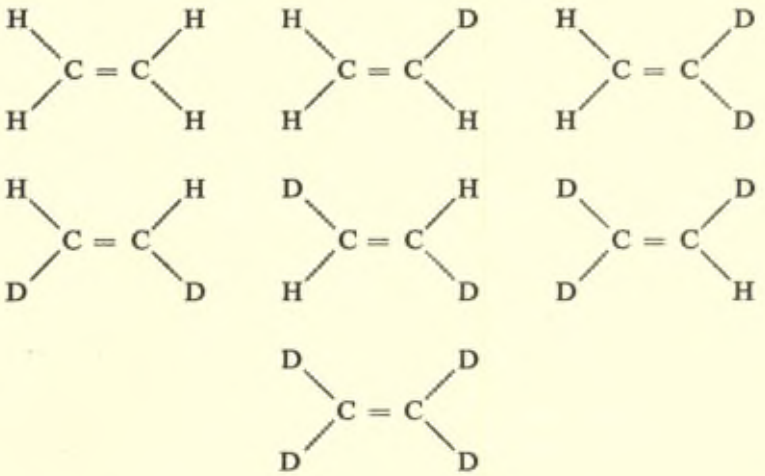
La cause des difficultés rencontrées réside dans le fait que d'une part la fréquence de torsion de la molécule d'éthylène légère est inactive aussi bien en Raman qu'en I. R. et que d'autre part un certain nombre d'autres vibrations d'intensités faibles, donc très difficilement observables, se trouvent dans la même région spectrale où elles peuvent se recouvrir l'une l'autre, ce qui rend leur observation difficile et complique l'analyse de leur structure.

Remarquons du reste qu'une étude en Raman et I. R. de la molécule C_2D_4, jointe à une étude Raman incomplète des autres dérivés

deutérés, telle qu'elle existe actuellement, ne permet pas encore de tirer des conclusions certaines. L'étude approfondie du spectre Raman et du spectre I. R. des mono et tri substitués s'impose, car elle semble pouvoir résoudre le problème, toutes les fréquences pour ces deux produits étant actives tant en Raman qu'en I. R.

Les résultats que nous possédons actuellement nous permettent cependant de proposer des attributions plausibles et surtout nous montrent la direction dans laquelle il faut diriger les recherches ultérieures.

La substitution des D aux H dans la molécule d'éthylène donne lieu, compte tenu de l'éthylène léger, à 7 molécules différentes.



Toutes ces molécules ont pu être préparées isolément par J.-C. Jungers à l'exception des 2 isomères *cis* et *trans* qui ont été obtenus mélangés l'un à l'autre.

On connaît le spectre I. R. de C_2H_4 et C_2D_4 gazeux (10) (19).

On connaît aussi le spectre Raman à l'état gazeux et liquéfié de C_2H_4; par contre, le spectre Raman de C_2D_4 n'est encore connu que pour ce corps à l'état liquide.

Enfin, on connaît le spectre Raman plus ou moins complet de chacun des autres éthylènes mais toujours à l'état de gaz liquéfié (24) (32).

Il faut remarquer que le spectre Raman, des corps à l'état liquide, diffère en général quelque peu de celui des corps à l'état gazeux. L'influence des molécules voisines peut, soit déplacer légèrement les raies, soit lever certaines interdictions, et donc faire apparaître des raies normalement interdites.

L'ensemble des données expérimentales a permis à Manneback (33) (52) (67) (74) et à ses collaborateurs d'établir une fonction

TABLEAU XI

	$H_2C=CH_2$ D_{2h}	$H_2C=CHD$ C_s	$HDC=CHD$ cis C_{2v}	$H_2C=CD_2$ C_{2v}	$HDC=CHD$ trans C_{2h}	$D_2C=CHD$ C_s	$D_2C=CD_2$ D_{2h}
Torsion	$\nu_4\ (a_u)$ (800) v ia [825]	$\nu_7\ (a'')$ (777) 730 R	$\nu_7\ (a_2)$ (450) -	$\nu_8\ (a_2)$ (495) -	$\nu_8\ (a_u)$ (627) -	$\nu_9\ (a'')$ (608) -	$\nu_4\ (a_u)$ (572) [580]
Gauche	$\nu_8\ (b_{2g})$ (943) ia 943 R	$\nu_{12}\ (a'')$ (884) 870 R	$\nu_{12}\ (b_2)$ (899) 864 R	$\nu_6\ (a_2)$ (734) -	$\nu_6\ (a_u)$ (836) -	$\nu_6\ (a'')$ (752) -	$\nu_8\ (b_{2g})$ (926) 755 R
Gauche	$\nu_7\ (b_{1u})$ (949) v 948,2 I	$\nu_6\ (a'')$ (949) 945 R	$\nu_6\ (a_2)$ (907) -	$\nu_{12}\ (b_1)$ (948) -	$\nu_8\ (b_g)$ (869) -	$\nu_{12}\ (a'')$ (873) -	$\nu_7\ (b_{1u})$ (781) 720 I
Déform. plane	$\nu_{10}\ (b_{2u})$ (949) 995 I	$\nu_5\ (a')$ (995) -	$\nu_5\ (a_1)$ (958) 963	$\nu_{11}\ (b_1)$ (941) -	$\nu_{12}\ (b_u)$ (934) -	$\nu_{11}\ (a'')$ (920) -	$\nu_{10}\ (b_{2u})$ (681) [712]
Déform. plane	$\nu_6\ (b_{1g})$ (950) 1050 R	$\nu_{11}\ (a'')$ (948) -	$\nu_{11}\ (b_1)$ (831) -	$\nu_5\ (b_1)$ (946) -	$\nu_5\ (a_g)$ (818) -	$\nu_5\ (a'')$ (811) -	$\nu_6\ (b_{1g})$ (930) 785 R
Déform. CH ou CD	$\nu_3\ (a_g)$ (1341) 1342,4 R$_{liq}$	$\nu_3\ (a')$ (1269) 1285 R	$\nu_3\ (a_1)$ (1222) -	$\nu_9\ (a_1)$ (1055) 1027 R	$\nu_3\ (a_g)$ (1219) 1215 R	$\nu_3\ (a')$ (1028) 1040 R	$\nu_3\ (a_g)$ (961) 981 R
Déform. CH ou CD	$\nu_{12}\ (b_{3u})$ (1444) 1443,5 I$_{liq}$	$\nu_9\ (a')$ (1393) 1396 R	$\nu_9\ (b_1)$ (1295) 1282 R	$\nu_3\ (a_1)$ (1361) 1379 R	$\nu_{10}\ (b_u)$ (1310) -	$\nu_9\ (a')$ (1256) 1285 R	$\nu_{12}\ (b_{3u})$ (1077) 1077,9 I$_{liq}$
Vibration C=C	$\nu_2\ (a_g)$ (1621) 1623,3 R	$\nu_2\ (a')$ (1600) 1600 R	$\nu_2\ (a_1)$ (1570) 1567 R	$\nu_4\ (a_1)$ (1582) 1579 R	$\nu_2\ (a_g)$ (1569) 1567 R	$\nu_2\ (a')$ (1548) 1546 R	$\nu_2\ (a_g)$ (1515) 1514 R
Valence CH ou CD	$\nu_{11}\ (b_{3u})$ (2988) 2989,5 I$_{liq}$	$\nu_{10}\ (a')$ (2272) 2265 R	$\nu_{10}\ (b_1)$ (2298) 2296 R	$\nu_4\ (a_1)$ (2252) 2220 R	$\nu_4\ (b_u)$ (2260) -	$\nu_4\ (a')$ (2227) 2213 R	$\nu_{11}\ (b_{3u})$ (2192) 2200,2 I$_{liq}$
Valence CH ou CD	$\nu_1\ (a_g)$ (3007) 3006 R liq 3013,3 R$_{sol}$	$\nu_4\ (a')$ (2986) 2964 R	$\nu_4\ (a_1)$ (2295) 2290 R	$\nu_{10}\ (b_1)$ (2322) 2326 R	$\nu_4\ (a_g)$ (2277) 2274 R	$\nu_1\ (a')$ (2294) 2271 R	$\nu_1\ (a_g)$ (2251) 2259 R
Valence CH ou CD	$\nu_9\ (b_{2u})$ (3107) 3105,5 I	$\nu_1\ (a')$ (3094) 3050 R	$\nu_1\ (a_1)$ (3056) 3047 R	$\nu_5\ (b_2)$ (3090) -	$\nu_9\ (b_u)$ (3053) -	$\nu_{10}\ (a'')$ (3045) 3037 R	$\nu_9\ (b_{2u})$ (2290) 2295 I
Valence CH ou CD	$\nu_5\ (b_{1g})$ (3092) 3075 R liq	$\nu_8\ (a')$ (3039) 3015 R	$\nu_8\ (b_1)$ (3033) -	$\nu_1\ (a_1)$ (3005) 3002 R	$\nu_1\ (a_g)$ (3038) 3035 R	$\nu_8\ (a')$ (2352) 2296 R	$\nu_5\ (b_{2g})$ (2304) 2307 R

Les valeurs entre parenthèses sont les valeurs théoriques, les autres, sont les valeurs expérimentales.

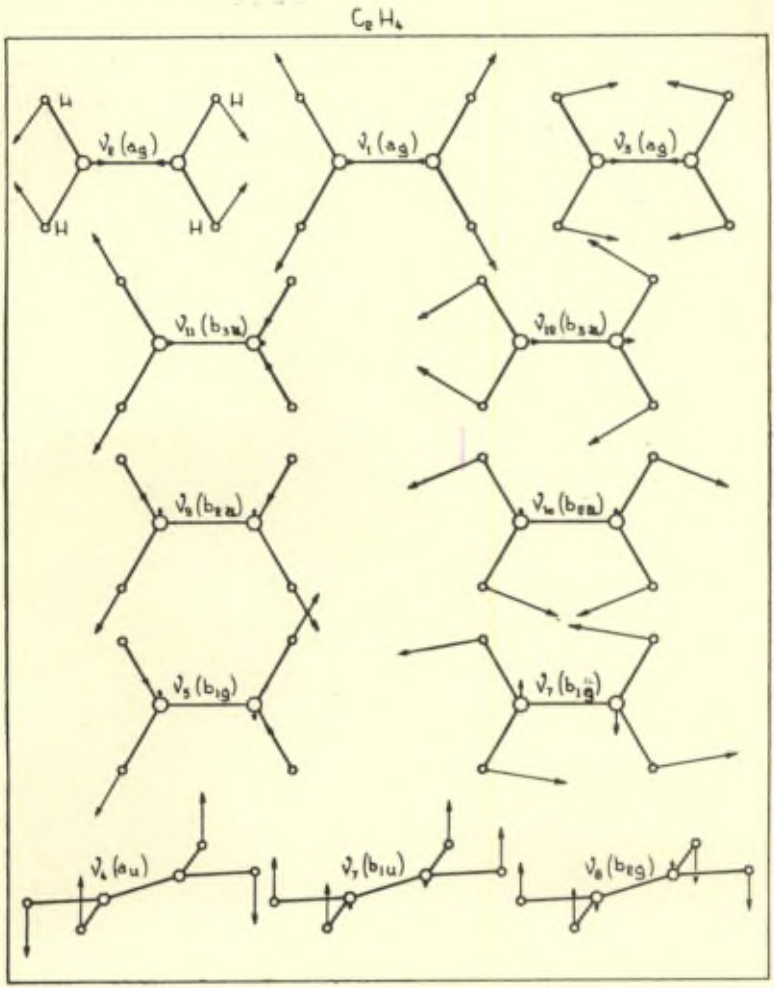


Fig. 23

potentielle capable de retrouver les fréquences expérimentales et de prévoir quelles devraient être les valeurs des fréquences gauches; Bernard (5) put en outre prédire l'ordre de grandeur des intensités des raies I. R.

Il est à remarquer que ces auteurs font usage dans leurs calculs de « masses spectroscopiques » qu'ils attribuent aux H et D en lieu et place des masses vraies; ceci est une façon commode d'introduire dans les calculs les corrections d'anharmonicité. L'utilisation des

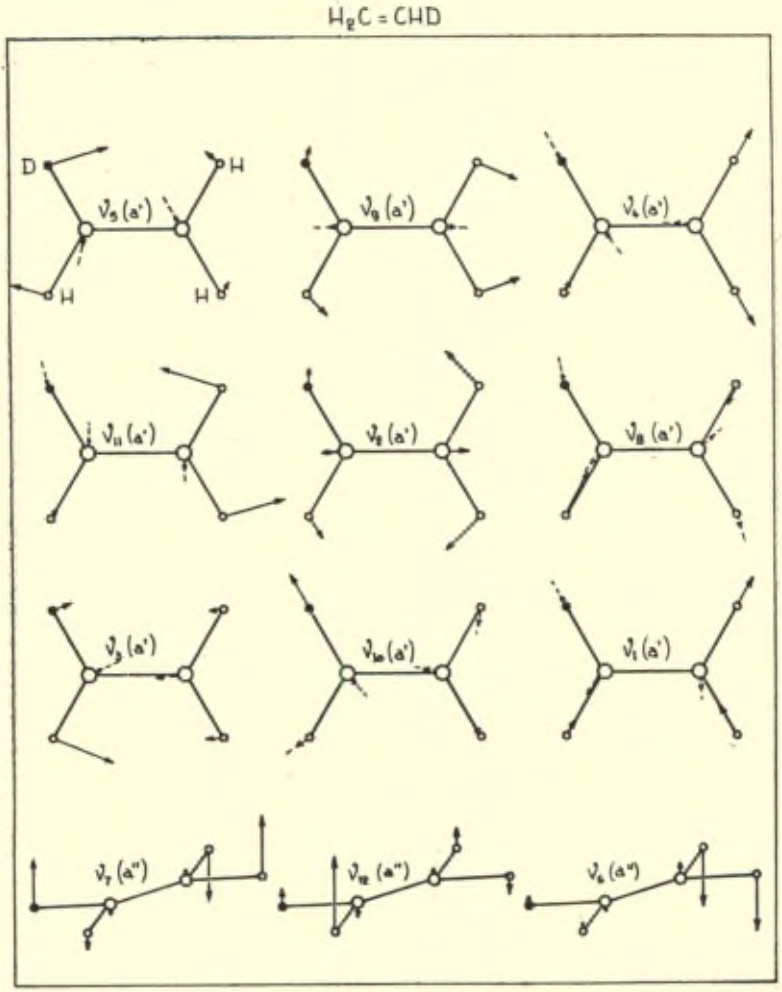


Fig. 24

masses vraies conduirait aux valeurs des fréquences harmoniques, légèrement différentes des fréquences expérimentales, qui elles seront entachées d'anharmonicité.

Les résultats théoriques et expérimentaux sont donnés dans le tableau XI. Les figures 23, 24, 25, 26, 27, empruntées aux travaux de Verleysen et Bernard, donnent les fréquences de vibration des différents types de molécules. Nous utilisons les notations de Herzberg.

Soulignons la concordance remarquable entre expérience et théorie

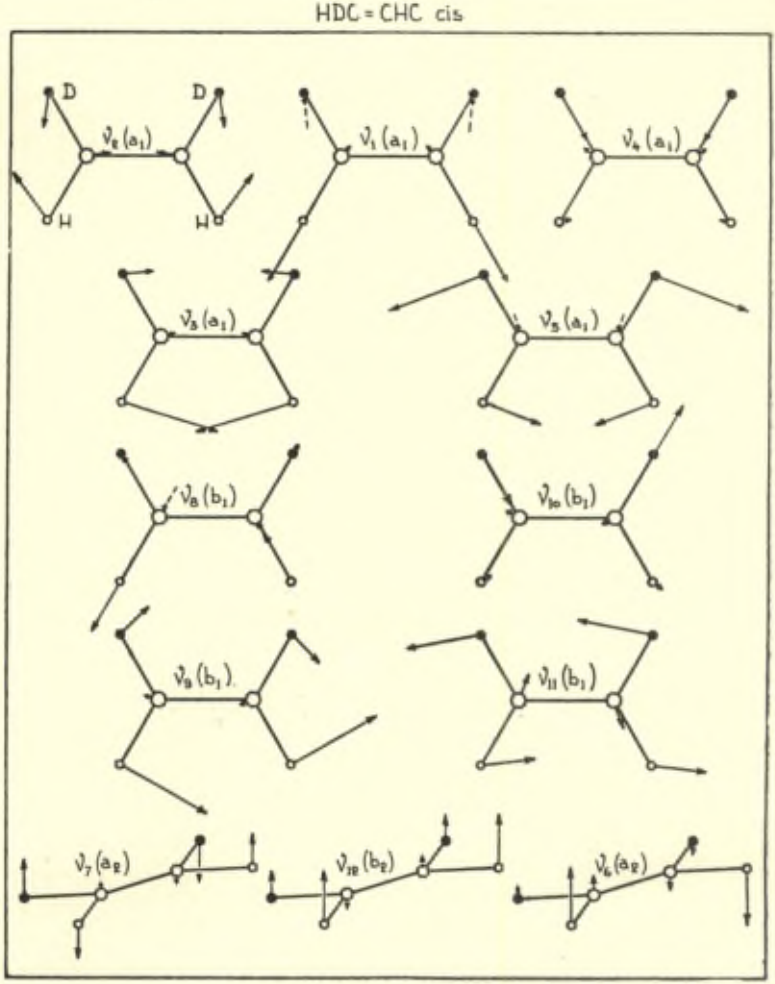


Fig. 25

en particulier pour les fréquences C-C, données dans la figure 28, qui sont particulièrement sensibles à une variation de la fonction potentielle.

L'interprétation diffère quelque peu de celle proposée par Herzberg; nous nous en expliquons par la suite. Le tableau que nous donnons diffère aussi de celui présenté précédemment par de Hemptinne et Manneback et ensuite par de Hemptinne et van Riet; le tableau actuel tient compte en effet des résultats obtenus en I. R., par

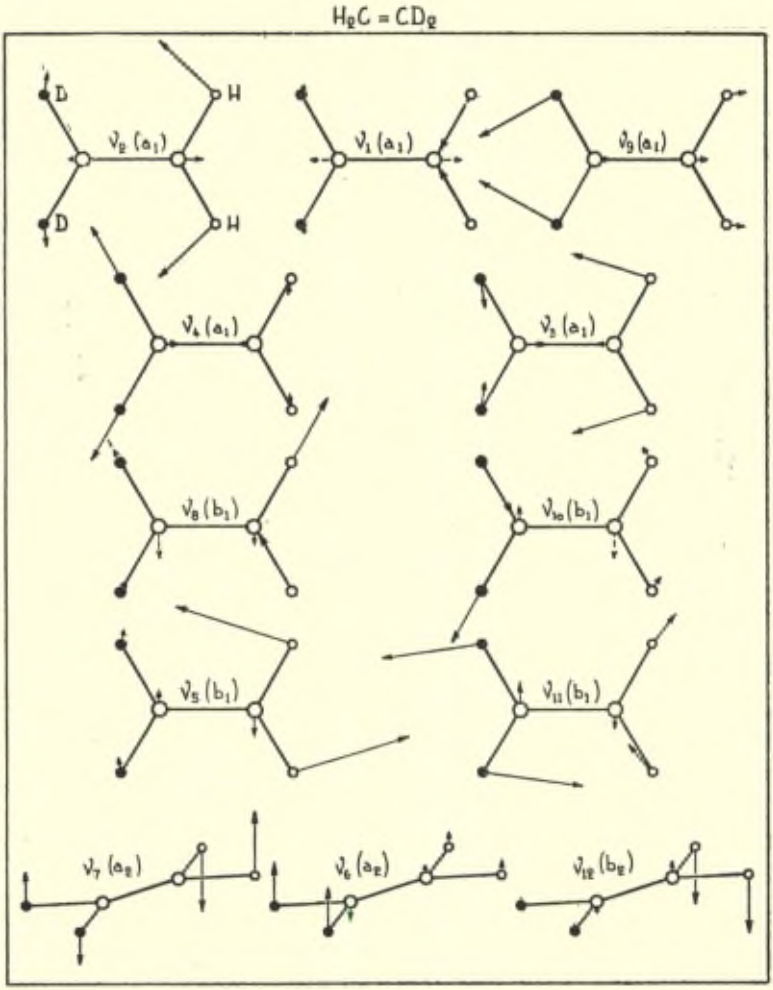


Fig. 26

Gallaway et Barker, qui n'étaient pas connus des auteurs précédents.

L'importance de l'étude des divers deutéro-éthylènes à l'état pur provient de ce que la symétrie, et partant les règles de sélection, varient lorsqu'un D est substitué à un H. Les molécules C_2H_4 et C_2D_4 présentent trois plans de symétrie perpendiculaires entre eux, ils sont du type D_{2h}. Elles présentent 9 fréquences propres planes dont 5 actives en Raman et 4 actives en I. R. et 3 fréquences gauches dont une active en Raman, une active en I. R. et une interdite dans les

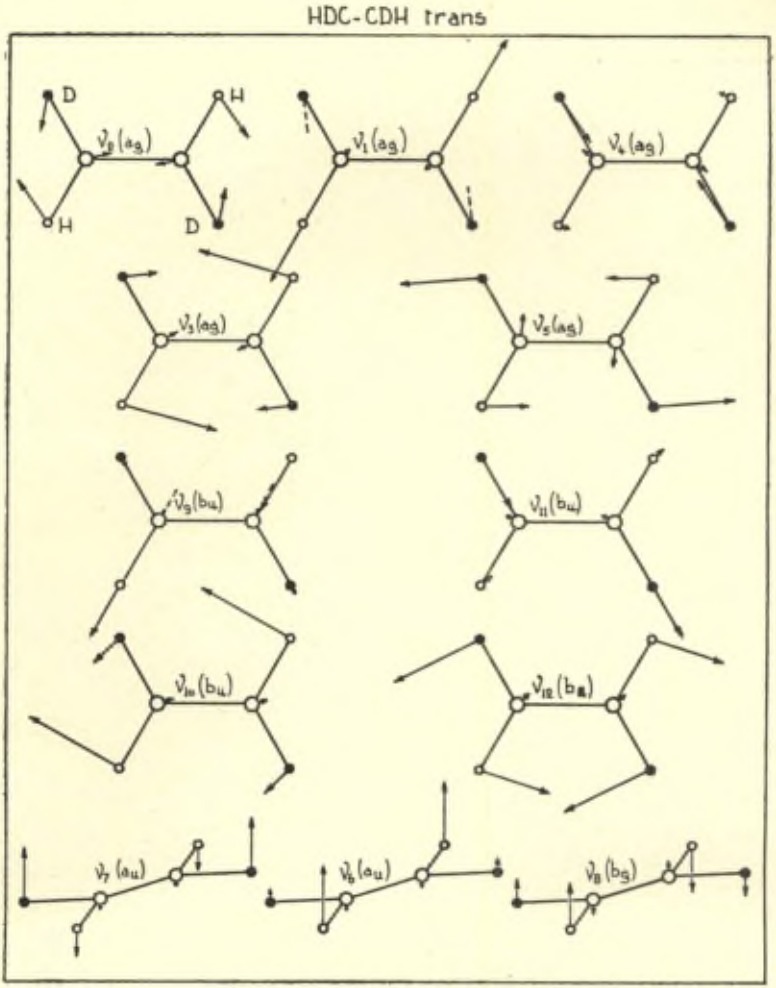


Fig. 27

2 spectres. Les molécules $H_2C = CD_2$ et HDC = CHD *cis*, au contraire, ne présentent plus que 2 plans de symétrie: le plan de la molécule et un plan perpendiculaire à celui-ci. Les règles de sélection seront dès lors modifiées : elles sont du type C_{2v}. La molécule HDC = CHD *trans* n'a plus qu'un plan et un centre de symétrie, elle est du type : C_{2h}.

Enfin le mono- et le trisubstitué n'ont plus que le plan de la molécule comme élément de symétrie, toutes les raies sont actives en Raman

et en I. R.; c'est ce qui constitue l'intérêt extraordinaire que présentent ces variétés; elles sont du type C_s.

L'ensemble des règles de sélection est donnée dans le tableau suivant :

TABLEAU XII

Groupe D_{2h}							
Type	C_2^z	σ_x	σ_y	C_z	i		
A_{1g}	s	s	s	s	s	p	ia
A_{1u}	s	as	as	s	as	v	ia
B_{1g}	s	as	s	as	s	dp	ia
B_{1u}	s	s	as	as	as	v	M_z
B_{2g}	as	as	as	as	s	dp	ia
B_{2u}	as	s	s	as	as	v	M_y
B_{3g}	as	s	as	s	s	dp	ia
B_{3u}	as	as	s	s	as	v	M_x

Groupe C_{2v}					
Type	C_2^z	σ_x	σ_y		
A_1	s	s	s	p	M_z
A_2	s	as	as	dp	ia
B_1	as	as	s	dp	M_x
B_2	as	s	as	dp	M_y

Groupe C_{2h}					
Type	C_2^z	σ_z	i		
A_g	s	s	s	p	ia
A_u	s	as	as	v	M_z
B_g	as	as	s	dp	ia
B_u	as	s	as	v	$M_\perp$

Groupe C_s			
Type	σ_z		
A'	s	p	$M_\perp$
A''	as	dp	M_z

L'application de la règle de Teller Redlich aux fréquences de C_2H_4 et C_2D_4 plaide en faveur de nos attributions, ainsi que le montre le tableau suivant qui donne, pour chaque groupe de fréquence d'un type particulier, la comparaison entre le rapport théorique et le rapport expérimental des produits des fréquences.

Nous ne croyons pas pouvoir suivre Herzberg dans sa suggestion d'attribuer la raie 3272, 3 cm^{-1} du C_2H_4 à la vibration $\nu_5^{CH}(b_{1g})$ car nous ne retrouvons aucune fréquence homologue dans aucun des autres dérivés deutérés. Nous ne pouvons malheureusement pas nous appuyer sur la valeur des rapports isotopiques pour déterminer cette fréquence car les fréquences B_{1g} de C_2H_4 et C_2D_4 ne sont pas connues avec certitude.

TABLEAU XIII

Type :	A_g	B_{1g}	B_{2u}	B_{3u}	A_u	B_{2g}	B_{1u}
R théorique	1,998	1,666	1,868	1,868	1,413	1,230	1,322
R expérimental .	1,959	—	(1,850)	1,818	1,422	1,201	1,318

Avec nos connaissances actuelles des spectres des éthylènes, il est extrêmement difficile de se prononcer sur l'attribution des basses fréquences, les 2 fréquences basses planes ayant, d'après les prévisions théoriques, des valeurs très voisines des fréquences gauches, d'où superpositions possibles. A défaut de plus amples informations, nous pouvons considérer les attributions adoptées par Herzberg comme tout à fait plausibles; elles sont confirmées dans une certaine mesure par les valeurs des rapports isotopiques, et par les mesures faites sur H_2C_2HD. Elles sont aussi confirmées par les valeurs obtenues pour les fréquences de torsion et les fréquences gauches des molécules $H_2C_2Br_2$ et $D_2C_2Br_2$, bien que les résultats donnés par ces dernières molécules semblent indiquer que les fréquences gauches de torsion et surtout les fréquences basses planes pourraient avoir des valeurs plus grandes que prévues.

Les valeurs proposées pour ν_6 sont déduites indirectement de la mesure de la fréquence de combinaison $\nu_6 + \nu_{10} = 2047$ et $\nu_6 + \nu_{10} = 1595$, respectivement pour C_2H_4 et C_2D_4.

Signalons un point noir au tableau : le spectre Raman du H_3C_2D présente une raie intense et nette de fréquence 1123 cm^{-1} et une raie intense de fréquence 1008 cm^{-1}; la première a son homologue dans le spectre Raman de D_3C_2H en 995; ces raies ne trouvent pas place dans le tableau proposé. Peut-être pourraient-elles être interprétées

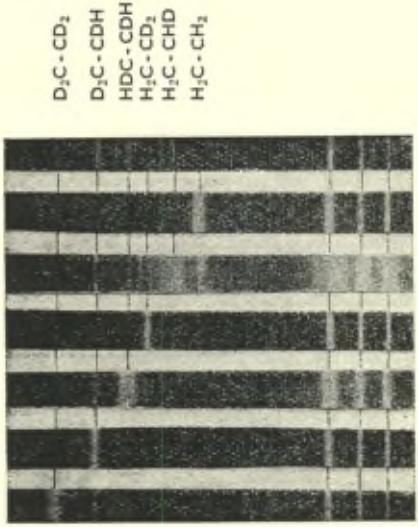


Fig. 28

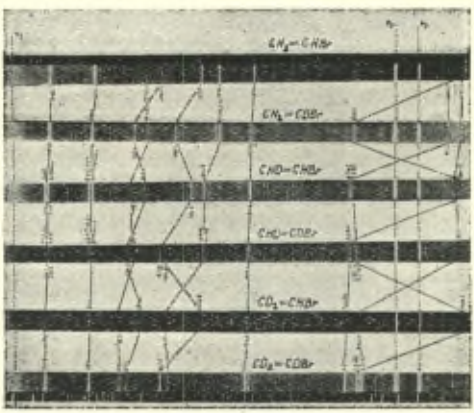


Fig. 29

comme fréquences ν_6; mieux vaut cependant attendre l'étude du spectre I. R. de ces molécules avant de se prononcer.

Les fréquences de combinaisons et les harmoniques donnent quelques indications supplémentaires. Nous avons déja parlé de $\nu_6 + \nu_{10}$; ajoutons que l'harmonique $2\nu_4$ de la fréquence de torsion apparaît pour C_2H_4 et C_2H_3D à 1654 et 1460. L'éthylène léger présente des bandes dans l'I. R. photographique; aucune étude n'a été faite dans cette région en ce qui concerne les dérivés deutérés: encore une lacune à combler.

L'éthylène présente un spectre d'absorption dans l'ultra violet lointain vers 1700 Å. Ce spectre est dû à la superposition de deux passages; un passage N... $(xx)_2 \rightarrow$ V... (xx) $(x\bar{x})$ et un passage N... $(xx)^2 \rightarrow$ R ... (xx) (r).

(Les notations employées sont celles adoptées par R. S. Mulliken) (*57bis*). L'éthylène normal N est une molécule plane, il en est de même de l'éthylène excité R; par contre les plans des 2 radicaux CH_2 sont perpendiculaires entre eux dans l'éthylène excité V. Les raies observées peuvent être classées suivant des séries de Rydberg.

$$(N \rightarrow R) \qquad \nu^h_{(C_2H_4)} = 84750 - \frac{R}{(n + 0{,}91)^2} \qquad n : 2, 3, 4.$$

W. C. Price et W. T. Tutte (64) ont pu mesurer les spectres d'absorption de C_2D_4 et C_2H_3D dans la région ultraviolette lointaine; ils trouvent pour C_2D_4 :

$$\nu^h_{(C_2D_4)} = 84850 - \frac{R}{(n + 0{,}92)^2} \qquad n : 2, 3, 4$$

Ils purent mettre en évidence les fréquences de vibration C = C et les fréquences de torsion ou plus exactement comme, le fait remarquer Mulliken (*57bis*), le double de cette fréquence pour l'état excité R de C_2H_4, C_2H_3D et C_2D_4 :

	C_2H_2	C_2H_3D	C_2D_4
C = C état N	1621	1600	1515
C = C état R	1375	1350	1290
C = C état V	8800	—	—
Torsion état R	470/2	415/2	290/2

On voit que la fréquence C = C dans l'état R est assez bien plus faible que dans l'état normal N et tombe encore davantage dans

l'état V. La fréquence de torsion de l'état R est, elle aussi, beaucoup plus basse que la fréquence de torsion supposée de l'état normal.

B. Dérivés halogénés de l'éthylène.

a) *Bromure de vinyle.* — Six deutéro-bromures de vinyle différents ont pu être préparés isolément; deux d'entre eux présentent une variété *cis* et une variété *trans*; ces deux variétés sont inséparables mais apparaissent sous forme de dédoublement des raies intenses.

Toutes ces molécules ne possèdent qu'un plan de symétrie, à savoir le plan de la molécule. Elles sont, tout comme la molécule H_2C_2HD, du type de symétrie C_s.

Toutes les raies sont donc, en principe, actives en Raman et en I. R. Les spectres Raman de ces deutéro-bromures de vinyle (25) sont reproduits ci-contre Fig. 29; la modification du spectre, consécutive à la substitution d'un D à un H, saute au yeux et permet d'identifier un grand nombre de fréquences presque à coup sûr. Ce travail se trouve encore facilité par le fait que les vibrations, dans lesquelles se trouve impliqué le Br correspondent généralement à des raies très intenses.

TABLEAU XIV

$H_2C=CHBr$	$D_2C=CDBr$	$H_2C=CDBr$	$D_2C=CHBr$	$HDC=CHBr$	$HDC=CDBr$	Attribution.
3103 (5)	2345 (7)	3101 (3)	2327 (5)	3057 (3)	3044 (3)	Valence CH ou CD
3017 (15)	2215 (15)	3010 (3)	2227 (15)	2247 (m) 2283 (7)	2283 (3) 2231 (7)	Valence CH ou CD
1370 (10)	1034 (2)	1349 (m)	1015 (2)	1263 (7)	1249 (10) 1258 (4)	Déformation CH ou CD
1586 (16)	1516 (12)	1597 (15)	1542 (15)	1564 (11)	1541 (15)	Vibration C=C
3095 (5)	2302 (10)	2301 (7)	3093 (3)	3075 (3)	2234 (3)	Valence CH ou CD
601 (10)	551 (15)	588 (15)	560 (10)	588 (10) 568 (10)	573 (10) 553 (m)	Valence CBr
1255 (10)	975 (12)	1087 (3)	1233 (7)	1135 (10)	978 (15) 1010 (10)	Déformation CH ou CD
348 (15)	306 (15)	345 (15)	309 (15)	355 (15) 379 (15)	332 (15) 317 (15)	Déformation CBr
905 (3)	740 (7)	908 (2)	725 (2)	808 (3)	805 (1)	-
1004 (3)	777 (5)	805 (2)	760 (2)	786 (3)	782 (2)	-
940						-
457						-

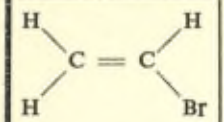
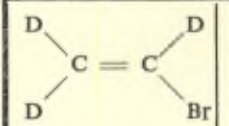
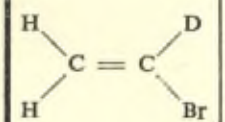
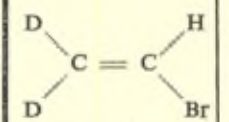
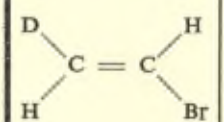
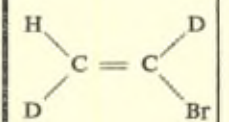
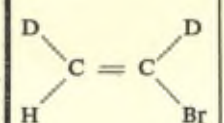
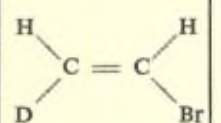
Le spectre Raman ne donne que 10 des 12 vibrations propres, une des vibrations manquantes a cependant été signalée par Kohlrausch dans le spectre Raman de C_2H_3Br; la deuxième vibration qui fait défaut a été trouvée en I. R. par Thompson (68). Ce dernier a pu identifier les vibrations, grâce au contour des bandes d'absorption.

L'identification faite par cette méthode corrobore les résultats obtenus grâce à la substitution.

Certaines conclusions générales se dégagent de l'ensemble des résultats obtenus. Les vibrations de déformation des molécules $H_2C = CHBr$ et $H_2C = CDBr$ sont très voisines, il en est de même pour les molécules $D_2C = CDBr$ et $D_2C = CHBr$. Ceci montre que l'atome d'hydrogène, ou de deutérium, du radical contenant Br, n'a qu'une influence minime sur cette vibration. Les fréquences où se trouvent impliqués les Br, ont des valeurs assez voisines pour des molécules $H_2C = CHBr$, $H_2C = CDBr$, $HDC = CHBr$ (trans) et $HDC = CDBr$ (cis) et d'autres valeurs, également voisines, pour les molécules $D_2C = CDBr$, $D_2C = CHBr$, $HDC = CDBr$ (trans) et $HDC = CHBr$ (cis).

Nous résumons ces faits dans le tableau suivant :

TABLEAU XV

	Def. CBr	val. CBr		Def. CBr	val. CBr
	348	601		306	551
	345	588		309	560
	336	588		317	559
	332	579		319	568

Il semble donc que ce soit l'hydrogène qui se trouve du même côté que le Br, par rapport à l'axe passant par les deux carbones, qui ait une influence sur le déplacement de la vibration.

La différence entre les fréquences des isomères *trans* et *cis* donne une mesure de la différence entre l'influence d'un atome H et d'un atome D. La différence entre les fréquences des molécules $H_2C = CHBr$ et $H_2C = CDBr$ de même que la différence entre les fréquences des isomères *trans* et *cis* de $HDC = CHBr$ et $HDC = CDBr$, donnent une mesure de la différence entre l'influence de l'hydrogène et du deutérium du radical contenant l'atome de brome. Toutes ces régularités justifient à posteriori l'attribution des fréquences à l'isomère *trans* et à l'isomère *cis*.

Le tableau montre aussi que, pour le premier groupe de molécules, la fréquence la plus basse de $H_2C = CHBr$ et de $H_2C = CDBr$ à savoir 348 et 345 est quasi la même. Il en est de même pour 336 et 332 basses fréquences des molécules $HDC = CHBr$ (trans) et $HDC = CDBr$ (cis). Par contre c'est la fréquence haute de $H_2C = CDBr$ et celle de $HDC = CHBr$ (trans) qui ont même valeur, à savoir 588. Les fréquences homologues du deuxième groupe de molécules présentent les mêmes régularités.

Les fréquences $C = C$ offrent également des régularités intéressantes. Les carrés des fréquences $C = C$ des molécules $H_2C = CHBr$, $HDC = CHBr$ et $D_2C = CHBr$ d'une part et $H_2C = CDBr$, $HDC = CDBr$ et $D_2C = CD\ Br$ d'autre part, varient très sensiblement de façon linéaire avec l'inverse de la masse réduite des 2 radicaux.

b) *Bromure de Vinylidène.* — Les 3 bromures $H_2C_2Br_2$, $C_2D_2Br_2$ et C_2HDBr_2 ont été préparés par J. Jungers et Verhulst (73), leurs spectres Raman ont été étudiés par de Hemptinne et C. Velghe (25). Les 2 premières molécules présentent 2 plans de symétrie, elles sont du type C_{2v}; la troisième est du type C_s, ne présentant plus qu'un plan de symétrie. Toutes les raies Raman sont actives, par contre la fréquence de torsion est inactive en I. R. pour les 2 premières molécules.

Le tableau XVI donne les résultats expérimentaux obtenus ainsi que les attributions des fréquences aux 12 modes de vibration des bromures de vinylidène. Les multiples sont dus à des résonances Fermi.

Les attributions sont confirmées entièrement par la théorie.

TABLEAU XVI

$H_2C = CBr_2$	$HDC = CBr_2$	$D_2C = CBr_2$	ATTRIBUTION
3108	3061	2339	Valence CH et CD
3023	2282	2230	» CH et CD
1379	1258	1019	Déform. CH et CD
1593	1563	1552 1536	Valence C-C
696	655	625	» CBr
467	458	553	» CBr
184	184	183	Déform. CBr
322	300	288	Vibration plane
668	545	478	Torsion
405	391	390	Vibration gauche
886	829	709	» »
1080 1049	949	932	» plane

Les fréquences de $H_2C = CBr_2$ et $D_2C : CBr_2$ peuvent être séparées en deux groupes.

5 modes π π' ou A_1
4 modes σ π' ou B_2 } fréquences planes.

1 mode σ σ' ou A_2
2 modes π σ' ou B_1 } fréquences gauches.

Les π' et σ' désignent des vibrations symétriques ou anti-symétriques par rapport au plan de la molécule et π et σ les vibrations symétriques ou antisymétriques par rapport au plan perpendiculaire au plan de la molécule.

Le rapport théorique des produits des fréquences des molécules isotopes homologues : $H_2C = CBr_2$ et $D_2C = CBr_2$ pour un groupe de symétrie déterminé peut être comparé au rapport des fréquences expérimentales.

Ceci donne comme à l'ordinaire un test très sensible pour l'attribution des raies aux différents modes de vibration.

Les résultats sont :

	Fréquences			Fréquences	
	Théoriques	Expérimentales		Théoriques	Expérimentales
A_1	1.987	1.972	A_2	1.410	1.397
B_2	1.957	1.923	B_1	1.302	1.297

La faible différence est due à l'anharmonicité. Un calcul semblable pour C_2HDBr_2 qui ne possède plus qu'un plan de symétrie et dont les fréquences ne peuvent plus se partager qu'en 2 groupes distincts, à savoir les fréquences planes d'une part et les fréquences gauches d'autre part, donne les résultats suivants :

	Calculs	Expérience
σ'	1.353	1.356
π'	1.964	1.961

Il est intéressant de remarquer qu'il est possible de déterminer la constante de torsion.

La fonction potentielle de torsion est :

$$2\ V = b\Phi^2 = D\ (S_{CC}\Phi)^2$$

où Φ est l'angle entre les plans CH_2 et CBr_2 et S_{CC} la distance C = C.

Le calcul donne :

$b = 0{,}45017.10^{11}$ erg, $D = 0{,}25837.10^5$ dynes/cm^2.

(Calculs effectués par C. Manneback et M^{lle} Jacmain.)

c) $C_2H_2\ Cl_2$ *cis et trans.* — La plupart des vibrations propres de ces molécules ont pu être identifiées grâce aux mesures de polarisation des raies Raman des molécules légères, de l'étude du profil des bandes I. R. de ces mêmes molécules et enfin grâce à la comparaison du spectre Raman de ces molécules avec le spectre Raman de leurs homologues deutérés. Les spectres Raman des $C_2D_2Cl_2$ *cis* et *trans* ont été mesurés par Trumpy (70) (71) et discutés ensuite par T. Y. Wu (82). Herzberg a repris la question et propose les attributions suivantes qui diffèrent quelque peu de celles de Trumpy et de Wu.

La molécule *trans* a la même symétrie que la molécule $H_2C_2D_2$ *trans*, la molécule *cis* présente la même symétrie que la molécule $C_2H_2D_2$ *cis*.

Les règles de sélection seront donc les mêmes et les figures de vibrations peuvent être représentées schématiquement par celles du tableau où les D seraient remplacés par des Cl. Il va de soi que les amplitudes des vibrations et les angles de déplacement seront différents.

Herzberg croit que la vibration de torsion ν_7 (a_u) inactive en Raman a échappé aux investigations en I. R. parce que sa valeur serait inférieure de 500; elle se trouverait donc dans une région qui n'a pas encore été étudiée. Il émet un doute sur l'attribution de 758 à la fréquence ν_8 (bg), car cette raie devrait être dépolarisée, alors qu'elle est polarisée; Wu avait écarté cette objection pour des raisons expérimentales.

TABLEAU XVII

	$C_2H_2Cl_2$ trans.		$C_2D_2Cl_2$ trans.
	Infra Rouge	Raman	Raman
ν_5 (a_g)	—	349 p.	346
ν_6 (a_u)	620	—	—
ν_8 (b_g)	—	758 p.	657
ν_{12}(b_u)	820	—	—
ν_4 (a_g)	—	844 p.	765
ν_{11}(b_u)	917	—	—
ν_{12} (b_u)	1200	—	—
ν_3 (a_g)	—	1270	992
ν_2 (a_g)	—	1576	1570
ν_1 (a_g)	—	3071	2325
ν_g (b_u)	3089	—	—
		3140	3150
ν_7 (a_u)	<500	—	—

La comparaison des spectres de la molécule légère et de la molécule lourde montre qu'il faut écarter l'hypothèse d'une attribution de 3160 à ν_8 (b_1), mais qu'il faut bien attribuer cette raie à $2\nu_2$, comme le proposent d'ailleurs Wu et Herzberg. La fréquence 406 semble bien basse pour être la fréquence de torsion; quoiqu'il en soit, l'attribution, proposée par Herzberg, paraît la plus plausible pour le moment.

Il serait très utile de pouvoir comparer les spectres de chlorures légers et lourds avec ceux des bromures légers et lourds.

Malheureusement ceux-ci ne sont pas encore connus.

TABLEAU XVIII

	$H_2C_2C_2$1cis		$D_2C_2Cl_2$cis
	Infra Rouge	Raman	Raman
ν_5 (a_1)	—	173 p.	171
ν_1 (a_2)	—	406 dép.	368
ν_{12} (b_1)	570	563 dép.	515
ν_{11} (b_1).....	694	—	—
ν_4^{cu} (a_1)	—	711 p.	689
$\nu_{1_o}^{cu}$ (b_1)	857	—	—
ν_6 (a_2)	—	876 dép.	—
ν_3 (a_1)	—	1179 p.	850
ν_9 (b_1)	1303	—	—
ν_2^{u} (a_1)	1591	1587 p.	1575
ν_1^{CH} (a_1).....	3086	3077 p.	2325
ν_8^{CH} (b_1) ou $2\nu_2$	—	3160 dép.	3150

V.

ÉTHANE ET DÉRIVÉS DE L'ÉTHANE

A. Éthane.

L'étude des spectres des dérivés substitués de l'éthane nous éclaire sur les vibrations caractéristiques du radical méthyle.

Les travaux de Kemp et Pitzer, Kistiakowsky, Lacher, Stilt et Wilson, sur l'entropie et la capacité calorifique de l'éthane, ont définitivement établi que, à la température normale, les groupes méthyle n'étaient pas en rotation libre l'un par rapport à l'autre autour de l'axe C-C. Le groupe méthyle aurait une barrière de potentiel de 3.000 cal. à franchir pour passer d'une position à une autre.

Cependant diverses positions réciproques sont possibles à priori. La position en éclipse conduirait à une symétrie du type D_3h.

Une position d'équilibre intermédiaire (position en zig-zag ou « staggered ») conduirait à une symétrie du type D_3d.

Les règles de sélection des raies Raman sont légèrement différentes pour ces deux types; le premier type admet 3 raies Raman dépolarisées de plus que le deuxième; les lois d'activité des raies Infra Rouge sont les mêmes dans les deux cas. Si donc, en principe, l'analyse des spectres de vibration doit pouvoir nous apprendre quelle est la symétrie de la molécule, il s'avère, qu'en fait, les résultats expérimentaux s'expliquent aussi bien en admettant l'une ou l'autre des configurations possibles.

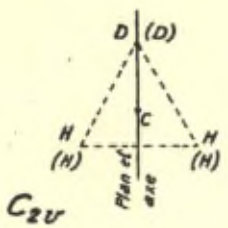
Herzberg, faisant la synthèse des travaux effectués sur C_2H_6 et C_2D_6, donne les attributions possibles pour les deux alternatives. Les raies de combinaison peuvent, elles aussi, s'expliquer également bien dans les deux hypothèses.

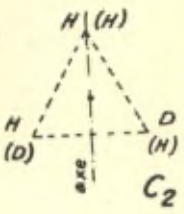
Langseth (48), à propos d'une étude consacrée à la rotation libre des molécules, donne pour l'éthane des arguments théoriques en faveur de la forme éclipse.

La stabilité d'une position serait, d'après lui, conditionnée par deux facteurs : le degré de superposition des fonctions d'ondes de la liaison carbone d'une part, qui agit en faveur de la position éclipse, et d'autre part l'action des dipoles ainsi que les empêchements stériques, qui établissent la position intermédiaire. Dans le cas de l'éthane le premier facteur serait prédominant et donc la forme éclipse serait la forme stable.

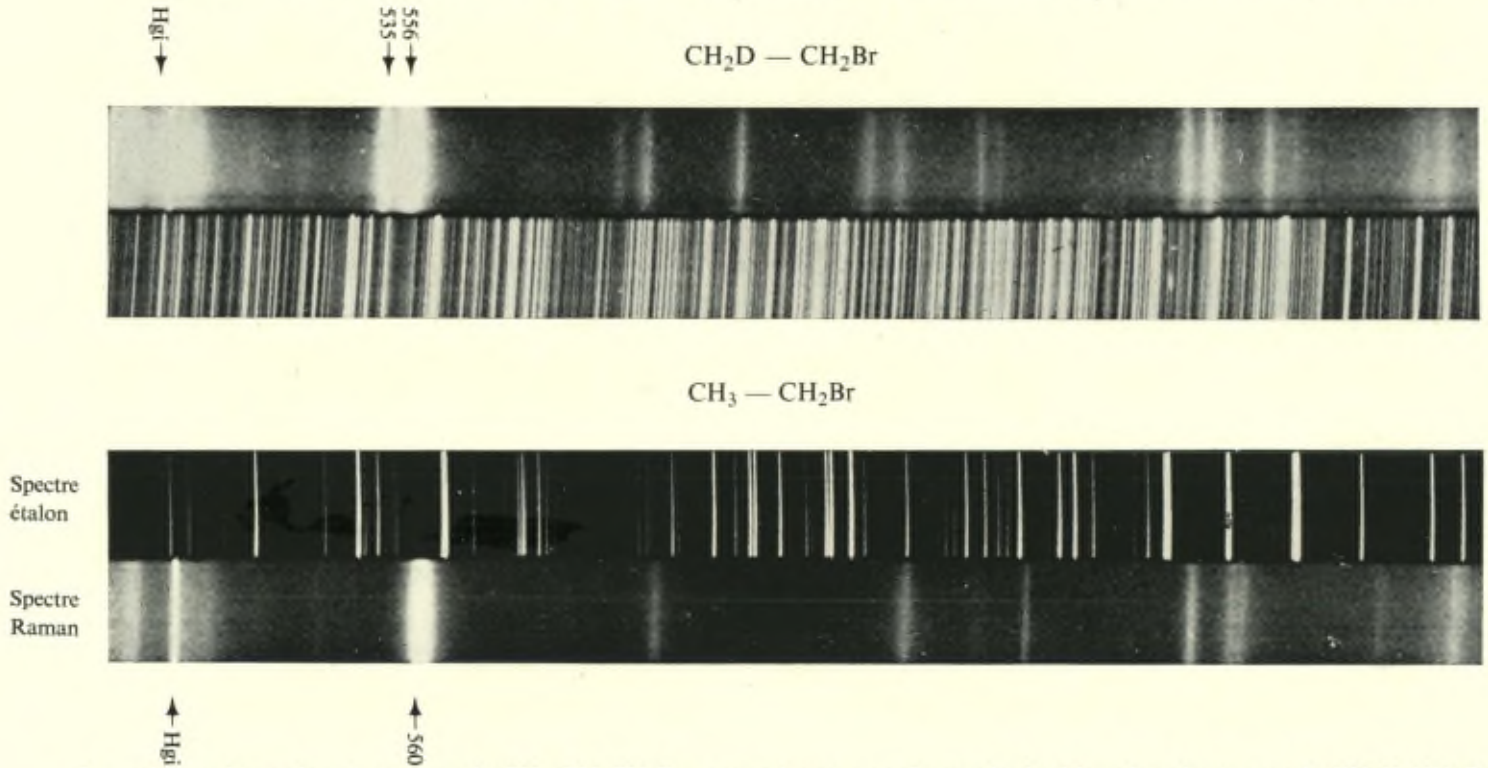
L'expérience n'a pas encore pu trancher le différent.

Une étude approfondie des molécules $H_2DC\text{-}CH_2D$ ou $D_2HC\text{-}CD_2H$ devrait pouvoir trancher en faveur de l'une ou l'autre configuration. En effet, si la forme éclipse est la forme à adopter, l'éthane disubstitué lourd symétrique peut présenter deux isomères de symétrie C_2v et C_2 dont l'un C_2 peut être obtenu de deux manières différentes et doit donc être, toutes autres choses égales d'ailleurs, deux fois plus fréquent que l'isomère de symétrie C_2v.





BASSES FRÉQUENCES DES SPECTRES DES BROMURES D'ÉTHYLE LÉGER ET MONO-LOURD



Les raies, uniques dans le spectre de CH_3-CH-CH_2Br, apparaissent sous forme de doublets dans le spectre de CH_2D-CH_2Br

Fig. 30

FREQUENCES C ⟷ Br DANS LE SPECTRE RAMAN DES BROMURES D'ETHYLE

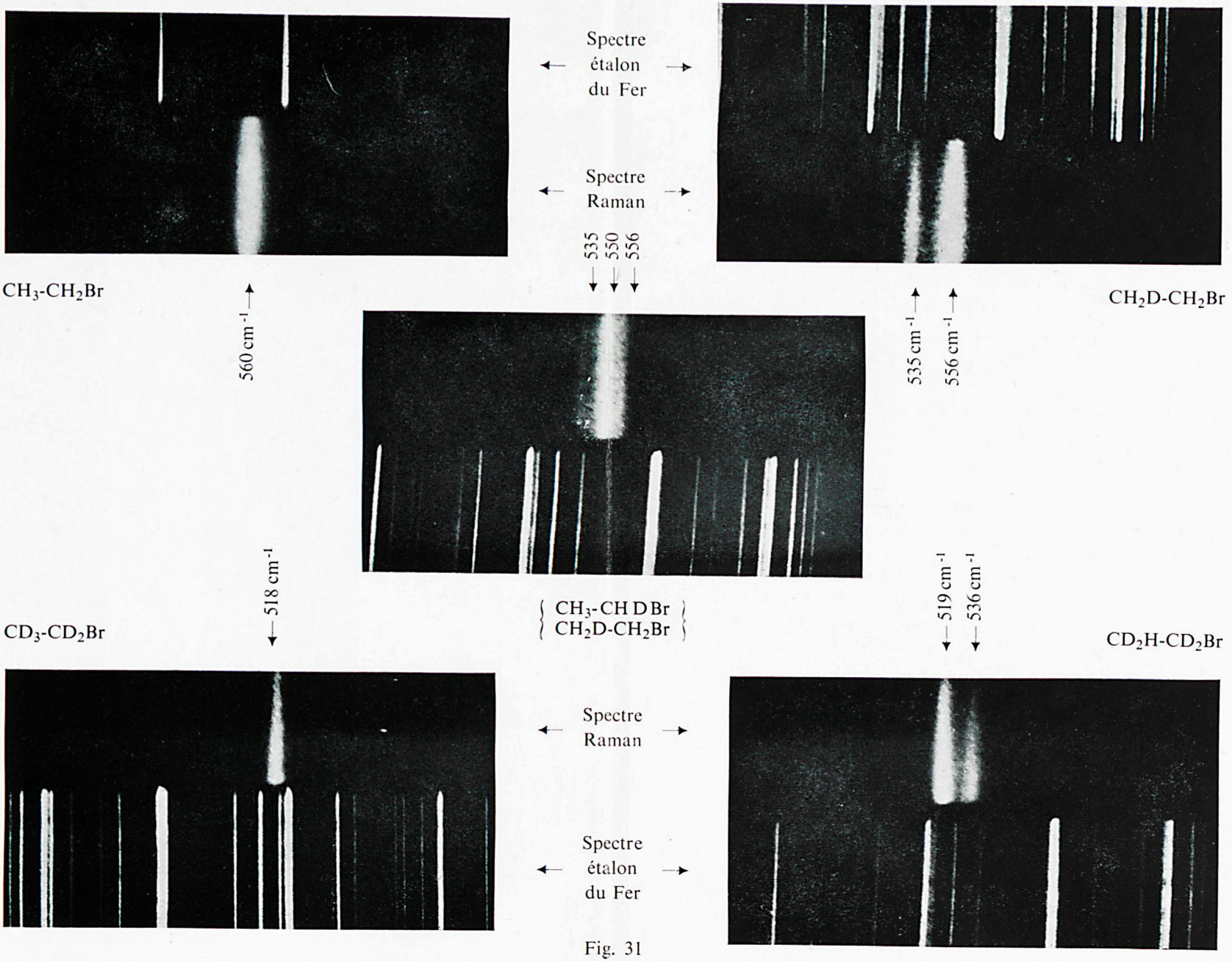
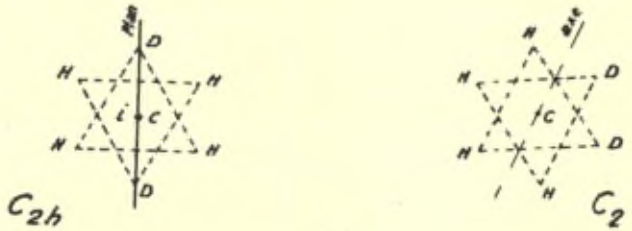


Fig. 31

Si au contraire il faut donner la préférence à la forme intermédiaire :



on aura encore deux isomères différents, mais la symétrie des deux formes sera respectivement C_{2h} et C_2. Les règles de sélection attachées à la symétrie C_{2h} et C_{2v} ont été données plus haut. La règle attachée à la symétrie C_2 est :

Groupe C_2		
Type	C_2	Activité
A	s	p M_z
B	as	dp $M_\perp$

Il faudra donc s'attendre à un beaucoup plus grand nombre de raies dans le spectre Raman et I. R. d'une molécule en éclipse, que d'une molécule en position intermédiaire en étoile.

B. Dérivés halogénés de l'éthane.

a) *Monobromure d'éthyle (bromure d'éthyle).* — L'étude du spectre Raman du monobromure d'éthyle et de ses dérivés deutérés montre de façon particulièrement spectaculaire l'existence d'isomères de position pour cette molécule. En effet alors que les spectres des $CH_3\ CH_2\ Br$, $CD_3\ CD_2\ Br$ et $CH_3\ CHD\ Br$ sont particulièrement simples, les spectres des $CH_2DCH_2\ Br$ et CD_2HCD_2Br présentent un nombre considérable de raies (fig. 30). Ces deux dernières molécules peuvent, en effet, présenter 3 isomères, dont 2 spectroscopiquement identiques, alors que les trois premières ne peuvent présenter qu'une seule variété du point de vue spectroscopique.

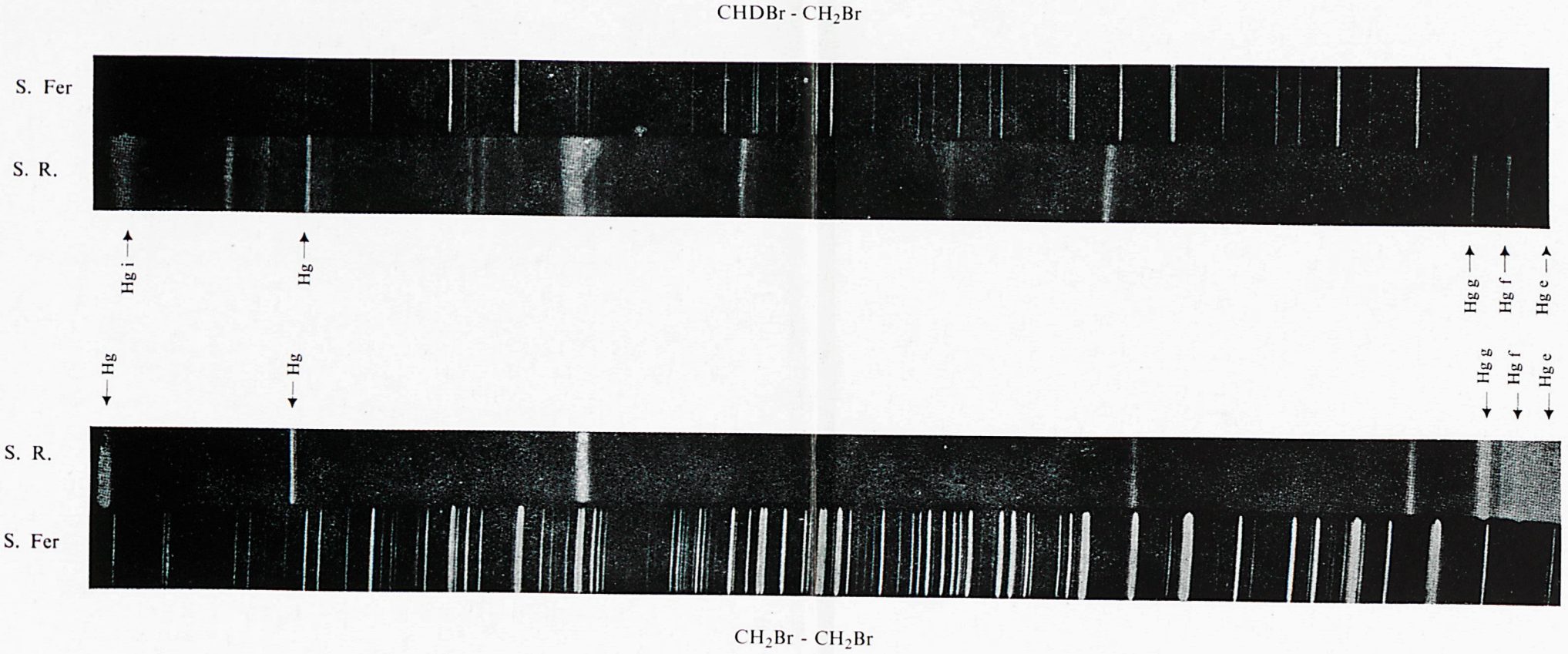
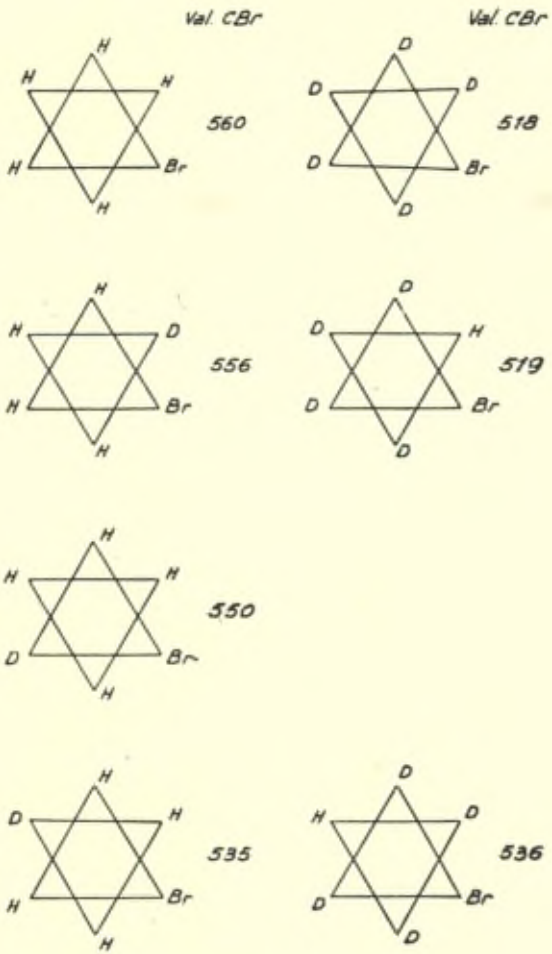


Fig. 32

Nombre de raies supplémentaires apparaissant dans le spectre du bibromure mono-substitué.

b) *Bibromures d'éthylène.* — L'étude du spectre Raman du $C_2H_4\,Br_2$, faite par Kohlrausch, et celle de la même molécule et de son homologue deutéré $C_2D_4Br_2$, faite par Morina et Mizushima (53) (54), conduisent à admettre pour ces molécules la forme *trans*



comme forme la plus stable; c'est en effet la forme *trans* qui subsiste à l'état solide.

La complexité des spectres à température ordinaire, de même que l'analyse des spectres de composés partiellement deutérés (fig. 32), démontrent l'existence d'une deuxième forme. Que la forme la

moins stable soit *cis*, en éclipse ou en étoile, peut encore prêter à discussion (fig. 33).

Suivant Langseth l'action des moments électriques et l'empêchement stérique exerceraient une action prépondérante en ces molécules; ce qui aurait pour effet de faire de la forme *trans* la forme la plus stable, au contraire de ce qui a lieu pour les tétra halogénés.

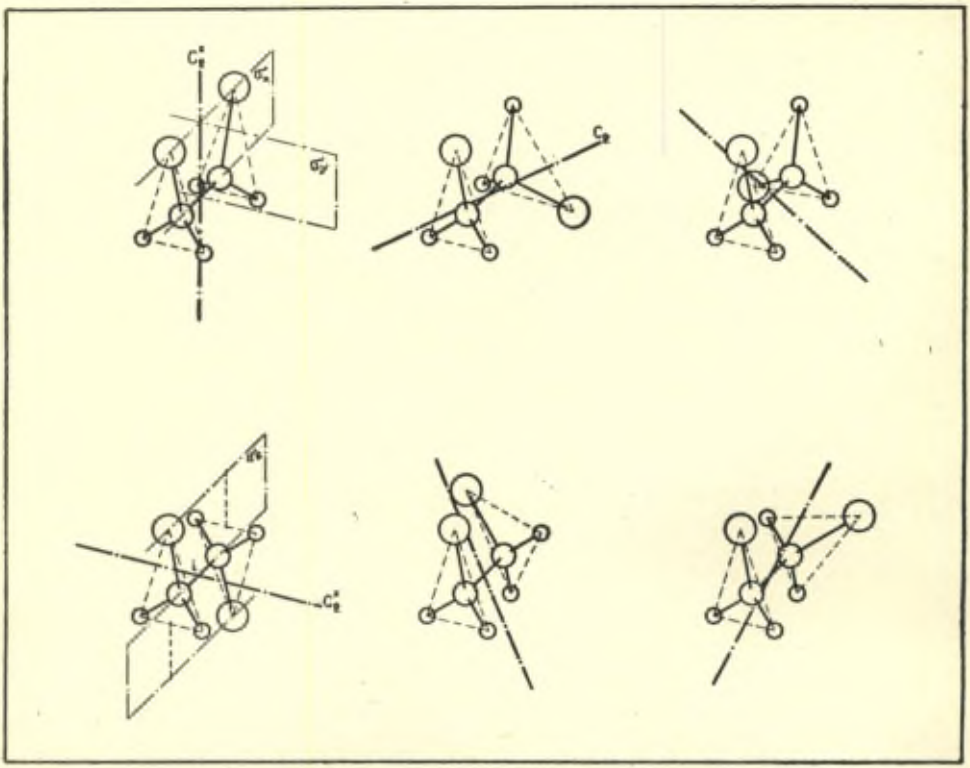


Fig. 33

L'étude approfondie, tant en Raman qu'en I. R., de la famille complète des bibromures deutérosubstitués, pourrait déterminer la symétrie de l'isomère coexistant à température ordinaire avec l'isomère *trans*.

c) *Tétrachlorure d'éthylène.* — Langseth et Bernstein (46) ont étudié le spectre Raman de $C_2H_2Cl_4$ et $C_2D_2Cl_4$ et ont examiné en parti-

culier comment variaient les spectres de ces corps sous l'influence d'une élévation de température.

Le présence de deux fois 5 raies fondamentales de basses fréquences les a amenés à la conclusion que ces molécules existent sous deux formes isomères: une forme de symétrie C_{2v} et une forme de symétrie C_2.

$C_2H_2Cl_4$		$C_2D_2Cl_4$	
Isomère le plus stable	Isomère moins stable	Isomère le plus stable	Isomère moins stable
88,2		88,2	
	88,2		88,2
173,0		172,6	
	183.6		180,4
241,7		239,8	
	226,0		226,2
288,8		287,1	
	294,6		295,1
352,9		350,2	
	366,7		362,1
546,4		531,2	
	546,4		531,1
648,1		628,0	
	765,1		703,1
801,6		741,0	
	812,1		758,1

La forme *cis* (C_{2v}) serait la plus stable, la forme intermédiaire moins stable et la forme *trans* correspondant au maximum de la barrière de potentiel s'opposant à la rotation libre, serait instable. La courbe potentielle en fonction de l'angle de rotation des pyramides aurait donc l'allure suivante (fig. 34) :

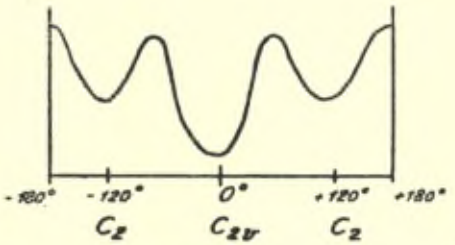


Fig. 34

VI.

ALDÉHYDE ET ACIDE FORMIQUE ET ACÉTIQUE, ACÉTONE

a) Le spectre I. R. de l'aldéhyde formique léger et lourd a été étudié par E.-S. Ebers et H.-N. Nielsen (17). Ces auteurs ont proposé, pour cette molécule, une fonction potentielle qui permet de retrouver théoriquement les fréquences propres observées. Le tableau suivant résume les résultats obtenus :

TABLEAU XX

	H_2CO	D_2CO
Valence symétrique : CH	(2780)	(2138)
CD	2780	2055,8
Valence antysymétrique CH ou CD. . . .	(2825)	(2118)
	2875	2159,7
Valence C	(1840)	(1625)
	1750	1700
Déformation symétrique CH ou CD . . .	(1470)	(1115)
	1503	1105,7
Déformation O-(CH_2) ou O-(CD_2) plane. .	(1267)	(997)
	1278	990
Déformation gauche.	—	—
	1165	938

Les chiffres entre parenthèses sont les valeurs calculées; les autres, les valeurs observées.

Bien que les résultats obtenus paraissent satisfaisants, Herzberg fait remarquer que le spectre v. V. de H_2CO semble indiquer d'autres valeurs pour les deux basses fréquences fondamentales.

b) J.-C. Morris (57) a publié en 1943 une discussion des spectres Raman et I. R. de l'adéhyde acétique entièrement léger et entière-

ment lourd. Il parvient à déterminer un certain nombre de fréquences fondamentales et détermine la hauteur de la barrière de potentiel s'opposant à la rotation libre. Elle serait de 2.000 cal.

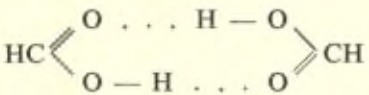
TABLEAU XXI

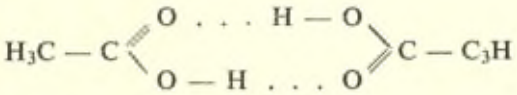
		CH_3COH	CD_3COH
CH_3	valence symétrique	2710	2033
CH_3	déformation symétrique	1370	1042
C C	valence	917	860
C = C	valence	1740	1730
C—H	wagging	1121	944
CH_3	valence asymétrique	2915	2128
C-H	valence	2788	2235
CH_3	déformation asymétrique	1414	1155
CH_3	rocking	890	755
C—C—O	déformation	426	419
CH_3	valence asymétrique	2964	2089
CH_3	déformation asymétrique	1445	1130
CH_3	rocking	760	570
C—H	rocking	515	479
CH_3	torsion	?	?

c) L'étude du spectre Raman et I. R. des acides formique et acétique est rendue difficile par suite de l'existence de polymères.

Herman et Hofstadter (34, 35, 36, 37) ont pu identifier les spectres des monomères et des dimères des composés légers et lourds en observant les variations d'intensité des raies du mélange en fonction de la température.

La structure proposée des dimères serait :





Les raies de valence OH et OD des groupes O------H — C et O------D — O se placent respectivement à 3080 et 2347 pour l'acide formique et 3125 et 2299 pour l'acide acétique.

d) Acétone. — L'acétone présente un spectre très fourni car la molécule n'est pas des plus simple. Cependant on peut obtenir des résultats intéressants en considérant séparément les deux radicaux méthyle et en traitant la molécule comme une molécule en Y du type $(Met)^2 C = O$. On connaît les spectres Raman et I. R. de l'acétone léger et totalement lourd mais ces résultats ne permettent pas à eux seuls de donner une attribution exempte de critique.

Les récents résultats (50) obtenus dans l'U. V. lointain pour la molécule légère et lourde (région s'étendant de 40.000 à 60.000 cm^{-1}), permettent, semble-t-il, de résoudre la question de manière satisfaisante, grâce à l'analogie qui existe entre les deux spectres.

La figure 35 donne les passages observés.

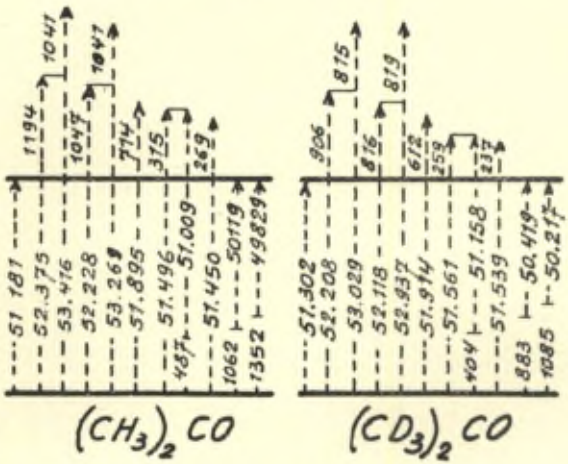


fig. 35

Le tableau donne les attributions proposées pour les fréquences fondamentales, données précédemment par Engler (19).

	$(CH_3)_2$ CO	$(CD_3)_2$ CO
Valence CO	1710	1710
» C (CH_3 ou CD_3)	1066	895
Déformation C (CH_3 ou CD_3)	787	700
» plan CO	530	483
» plan CO	488	413
» gauche CO	391	335
» H ou D	1357	1087

CONCLUSION

Les quelques exemples, donnés plus haut, montrent toute l'utilité de l'étude parallèle des spectres des dérivés substitués deutérés.

Nous avons vu que la substitution du deutérium à l'hydrogène permet, non seulement de résoudre des problèmes d'identification de fréquences, mais permet aussi d'aborder des problèmes de structures.

Le fait que la masse de D est double de celle de l'H, a pour conséquence que les spectres des molécules substituées diffèrent notablement entre eux, ce qui est un cas particulièrement favorable. Cependant, la substitution d'autres atomes de la molécule par leurs isotopes et en particulier du C^{12} par le C^{13}, tout en ne donnant pas lieu à de grands écarts, est susceptible, pour les mêmes raisons que celles développées dans l'introduction, d'apporter aussi nombre de renseignements précieux. Les progrès, faits dans les méthodes de concentration des isotopes stables, ouvrent la voie à de tels travaux. La spectroscopie I. R. est particulièrement favorable pour ce genre de recherches, car elle n'exige généralement que des quantités minimes de substances à mettre en action. Les méthodes de Clusius et de Hertz (pompes à vapeur de Hg) se prêtent particulièrement bien à l'obtention en laboratoire des isotopes en question.

Juin 1947.

Université de Louvain.

LITTÉRATURE

1. G.-M. Alony et H.-A. Schulz, *Phys. Rev.* **51,** 62 (1937) (CD_4).
2. E.-F. Barker et G. Bosschieter, *J. Chem. Phys.* **6,** 563 (1938), (CH_3OD, CH_2DOD).
3. E. Bartholome et H. Sachsse, *Z. Phys. Chem.* (B) **30,** 40 (1935) (CH_3OD).
4. W.-S. Benedict, K. Morikawa, R.-B. Barnes et H.-S. Taylor, *J. Chem. Phys.* **5,** 1 (1937) (CD_4).
5. E. Bernard et C. Manneback, *Ann. Soc. Sci. Brux.* **159,** 113 (1939).
6. G. Bosschieter, *J. Chem. Phys.* **5,** 992 (1937) (CD_3OD).
7. C.-A. Bradley et A. McKellar, *Phys. Rev.* **46,** 236 (1934) (CD_2HD I. R. phot.).
8. C.-A. Bradley et A. McKellar, *Phys. Rev.* **47,** 914 (1935) (C_2D_2 I. R. phot.).
9. W.-H.-J. Childs et H.-A. Jahn, *Proc. Roy. Soc.* (London) A **169,** 428 (1939) (CH_3D).
10. G.-K.-T. Conn et G.-B.-B. Sutherland, *Proc. Roy. Soc.* (London) A **172,** 172 (1939).
11. C. Courtoy, *Ann. Soc. Sci. Brux.* **60,** 122 (1946); **61,** 139 (1947).
12. A. Dadieu et W. Engler, *Naturwissenschaften* **23,** 335 (1935) (CD_4).
13. J.-M. Delfosse, *Ann. Soc. Sc. Brux.* **55,** 114 (1935).
14. D.-M. Dennison, *Rev. Mod. Phys.* **12,** 175 (1940).
15. D.-M. Dennison et M. Johnston, *Phys. Rev.* **47,** 93 (1935) (CD_4).
16. Th. Doehaerd, *Bull. Acad. Sc. Belg.* **30,** 430 (1944).
17. E.-S. Ebers et H.-H. Nielsen, *J. Chem. Phys.* **6,** 311 (1938).
18. W.-F. Edgell, *J. Chem. Phy.* **13,** 539 (1945).
19. W. Engler, *Z. Phys. Chem.* (B) **35,** 433 (1937) (CD_3COH).

19b. W.-S. Gallaway et E.-F. Barker, *J. Chem. Phys.* **10,** 88 (1942) (C_2D_4).

20. G. Glockler et C.-E. Morell, *Phys. Rev.* **4,** 569 (1935) (C_2D_2).
21. G. Glockler et C.-E. Morell, *J. Chem. Phys.* **4,** 15 (1936) (C_2D_2).
22. G. Glockler et H.-M. Darri, *Phys. Rev.* **46,** 535 (1934).
23. J.-D. Halford, L.-C. Anderson et G.-H. Kessin, *J. Chem. Phys.* **5,** 927 (1937) (CH_2DOD).

23*bis*. F. Halverson, *Rev. Mod. Phys.* **19,** 87 (1947).

24. M. de Hemptinne et R. Van Riet, *Bull. Acad. Sc. Belg.* **29,** 79 (1943) ($C_2H_xD_y$).
25. M. de Hemptinne et C. Velghe, *Bull. Acad. Sc. Belg.* **30,** 75 (1944) (C_2D_3Br).
26. M. de Hemptinne, C. Velghe et R. Van Riet *Bull. Acad. Sc. Belg.* **30,** 40 (1944) ($C_2D_2Br_2$).
27. M. de Hemptinne et Th. Doehaerd, *Bull. Acad. Sc. Belg.*, **29,** 447 (1943) (CD_3Cl, CD_3Br).

28. M. de Hemptinne et Th. Doehaerd *Bull. Acad. Sc. Belg.* **31,** 497 (1944) (CH_2D, CD_2H).
29. M. de Hemptinne et Th. Doehaerd **30,** *Bull. Acad. Sc. Belg.* 189 (1944) (CD_3OD).
30. M. de Hemptinne, *Trans Farad. Soc.* XLII, 5 (1946).
31. M. de Hemptinne et C. Velghe, *Physica*, V, 958 (1938) ($C_2D_4Br_2$) (C_2D_5Br).
32. M. de Hemptinne, J. Jungers, J.-M. Delfosse, *J. Chem. Phys.* **6,** 319 (1938) $C_2H_xD_y$. $x + y = 4$).
33. M. de Hemptinne et C. Manneback, *Proc. Ind. Acad. Sc.*, IX, 286 (1939).
34. R.-C. Herman et V. Williams, *J. Chem. Phys.* **8,** 447 (1940).
35. R.-C. Herman et V. Williams, *J. Chem. Phys.* **8,** 252 (1940).
36. R.-C. Herman et R. Hofstadter, *J. Chem. Phys.* **7,** 460 (1939) (CD_3COOD).
37. R.-C. Herman et R. Hofstadter, *J. Chem. Phys.* **6,** 534 (1938).
38. G. Herzberg, *Infra Red and Raman Spectra of Polyatomic Molecules* (D. Van Nostrand Cg, New-York) (1945).
39. G. Herzberg, F. Patat et J.-W.-T. Spinks, *Z. Phys.* **92,** 87 (1934) (C_2HD).
40. G. Herzberg, F. Patat et J.-W.-T. Spinks, *Nature*, **133,** 951 (1934) (C_2D_2).
41. G. Herzberg, F. Patat et H. Verleger, *Z. Phys.* **102,** 1 (1936) (C_2DH).
42. R. Hofstadter, *J. Chem. Phys.* **6,** 540 (1938).
43. R. Hofstadter, *Phys. Rev.* **53,** 940 (1938).
44. J.-H. Hibben, *The Raman Effect and its Chemical Applications* (Reinhold Publ. Co., New-York) (1939).
45. K.-W.-F. Kohlrausch, *Der Smekal-Raman-Effect, Ergänzungsband* (J. Springer, Berlin) (1938).
46. A. Langseth et H.-J. Bernstein, *J. Chem. Phys.* **8,** 410 (1940) ($C_2D_2C1_4$).
47. A. Langseth, H.-J. Bernstein et B. Bak, *J. Chem. Phys.* **8,** 430 (1940) (CH_2DCH_2Br).
48. A. Langseth, H.-J. Bernstein et B. Bak, *J. Chem. Phys.* **8,** 415 (1940).
49. J. Lecomte, *Ann. Phys.* **15,** 258 (1941).
50. M. Lawson et A.-B.-F. Duncan, *J. Chem. Phys.* **12,** 329 (1944) $(CD_3)_2CO$).
51. J.-W. Linnett, *J. Chem. Phys.* **8,** 91 (1940).
52. C. Manneback et A. Verleysen, *Ann. Soc. Sc. Brux.*, LVI, 349 (1936).
53. S.-I. Mizushima et Y. Morino, *Z. Phys.* **119,** 188 (1942) ($BrCD_2$-D_2C Br).
54. S.-I. Mizushima, Y. Morino et A. Suziki, *Sci. Papers Inst. Phys. Chem.* (Tokio), **36,** 281 (1939) (1.2 $C_2D_4Br_2$).
55. Y. Morino, *Sci. Papers Inst. Phys. Chem.* (Tokio) **25,** 232 (1934) (C_2D_2).
56. Y. Morino et S. Mizushima, *Phys. Z.* **36,** 600 (1935) (C_2D_2).
57. J.-C. Morris, *J. Chem. Phys.* **11,** 230 (1943) (CD_3COD).
57*bis* R.-S. Mulliken, *Rev. Mod. Phys.* **14,** 265 (1942).
58. H.-H. Nielson, *Phys. Rev.* **55,** 299 (1939) (D_2CO).
59. H.-D. Noether, *J. Chem. Phys.* **10,** 664 (1942) (CD_3C1 et CD_3Br).
60. *J. Chem. Phys.* **10,** 693 (1942) (CD_3OD et CD_3OH).
61. A.-H. Nielsen et H.-H. Nielsen, *Phys. Rev.* **54,** 118 (1938) (CD_4).
62. A.-H. Nielsen et H.-H. Nielsen, *Bull. Amer Phys. Soc.* **11,** 10 (1935) (CD_4).
63. A.-H. Nielsen, *Phys. Rev.* **57,** 346 (1941) (C_2D_2).
64. W.-C. Price et W.-T. Tulle, *Proc. Roy. Soc.* (London) A **174,** 207 (1940).
65. J.-E. Rosenthal, *Phys. Rev.* **46,** 730 (1934).
66. F. Stitt, *J. Chem. Phys.* **7,** 297 (1939) (C_2D_6).

67. Y.-L. Tchang, *Ann. Soc. Sc. Brux.* **58,** 87 (1938).
68. P. Torkington et H.-W. Thompson, *J. Chem. Soc.*, p. 303 (1944).
69. H.-W. Thompson, *J. Chem. Soc.*, p. 183 (1944).
70. B. Trumpy, *Nature*, **135,** 764 (1935).
71. B. Trumpy, *Kgl. Norske. Vind. Selsk. Skrif.* **29,** 14 (1935).
72. B. Trumpy, *Z. Phys.* **100,** 250 (1936).
73. J. Verhulst, F. Van Hemelrijck et J.-C. Jungers, *Natuurw. Tijds.* **8,** 203 (1943).
74. A. Verleysen et C. Manneback, *Ann. Soc. Sc. Brux.*, **LVII,** 31 (1937).
75. G.-E. McWood et H.-C. Urey, *J. Chem. Phys.* **4,** 402 (1936).
76. G.-E. McWood et H.-C. Urey, *J. Chem. Phys.* **3,** 650 (1935) (CD_4).
77. J. Wagner, *Zeit. Phys. Chem.* (B) **40,** 36 (1938).
78. J. Wagner, *Zeit. Phys. Chem.* (B) **45,** 69 (1939).
79. J. Wagner, *Zeit. Phys. Chem.* (B) **40,** 36 (1938).
80. E.-B. Wilson Jr., *J. Chem. Phys.* **7,** 1047 (1939).
81. E.-B. Wilson Jr., *J. Chem. Phys.* **9,** 76 (1941).
82. T.-Y. Wu, *J. Chem. Phys.* **10,** 116 (1942) (CH_2X_2).

Discussion du rapport de M. de Hemptinne

M. Ingold. — I think that the work of M. de Hemptinne and his colleagues on the vibrational spectra of ethylene and its deuterium and halogen derivatives has an elegance and completeness which will cause it to become one of the classics of molecular spectroscopy.

I would like to call attention, as Teller has done already, to the theoretical problems presented by the bending frequencies of acetylene. There are two, and we may call them «gerade» and «ungerade», the former being allowed in the Raman spectrum only, and the latter in the infra red spectrum only.

ungerade gerade

If we adopt a potential energy function of the Valency force type, these two vibrations depend on only one common force constant *viz.*, the force constant (or more correctly, the moment constant) for the bending of the H-C-C angle from its equilibrium value of 180°. If now we take the ratio of the calculated expressions for the two frequencies, this common force constant cancels, the ratio involving only the molecular dimensions and the atomic masses. This calculated ratio is, in fact :

$$\frac{\nu_{\text{ungerade}}}{\nu_{\text{gerade}}} = \sqrt{\frac{13\ a^2}{A^2 + 12\ a^2}}$$

where a is the C-C distance and A is the H-H distance. The value

of this ratio is about 0.8. The observed frequencies are $\nu_{ungerade} = 729\ cm^{-1}$ and $\nu_{gerade} = 612\ cm^{-1}$. The ratio of these figures is about 1,2. The large disperepancy means that the forces resisting bending of the valencies at one carbon atom are strongly dependent on whether, in what direction and by how much, the valencies at the other carbon atom are bent. This dynamical interconnection can be formally described by a cross term, but that gives no insight into physical mechanism, which I should have thought it would be possible to discuss, semiquantitativly at least, on the basis of what is known to-day concerning the nature of valencies and the factors controlling their orientation in the atom.

An other theoretical problem of great importance arises in connexion with hindered rotation of ethane. The presence of barriers of 2,8 kilocalories per gram-mol., which are indicated by the specific heat data to be restricting the relative rotations of the methyl groups of ethane, is universally accepted; but, as M. de Hemptinne says, spectroscopic investigations have not yet been succesful in determining whether the stable configuration separated by such barriers have the hydrogen in the « eclipsed » or « staggered » positions. However, a recent thermochemical investigation by Spitzer and Huffman (1) seems to me to point rather definitly to the staggered configuration, at least if the measurements are accurate, a fact which will only be known when they have been reproduced by independent workers. Spitzer and Huffman measured the heats of combustion of *cyclo*pentane, *cyclo*hexane, *cyclo*heptane and *cyclo*octane. The values found for the heat per methylene group were 158,7 - 157,41 - 58,3 and 158, 6 kilo-calories. The *cyclo*hexane ring is thus the most stable of the series. The argument is that if the eclipsed arrangment of the hydrogen atoms attached to adjacent carbon atoms were the more stable arrangment, then *cyclo*pentane should be the most stable of the four hydrocarbons, because it is the only one of the series that (by being planar) can simultaneously achieve a practically strainless ring and a fully eclipsed arrangment of all hydrogen atoms. On the other hand, if the staggered arrangment is the more stable, then the *cyclo*hexane ring should be the most stable of the series, because it is the only one that (by being trigonally puckered) can simultaneously achieve a strainless ringform and a fully staggered arrangment of all hydrogen atoms. On either hypothesis, *cyclo*heptane and *cyclo*octane

(1) R. Spitzer and H.-M. Huffman, *J. Amer. Chem. Soc.* **69**, 211 (1947).

must accept either some ring strain or some departure from the least energised arrangement of hydrogen atoms; and presumably they will strike a compromise by accepting a certain amount of potential energy of each of these kinds. The observations, therefore, seem consistent with the theory that staggered arrangement is favoured : it would be hard to reconcile them with the view that the eclipsed arrangement is the stable one. I think that the degree of importance to be attached to such a conclusion is a matter for individual judgement, and I draw attention to the argument only because it seems to me to be a weighty one though applying to a question which is not yet closed.

M. Langseth. — First of all I should like to thank Prof. de Hemptinne for reporting on his very important and extensive work on the spectroscopy of simple organic molecules.

I was particulary interested in his remarks on the hindered rotation about the C-C bond in ethane, as I myself have recently been working on the same problem.

Together with B. Bak (1) I have prepared and taken the Raman spectra of CH_2D-CH_2Br, CH_3-$CHDBr$, CH_3-CH_2Br and CD_3-CD_2Br. According to selection rules, 13 Raman lines are to be expected in the frequency region below 1600 cm^{-1} corresponding to Raman active fundamentals in all these molecules. Actually 12 lines were observed in CH_3-CH_2Br, CH_3-$CHDBr$ and 13 lines in CD_3-CD_2Br. As one of these lines found in deutero-ethyl bromide probably is an overtone this means that only one fundamental frequency in the region remains unobserved. CH^2D-CH^2Br however has a much more complicated spectrum showing 24 lines. A closer examination of the frequencies reveals that this seems due to a splitting into pairs of all the lines corresponding to those found in other compounds. This is exactly the kind of spectrum to be expected if CH_2D-CH_2Br is a mixture of two slightly different species. Evidently it is the destruction of the trigonal symmetry of the methyl groups by the deuterium atom CH_2D-CH_2Br which is responsible for the isomerism. The only reasonable explanation seems to be rotational isomerism.

It is easily seen that one of the rotational isomers must have a double statistic weight compared with the other, whether the configuration corresponds to the staggered or the eclipsed form. The

(1) *D. Kgl. Danske Vidsk. Salsk. Mat.-Fys. Medd.*, Bd **24**, 3 (1947).

relative abundance of the two isomers should therefore be approximately 1/2. Moreover the intensities of the component lines should be different in each of the observed pairs. It will depend on the difference in the modes of the vibration of the two rotational isomers how close the observed intensity ratio will be to 1/2. For two of the strongest low frequency pairs the observed intensity ratios are 3/5 and 5/10 in good accordance with the expected value. As these lines have a fairly isolated position in the spectrum, we are confident of their assignment as pairs. As it is the line with the highest frequency in each pair which is the most intense, I have previously interpreted the spectrum in favour of the eclipsed position on the basis of qualitative considerations (1). However, the only statement which can be made quite safely from the observed Raman spectrum, is that CH_2D-CH_2Br must be a mixture of two different molecular species. Until a complete analysis of the spectra has been carried out we will not be able to decide between the two possible configurations.

M. Ingold. — If it should eventually be agreed that the staggered arrangement of hydrogen atoms in ethane is the more stable arrangement, the problem will arise as to where the forces come from which make this configuration more stable than the alternative one in which the hydrogen atoms are in eclipsed positions. For it will be implied that the hydrogen atoms, when in eclipsed positions, repel their opposite partners. But according to Pauling's values of Van der Waals atomic radii, two opposed hydrogen atoms in the eclipsed configuration of ethane should be far enough away from each other for repulsion to have already ceased. The Van der Waals radii of hydrogen at least will then have to be revised; and because of the flatness of the minima in the mutual energy curves of non-bonded atoms, the revision necessary to provide a rotation-restricting barrier of the height of that present in ethane will be quite large, probably amounting to 0.4 A at least. It is already certain that in ethane nett forces of *some* kind exist at the accepted Van der Waals radii, where there should be no nett forces according to the meaning and definition of such radii.

M. de Hemptinne. — Nous avons pu préparer à Louvain l'éthane disubstitué symétrique DH_2C — CH_2D à partir de H_2DC — CH_2Br en utilisant la méthode de Grignard. Le spectre Raman de cet éthane

(1) A. Langseth, H.-J. Bernstein and B. Bak, *J. Chem. Phys.*, **8**, 430 (1940).

fut d'une pauvreté surprenante ce qui plaide tout d'abord en faveur de la pureté du produit obtenu et ensuite en faveur de la configuration étoile (staggered) ce qui confirmerait les conclusions développées par MM. Ingold et Langseth.

M. Karrer. — Quelle est la stabilité des deux formes qui se distinguent par leur vibration ou constellation?

Est-ce que la température et les autres conditions extérieures ont une grande influence sur l'équilibre des deux formes?

M. Langseth. — The two rational isomers of CH_2D-CH_2Br have to a high approximation the same thermodynamical stability and the relative abundance of the two forms (approximately 1/2 for statistical reasons) can therefore not be influenced by a change in temperature. .

M. Timmermans. — Pour quelques substances la littérature renseigne l'étude de la configuration par les méthodes spectroscopiques de l'Infra-Rouge et du Raman en exécutant les expériences sur des cristaux.

En général il en résulte que la structure privilégiée à l'état solide correspond exclusivement à la configuration TRANS ce qui, pour une molécule à deux atomes de carbone du type de l'éthane, correspond à la configuration en étoile (« staggered ») par opposition à la configuration en éclipse.

Je voudrais savoir :

1° Si des expériences de cette nature ont également été exécutées sur des substances contenant du deutérium?

2° Si la configuration TRANS, qui existe seule à l'état cristallisé correspond toujours à une prépondérance de cette configuration dans le fluide liquide ou gazeux?

3° Si aucune expérience n'a été réalisée par ces méthodes pour nous renseigner sur la configuration de formes polymorphes de telles substances?

Exemple : les deux formes du bromure d'éthylène cristallisé dont la température de transformation est — 26°.

M. de Hemptinne. — Nous avons examiné les spectres Raman d'un certain nombre de bibromures lourds à l'état solide. Pour tous ces corps, comme pour le bibromure léger, seul le spectre correspondant à la forme trans subsiste à l'état solide.

Dans le cas particulier de HDBrC — CHDBr la raie de valence

CBr apparaît double dans le spectre du solide ce qui correspond vraisemblablement à l'existence de deux isomères trans :

Les raies Raman des bibromures caractéristiques de la forme trans sont plus intenses que les autres dans le spectre de ces corps à l'état liquide.

Aucune expérience par méthode spectroscopique n'a été réalisée à ma connaissance sur les différentes formes polymorphes des composés deutérés solides.

M. Backer. — En rapport avec les discussions sur la structure spatiale des dérivés simples de l'éthane je voudrais attirer l'attention sur un cas où il y a un argument chimique en faveur de l'une des deux structures limites possibles.

Les 2, 3- ditertiobutylbutadiène ne donnent pas la réaction diénique avec l'anhydride maléique. On doit en conclure que les deux groupes volumineux butyle-tertiaire forcent les doubles liaisons à prendre une « position éloignée ».

M. de Hemptinne. — L'étude des spectres d'une telle molécule serait certes intéressante mais il faut s'attendre à un spectre compliqué d'interprétation difficile.

M. Briner. — Au sujet de la rotation libre des deux groupes méthyle de l'éthane autour de l'axe C-C, il est dit (p. 199) que la barrière de potentiel à franchir par le groupe méthyle pour passer d'une position à l'autre est de l'ordre de 3000 cal. à la température normale.

Peut-on connaître l'élévation de température nécessaire à la libération de la rotation?

Peut-on déduire une valeur pour cette grandeur énergétique des mesures spectroscopiques, notamment de la fréquence de torsion? Une confirmation d'ordre spectrographique serait d'une grande importance.

M. Ingold. — From the height, 2,8 kilocals/g.mol, of the energy barrier which restricts rotation in ethane, it can readily be calculated that temperatures above the limits of the thermal stability of ethane would be needed in order to allow any high proportion of the ethane molecules simultaneously to enjoy free relative rotation of the methyl groups. From the same basic datum, it can be deduced that, although at practicable experimental temperatures any molecule spends very nearly all its life in the most energetically favoured configuration, and only an insignificant time in other configurations, the intantaneous jumps from one favoured configuration to an equivalent one occur with great frequency; in particular, the average time interval between such jumps is much shorter than would be required for any relevant experimental observations.

M. Bjerrum. — Permettez-moi de poser une question. A-t-on pensé à la possibilité que l'éthane et son dérivé bromé n'ont pas nécessairement la même configuration et cela notamment à cause de l'attraction entre l'hydrogène et le brome?

M. Langseth. — Prof. Bjerrum may be right in his remarks. It is conceivable that the introduction of a bromine atom into the ethane molecule may change the equilibrium configuration from the staggered to the eclipsed which from qualitative considerations on the Raman spectrum of CH^2 D-CH^2Br seems to be most likely.

M. Calvin. — Have you tried to calculate an energy of activation for the *cis-trans* isomerization for an unsubstituted double bond from the torsion frequency and some anharmonicity function?

M. de Hemptinne. — Nous n'avons pas fait de calcul de ce genre.

The Preparation of Radioactive Tracers

by

M. F. A. PANETH

In view of the extensive use which to-day is made of radioactive tracers in biological and non-biological sciences, the question of their preparation assumes an ever growing importance. For many problems more than one tracer substance can be advantageously applied, and many tracer substances can be obtained in more than one way. To deal with the whole wealth of information published during the last few years would require a volume. We shall confine ourselves here to a description of the principles underlying the preparation of radioactive tracers, and illustrate them by a few examples.

Generally four stages can be distinguished whenever a radioactive tracer is prepared. I. The selection of a suitable tracer substance. II. The production of the chosen radioactive atoms. III. The analytical separation and concentration of these atoms. IV. The synthesis of the tracer substance incorporating the radioactive atoms. We shall deal with each of these stages in turn.

I.

SELECTION OF A SUITABLE TRACER SUBSTANCE

The choice of the tracer substance has, of course, to be made by the worker who is going to use it; but he should be acquainted with the points of view of the physicists and chemists who will be responsible for the stages II, and III and IV, respectively.

In the interest of his own work he will try to obtain for his tracer substance an isotope—or, as we shall better say, a « nuclide » (1)

(1) In the literature on radioactivity, it has recently become customary to refer to the seven hundred odd atomic species of the 96 known elements as « isotopes », thus neglecting the original meaning of the word which designated the atomic species of one and the same element which occupy « the same place » in the Periodic Table. As to-day, as well as thirty years ago when Soddy coined it, we need a word for describing this special relationship of two atomic species, we agree with T. P. Kohman [1]) that this loose usage of the term isotopes is to be discouraged; and since the expression « atomic species » is awkwardly long, we adopt here the new word « nuclide » introduced by Kohman.

—which emits sufficiently energetic rays to make the quantitative measurements easy, and which has a convenient half-life. It is obvious that too short a life tends to make the experiments difficult or impossible; but also too slow a disintegration rate may be unwelcome, e. g. if the tracer is to be used in biological experiments where the introduction of a permanent radioactivity into the organism may have to be avoided. Moreover, it follows from the disintegration laws that the time needed for attaining the necessary activity of a long-lived artificial radioelement may be prohibitively long.

In table I we have brought together the radioactive nuclides, with their half-lives, which seem to be the most useful ones for tracer work.

For the meaning of the asterisks see below (p. 235).

TABLE I

Element	Isotope	Half-life	Element	Isotope	Half-life	Element	Isotope	Half-life
1	^{3}H	31 y	27*	^{60}Co	5.3 y	51*	^{122}Sb	2.8 d
6	^{11}C	20.5 m	29*	^{64}Cu	12.8 h	—*	^{124}Sb	60 d
—*	^{14}C	10000 y	30*	^{65}Zn	250 d	—*	^{125}Sb	2.7 y
9	^{18}F	2 h	—*	^{69}Zn	13.8 h	52	^{129}Te	32 d
11	^{22}Na	3.0 y	33	^{74}As	16 d	53*	^{131}I	8 d
—*	^{24}Na	14.8 h	—*	^{76}As	26.8 h	54	^{135}Xe	9.4 h
14	^{31}Si	170 m	—*	^{77}As	40 h	55	^{137}Cs	33 y
15*	^{32}P	14.3 d	35*	^{82}Br	34 h	56	^{140}Ba	12.8 d
16*	^{35}S	87.1 d	36	^{85}Kr	4.5 h	57	^{140}La	40 h
17*	^{36}Cl	10^{6} y	37	^{86}Rb	19.5 d	58	^{141}Ce	30 d
—	^{38}Cl	37 m	38*	^{89}Sr	55 d	59	^{143}Pr	14 d
18*	^{37}A	34 d	39	^{91}Y	57 d	73	^{182}Ta	97 d
19*	^{42}K	12.4 h	40	^{95}Zr	63 d	74	^{185}W	77 d
20*	^{45}Ca	180 d	41	^{95}Nb	36 d	79*	^{198}Au	2.7 d
21	^{45}Sc	85 d	42	^{99}Mo	67 h	—*	^{199}Au	3.3 d
22	^{51}Ti	72 d	44	^{103}Ru	42 d	80*	^{197}Hg	64 h
24	^{51}Cr	26.5 d	—	^{106}Ru	1 y	—*	^{203}Hg	51.5 d
25	^{54}Mn	310 d	47	^{108}Ag	225 d	81	^{206}Tl	3.5 y
26*	^{55}Fe	4 y	—	^{110}Ag	—	85	^{211}At	7.5 h
—*	^{59}Fe	47 d	—	^{111}Ag	7.6 d	93	^{239}Np	2.3 d
						94	^{238}Pu	50 y

Only artificial radioelements are included in the above table. In rarer cases, however, some of the natural radioactive substances may be helpful in tracer experiments, and we give, therefore, here a list of those most likely to be employed.

TABLE II

Element	Radioactive isotopes	Half-lives
81 Thallium	Thorium C''	3.1 m
82 Lead	Radium D	22 y
	Thorium B	10.6 h
83 Bismuth	Radium E	5.0 d
	Thorium C	60.5 m
84 Polonium	Polonium	140 d
86 Emanation	Radon	3.82 d
87 Francium	Actinium K	21 m
88 Radium	Radium	1590 y
	Thorium X	3.64 d
89 Actinium	Actinium	13.5 y
	Mesothorium 2	6.13 h
90 Thorium	Radiothorium	1.9 y
	Uranium X	24.5 d
91 Protactinium	Protactinium	3.2×10^4 y
	Uranium Z	6.7 h

Some investigations, especially those of a biological nature, require often tracers of negligible weight because the incorporation of any appreciable amount of matter would create non-physiological conditions. The demand for a high « specific activity » (1) of the tracer is likely to increase the difficulties of its production.

Sometimes an otherwise suitable nuclide may not make a convenient tracer because of complications in its assaying arising from the activity of its disintegration products; in other cases, however, the measurement may be facilitated by the stronger radiation of a daughter substance which quickly reaches radioactive equilibrium with the tracer.

(1) By specific activity we understand the number of disintegrations per second, per gram of the tracer substance.

II.

PRODUCTION OF THE TRACER NUCLIDES

There are many processes known for the creation of artificial radioelements, but only the following are of universal importance in tracer production.

A. Bombardment of stable atoms with neutrons.

B. Bombardment of stable atoms with deuterons.

C. Splitting of uranium into active nuclides.

Process A can be carried out in three different ways : with a radium-beryllium source, with a cyclotron, and in a uranium pile, while B is restricted mainly to the cyclotron, and C to the pile.

A. Neutron bombardment.

The three methods of neutron bombardment mentioned above differ by several orders of magnitude in their efficiency.

A radium-beryllium source, containing 1 g of radium carefully mixed in the form of its chloride with 5 g of the finest beryllium powder, emits per second about 10^7 neutrons. A cyclotron of the size of the Berkeley machine (diameter of the magnet about 60''), running with an output of 50 μA, can produce 4.10^{12} neutrons per second. An uranium pile, operated at 1.000 kW, yields as much as 6.10^{16} neutrons per second.

The differences in efficiency are, however, not quite so great as suggested by the above figures. Owing to the smallness of a radium-beryllium source it will usually be possible to utilise its total neutron radiation; and most of the neutron flux of a cyclotron is also, at least theoretically, available for experiments. In the pile, however, almost all of the neutrons produced by the fission of uranium 235 atoms are used up in either splitting other uranium 235 atoms or in being captured by uranium 238 atoms; the pile can only be operated safely if these two processes account for most of the neutrons liberated, and no more than some 10^{15} neutrons per second are available for tracer production. Even so, the efficiency of the pile is about a thousand times that of a big cyclotron, and hundred million times that of a fairly « strong » radium-beryllium source.

It would seem, therefore, that, as far as quantity of output is concerned, the uranium pile could take over the world production of tracers. There is in fact, no doubt that during the next years the tracers to be used in laboratories all over the world will mainly come from the uranium piles of the United States and, to a lesser extent, from the British and Canadian uranium piles. But it would be erroneous to suppose that the other methodes of production will be insignificant. First of all, most of the radionuclides formed in the pile must belong to the group which have more neutrons than their stable isotopes; they may be produced by an (n, γ), or an (n, p), or an (n, α) reaction, examples of which are given in the following table :

$$^{31}_{15}P + ^{1}_{0}n \rightarrow ^{32}_{15}P + \gamma$$
$$^{31}_{15}P + ^{1}_{0}n \rightarrow ^{31}_{14}Si + ^{1}_{1}H$$
$$^{6}_{3}Li + ^{1}_{0}n \rightarrow ^{3}_{1}H + ^{4}_{2}He$$

The first two reactions obviously lead to nuclides in which the proportion of neutrons to protons is increased and which are, therefore, heavier than their stable isotopes. If we consider that the same is true of the fission products, we realize that for the production of the second group of nuclides, those which are too light to be stable, another mode of production is necessary. For this group, the cyclotron will be indispensable.

But also in some cases where both methods are applicable, the cyclotron may have the advantage of yielding radionuclides of higher specific activity. There are limits to what can be obtained in this respect in the pile, because here in the central region, where the densest neutron flux obtains, a high proportion of the neutrons are not yet slowed down and, therefore, for some reactions unsuitable; further away from the center, the neutron density is smaller. In the cyclotron, concentration of the beam on a small target is frequently able to produce the desired specific activity where chemical concentration is impossible, as in the case of (d, p) reactions (see below).

Furthermore, short-lived tracers can not be shipped far away from the place of their origin; those produced in a pile will have to be used on the spot, and countries not possessing an uranium pile will continue to prepare these tracers by cyclotrons.

For many laboratories also the radium-beryllium source will retain its value as an extremely simple and constant producer of

neutrons; instead of radium, radon can be used. The neutrons are generated according to the equations :

$$^{9}_{4}\mathrm{Be} + {}^{4}_{2}\mathrm{He} \rightarrow {}^{12}_{6}\mathrm{C} + {}^{1}_{0}\mathrm{n}$$

$$\text{and } 3\ {}^{4}_{2}\mathrm{He} + {}^{1}_{0}\mathrm{n}$$

B. Deuteron bombardment.

Deuteron bombardment can only be effected by a cyclotron or a similar instrument which accelerates charged particles. Its application in tracer production is twofold. The deuterons may either be made to hit directly the element from which the tracer is to be obtained, or they may be used to create neutrons which then, in their turn, produce the tracer nuclide. The usual targets for the second application are beryllium and lithium, according to the reactions :

$$^{9}_{4}\mathrm{Be} + {}^{2}_{1}\mathrm{H} \rightarrow {}^{10}_{5}\mathrm{B} + {}^{1}_{0}\mathrm{n}$$

$$^{7}_{3}\mathrm{Li} + {}^{2}_{1}\mathrm{H} \rightarrow {}^{8}_{4}\mathrm{Be} + {}^{1}_{0}\mathrm{n} \quad \text{or } 2\ {}^{4}_{2}\mathrm{He} + {}^{1}_{0}\mathrm{n}$$

Beryllium targets can better withstand the high deuteron beam currents than lithium targets, but the lithium reaction gives neutrons of higher energy. The number of neutrons produced is no more than a fraction of the primary deuterons and even at high energies does not exceed 0.2%. This explains the relative inefficiency of the cyclotron as neutron source (see above).

For the direct production of tracer nuclides the target to be hit by the deuteron beam can either be placed inside the cyclotron vacuum chamber, or outside. In the first case we make better use of the whole deuteron beam, but the temperature created in the vacuum will be too high for many materials, even if we try to cool the target from one side by water, or use other cooling devices; if, on the other hand, the sensitive target material is placed outside the cyclotron in a special chamber which the deuteron beam enters through a metal foil window, we may cool the target much more efficiently by surrounding it by helium gas. For inside bombardment we have frequently to use chemical compounds instead of the elements; for instance a phosphide instead of elementary phosphorus, thus reducing in the target area the number of the phosphorus atoms, but gaining not only higher heat resistance but also the advantage of a better-conducting contact with the water cooled target support.

Several of the deuteron reactions in the cyclotron permit the production of nuclides which are not obtainable in the pile; e. g.

^{22}Na and ^{55}Fe, with half-lives of 3 and 4 years, respectively; other isotopes of the same elements, ^{24}Na and ^{59}Fe, with half-lives of 14.8 hr and 47 d, are better produced in the pile. The reactions are :

$$^{24}_{12}\mathrm{Mg} + {}^{2}_{1}\mathrm{H} \rightarrow {}^{22}_{11}\mathrm{Na} + {}^{4}_{2}\mathrm{He}$$
$$^{55}_{25}\mathrm{Mn} + {}^{2}_{1}\mathrm{H} \rightarrow {}^{55}_{26}\mathrm{Fe} + 2{}^{1}_{0}\mathrm{n}$$
$$^{23}_{11}\mathrm{Na} + {}^{1}_{0}\mathrm{n} \rightarrow {}^{24}_{11}\mathrm{Na} + \gamma$$
$$^{59}_{27}\mathrm{Co} + {}^{1}_{0}\mathrm{n} \rightarrow {}^{59}_{26}\mathrm{Fe} + {}^{1}_{1}\mathrm{H}$$

C. Uranium fission.

The fission products of uranium 235 comprise radioactive isotopes of the elements $_{30}$Zn to $_{63}$Eu. Many of them are valuable as tracers thanks to the convenient properties of their radiations and half-lives. Some, however, are followed by a long chain of disintegration products which limit their usefulness (see above). The total activity of all the fission products in a pile is tremendous; from a 1000 kW pile more than 1 million curie are available. So the problem is here mainly one of safeguarding the workers against health dangers if — after most of the activity has decayed — the separation of pure tracer material is attempted.

It ought to be mentioned that fission products can also be obtained by the neutron bombardment of uranium in a cyclotron, but the yield is, of course, much smaller.

III.

ANALYTICAL SEPARATION AND CONCENTRATION OF THE NUCLIDES

After the desired nuclide has been produce dby one of the methods outlined in the preceding chapter, it will be the task of the chemist to free it as far as possible from the accompanying substances and thus prepare it in the highest specific activity obtainable. The chemical procedure, and the results, will be quite different according to whether or not the tracer nuclide is isotopic with the stable atomic species from which it has been produced.

Most of the slow neutron reactions consist in the capture of the neutron in the nucleus without the emission of a charged particle;

consequently the new radioactive atom is isotopic with its parent, and no chemist is able, by the usual laboratory processes, to separate the two, once complete mixing has taken place. The maximum specific activity in these cases is determined by the percentage of atoms converted during the bombardment; and all the chemist can do is to separate the two isotopes together from the other elements. Formally equivalent to these (n, γ) reactions are the (d, p) reactions in the cyclotron.

If we speak of the chemical inseparability of isotopes, it is always understood that they have been completely mixed; if, however, from the start they have been in a different physical or chemical state, then their reactions may be different and allow of a partial or complete separation. Szilard and Chalmers [2]) have pointed out that in most cases the neutron capture by an atom will not only cause a change in its nuclear structure, but also the breaking of its chemical linkage; the resulting new atoms, although isotopes of the same element, will then no longer be present as part of the same molecules; a separation, and consequently an increase of the specific activity, should, therefore, be possible. This consideration will, however, not be valid, if the chemical linkage in which the old atoms are held is of the kind which permits of a quick interchange with the new isotopes; therefore, if we intend to make use of the Szilard-Chalmers effect for obtaining a high specific activity of an isotopic tracer, we have to choose the target material carefully so as to avoid the possibility of such exchange processes. Unfortunately, the chemist will not always be able to give here good advice; more study will be necessary before full use can be made of the possibilities opened up by Szilard and Chalmers.

The situation is quite different in all the other cases where the tracer substance produced is not isotopic with its parent. The task of the chemist is then the separation of two different chemical elements, and ordinary analytical chemistry should provide the necessary knowledge. As a matter of fact, the well-known analytical processes actually form the basis of all the operations of tracer purification, but in two respects the chemist handling a radioactive substance is likely to come up against special difficulties.

First of all, the radiation emitted by the material may introduce complications. We are not concerned here with the important question of protection against health hazards. To devise laboratories with appropriate shielding by lead or concrete, and with machinery for

the performance of the chemical operations by remote control, is largely a problem for the builder and engineer; the chemist will, however, be compelled to select the simplest possible processes in order not to make the engineer's task too difficult. But even when the operator is safe-guarded, the radiations will still be efficacious inside the solutions or gases the chemist has to deal with, causing here a variety of chemical changes. For instance, in any aqueous solution hydrogen and hydroxyl radicals will be produced, and these radicals will then act in different ways upon the solute [3]). A knowledge of « radiation chemistry » (i. e. chemical changes under the influence of radiations), is, therefore, important in « radiochemistry » (i. e. the chemistry of the radioactive elements).

While this first disturbance becomes the more disagreeable, the greater the amount of radioactive material and, consequently, the strength of its radiation, quite the opposite is true of the second complication; here the difficulties are, generally speaking, the more marked, the smaller the number of atoms the radiochemist has to deal with. Compared with ordinary chemistry, the quantity of matter he has to handle is almost invariably insignificant, but it is expedient to distinguish three different ranges of weight to which it may belong, the limits being roughly the following :

1. Microchemical quantities : from 10^{-2} g. to 10^{-4} g.
2. Ultramicrochemical quantities : from 10^{-4} g. to 10^{-7} g.
3. Invisible quantities : less than 10^{-7} g.

In the first range, the methods are still those of ordinary chemistry, with a somewhat refined technique. For the analysis of organic substances, these improvements are well known thanks to the work of F. Pregl; he introduced to wider circles the micro-balance which can measure with an accuracy of 10^{-6} g. On similar lines, but on a broader basis, the inorganic microchemistry of this region was worked out in Graz by F. Emich [4]) and his school.

Operations in the ultramicrochemical region were attempted somewhat later by chemists of the same school, especially A. A. Benedetti-Pichler (now in New York) and his pupil M. Cefola [5]), and during the last war were taken up by radio-chemists (P. L. Kirk, and others in G. T. Seaborg's group [6]). They took their lead partly from biologists who had previously developed the technique of working under the microscope with the help of a micro-manipulator.

For weight determinations quartz-fibre balances of different patterns are used; the accuracy of some of them reaches 2.10^{-8} g.

Even in the ultramicrochemical region the chemical processes are the familiar ones; by reducing the size of the vessel and the quantity of the liquid to, say, 10^{-5} c. c., it is for instance possible directly to determine in the usual way the solubility of a salt of which only a few micrograms are available. This technique proved most valuable in the exploration of the chemistry of the new radioelement plutonium after it had been fabricated in barely visible quantities. But the real peculiarities of radiochemistry are only revealed if we go down to invisible and unweighable quantities of matter; their behaviour depends, naturally, largely on that of the other substances present, but contrary to the expectations of many, even quantities of matter of 10^{-12} g. or less per litre have a definite chemistry of their own, as emphasized by F. Soddy already in the early days of radiochemistry.

In dealing with invisibly small amounts of a radioelement the chemical operations can usually be much simplified by the addition of a trace of an inactive isotope; by this artifice we can without any difficulty raise the total concentration of the element (consisting now of two isotopes) into the microchemical or even macrochemical region. But it must be remembered that such a procedure reduces the specific activity of the radioactive substance irreversibly, since isotopes are inseparable by ordinary methods. The problem of concentrating the radioelements *without* the addition of an isotope is therefore the fundamental problem of radiochemistry.

No generally valid way to solve this problem can be told to the analytical chemist. It can, however, be stated that invisible amounts of a metal are generally carried down with any precipitate whose anion forms an insoluble compound with this metal [7]). If, e. g. traces of bismuth are to be removed from a solution, it suffices to precipitate therein lead sulfide; the anions of the PbS precipitate then carry down the bismuth, because bismuth sulfide is insoluble; a precipitate of $PbSO_4$ on the other hand would not act in the same way as a « carrier » because bismuth sulphate is soluble. This precipitation rule was established as early as 1914; since then many attempts have been made to replace it by a stricter « precipitation law » [8]), but with little success.

The advantage of a non-isotopic carrier is that we retain the possibility of a further concentration of the radioelement in later stages of the analytical process; this operation may be effected by

attaching the radioelement to smaller quantities of another carrier, or by completely changing the procedure. Very often electrochemical deposition is the most satisfactory final method for obtaining a radioelement with the minimum amount of foreign matter. The same result may sometimes be achieved by solvent extraction, or by distillation. The choice of the method will frequently be influenced by the time factor; it is obvious that for rapidly decaying tracer substances only quick-working processes are suitable.

As the efficient chemical extraction of the radioactive nuclide is a very essential condition for the success of the whole tracer experiment, the voice of the chemist ought to be heard already in the discussion on the best process for its production. For instance, the important tracer ^{11}C has a half-life of only 20 mins.; it can be produced by deuteron bombardment of boron atoms :

$$^{10}_{5}B + {}^{2}_{1}H \rightarrow {}^{11}_{6}C + {}^{1}_{0}n$$

Now from a physicist's point of view, elementary boron would seem to be the best target because it possesses the densest packing of boron atoms; but the ^{11}C atoms produced therein can not be recovered without the time-consuming process of oxidation at high temperature. If, however, boron oxide B_2O_3, is used as a target, the ^{11}C atoms, expelled by recoil and present in the highly reactive state of free, or « hot » atoms, have a good chance of combining with one of the O atoms; and as the B_2O_3 target melts under the heat of the deuteron beam, the majority of the ^{11}C atoms escape already during the bombardment as gaseous ^{11}CO, which can be easily collected [9]).

Usually a compromise will have to be reached between the physicist's and the chemist's point of view. One of the tracers most used in biological work is ^{32}P; if no uranium pile is available, the following deuteron reaction of the cyclotron is one of the most convenient ways :

$$^{31}_{15}P + {}^{2}_{1}H \rightarrow {}^{32}_{15}P + {}^{1}_{1}H$$

For reasons given above, elementary phosphorus which would be ideal for the subsequent chemical treatment, can not be used as internal target; in its stead ferrous phosphide, soldered to a water-cooled copper target is bombarded by the deuterons, with the consequence that the chemist has later the job of separating the phosphorus from the iron, and purifying it from traces of copper and various radioelements resulting from the deuteron bombardment of the iron.

Instead of the rather laborious process described in the literature [10], K. F. Chackett and F. Morgan, in the Londonderry Laboratory for Radiochemistry in Durham, have found the following procedure very satisfactory as regards speed as well as efficiency. If the target is heated in a stream of chlorine gas, the phosphorus, as PCl_5, and the iron, as $FeCl_3$, are distilled off; after removal of the chlorine and reduction, by hydrogen, of the $FeCl_3$ to $FeCl_2$, the phosphorus alone, as PCl_3, is volatile and can be collected in water and then converted into phosphoric acid or sodium phosphate, whichever is desired.

One point which the chemist is anxious to stress is the necessity of using in the targets material of the highest chemical purity. Mere traces of unexpected impurities may cause considerable trouble, especially if the chemist has to attain a prescribed specific activity and is not permitted to overcome difficulties by the addition of an isotopic carrier (see above). Purification of the target material is always much easier before the irradiation than afterwards when minute amounts of radioactive substances contained therein have to be preserved, and the time allowed for the work may be limited by the short half-life of some of them.

IV.

SYNTHESIS OF THE TRACER SUBSTANCE

After the radioactive nuclide in the desired specific activity has been produced by the physicist and extracted by the analytical chemist, it has to be incorporated in the proper tracer substance. If only the path, or the distribution, of the whole tracer molecule has to be studied, it will suffice to know that the radioactive atom is contained therein; but very frequently, especially in the field of biochemistry, changes in the molecule itself are the object of study, and then the introduction of the active atom into a well-defined position of the molecule becomes imperative. For this reason the synthesis—often on a very small scale—of compounds of biological importance containing the active nuclide has become an integral part of tracer technique.

One of the major difficulties encountered here is the necessity of performing all the chemical operations within a period not much in

excess of the life-time of the radioactive atom; only if at the beginning activities of a much higher order than needed for the experiment are available, is it possible to spend, say, ten life-times of the radioelement on chemical manipulations, thus reducing its activity to less than 1 per thousand. It is clear that a saving of time will frequently much more than compensate for a loss of material in the synthesis, and the chemist will have to devise his method accordingly.

In some fortunate cases the synthesis of the described tracer substance, or at least of an intermediate compound, takes place automatically during the production of the radionuclide. We have mentioned above the formation of the useful ^{11}CO from bombarded boron oxide. The ^{11}C atoms, after their formation out of boron, react preferably with the abundant oxygen atoms, though some of them apparently combine with the boron and form boron carbide; this explains the difficulty of removing a fraction of the ^{11}C atoms from the target. Such reactions of « hot » atoms play also an important role in liquid systems and are likely to obscure the scope of the Szilard-Chalmers effect. According to their ideas it was to be expected that if, e. g. ethyl or methyl bromide is irradiated with neutrons, every bromine atom capturing a neutron and consequently emitting a gamma-ray, would acquire sufficient recoil energy to become detached from the alkyl group. Such bromine atoms can be extracted by simply shaking the ethyl bromide with water; but the yield found was rather disappointing and it appeared as though many bromine atoms, although activated, were still « retained » by their original molecules. A closer study has shown [11], however, that beside the alkyl bromide there were now new bromine compounds present in the organic phase, and it is very likely that also the methyl and ethyl bromide molecules containing active bromine were not the original molecules, but had their inactive bromine dislodged by a radioactive and chemically « hot » bromine from another molecule. This interpretation receives strong support from the fact that in gaseous ethyl bromide more than 97 % of the activated bromine atoms could be proved to be separated from the ethyl groups [12].

We are mentioning these observations here because some of the « new » bromine molecules which are formed as an immediate consequence of the bromine activation, may be valuable as tracer substances; likewise, we may directly obtain several different organic iodine compounds suitable for tracer experiments by treating alkyl iodides with neutrons.

Somewhat similar observations were made on the chemical combinations into which hot ^{14}C atoms enter. This very important tracer material is produced from nitrogen by neutron bombardment :

$$^{14}_{7}N + ^{1}_{0}n \rightarrow ^{14}_{6}C + ^{1}_{1}H$$

Usually, as nitrogen containing target, saturated solutions of ammonium nitrate in water are chosen; if so, most of the ^{14}C appears as CO and CO_2; but CH_4, CH_3OH, HCOOH and other simple molecules containing ^{14}C were also found [13]). If beryllium nitride is used as a target, the hot ^{14}C atoms apparently combine partly with the beryllium, and the radio-carbide $Be_2{}^{14}C$, on treatment with water, gives active methane.

Interesting though these automatic syntheses are, they do not lead very far and for all the more complicated organic molecules the skill of the chemist is necessary. Since both ^{11}C and ^{14}C are easiest obtained as carbon monoxide or dioxide, as a first step the conversion of these gases into a compound more useful for organic syntheses is desirable. An elegant method has recently been devised [15]) which permits work on as little as 50 microlitres of CO_2 and to obtain within a few minutes almost complete conversion into C_2H_2.

A more complicated way for the change of CO_2 into C_2H_2 had to be used previously in connection with the successful attempt to introduce ^{11}C into different positions in the lactic acid molecule, labelling either the carbon atoms in the carboxyle group, or those in the alpha- and beta-positions. The methods employed [16]) can be seen from the following two schemes :

$$\overset{*}{CO_2} \xrightarrow{K.NH_3} KC\overset{*}{N} \xrightarrow{CH_3.CHO} CH_3.CHOH.C\overset{*}{N} \xrightarrow{\text{hydrolysis}} \mathbf{CH_3.CHOH.C\overset{*}{O}OH}$$

$$\overset{*}{CO_2} \xrightarrow{NaOH;\ BaCl_2} \xrightarrow{Mg} Ba\overset{*}{C}O_3 \xrightarrow{H_2O} Ba\overset{*}{C}_2 \xrightarrow{} \overset{*}{C_2}H_2 \xrightarrow{HgSO_4;\ H_2SO_4} CH_3.C\overset{*}{H}O \rightarrow$$

$$\rightarrow \xrightarrow{KCN} CH_3.CHOH.C\overset{*}{N} \xrightarrow{\text{hydrolysis}} \mathbf{CH_3.CHOH.C\overset{*}{O}OH}$$

Even more complicated organic syntheses have been performed with isotopic nuclides; attention may be drawn to a paper [17]) in which the fate of methionine in the animal body was studied with the help of molecules of this substance containing sulphur 33 in addition to two ^{13}C atoms in the following positions :

$$CH_3\overset{*}{S} . \overset{*}{C}H_2 . \overset{*}{C}H_2 . C(NH_2)H . CO_2H$$

In some cases biological action can be made use of in the preparation of complicated tracers. Robley D. Evans and his collaborators [18]), in their studies on red blood corpuscles, started by labelling the iron in ferric ammonium citrate by mixing it with both ^{55}Fe and ^{59}Fe; a solution of this active citrate was injected intravenously into humans; both tracer substances entered by exchange processes into the iron content of the blood; so later the radioactive red blood cells of these « donors » could be transfused and traced in the bloodstream of other humans. The combined use of two tracers of halflives of 4 years and 47 days made this tracer method particularly flexible and valuable.

Such « biosyntheses » can be performed also by microorganisms. The so-called methanobacterium reduces carbon dioxide to methane in the fermentation of ethyl alcohol to acetic acid :

$$2\ C_2H_5OH + \overset{*}{C}O_2 \longrightarrow 2\ CH_3COOH + \overset{*}{C}H_4$$

In an hour or so a few millimoles of labelled CH_4 can thus be formed [19]). Other bacteria, in the presence of active CO_2, produce labelled lactic, acetic and succinic acids, and as there is a great variety of microorganisms available, the method seems well worth further study.

Availability of tracers.

As this report had to deal only with the preparation of radioactive tracers, no mention was made of any problem which we may hope to solve by their application; however, in most cases the real scientific interest of the work starts after the preparation of the tracer, at the moment when we begin to use it. For this reason scientists will be the happier, the less they have to worry about the manufacture of the tracers they need, and it is very desirable that central organisations should be built up to carry out the preparation of tracers for the benefit of all the laboratories that want them. In this way also the costs for the tracers can be very substantially reduced. As an example it may be quoted that the price for 1 mc. of $^{14}_{6}C$ which is of such inestimable value for biochemical research, if produced by a cyclotron, amounts to about $ 1.000.000; as a product of the Clinton uranium pile it is sold in the U. S. A. for $ 50.

Until recently other countries could not benefit from this development, because the wealth of radioactive tracers produced in the

U. S. A. uranium piles, and distributed in that country, was excluded from export, and even the scientific experience gained by those who had been engaged in making and separating the various tracer substances was not completely available to other scientists. The restrictions, however, on the distribution of scientific information are slowly but surely diminishing, and it was most welcome news when—as already mentioned by Prof. Joliot in his report—at the beginning of this month President Truman in a telegram to the International Cancer Congress announced that from now on most of the tracer substances available to U. S. A. scientists may also be sent abroad. In Table I the tracers whose export has thus been allowed, are marked by an asterisk*. It is true that there are still several conditions attached to the sending of tracer material from the U. S. A. to other countries, but anyone acquainted with the spirit in which during the war American scientists observed the unavoidable security restrictions in their dealings with their colleagues from abroad, will have no doubt that the relevant paragraphs will be interpreted in a liberal sense. Professor Bainbridge also has assured us of that.

There is another good piece of news with respect to the availability of radioactive tracers in the future: about a fortnight ago the low-energy pile at the Atomic Research Establishment of the British Government at Harwell (Berkshire) started to function. It is meant primarily as a pilot plant for obtaining information in connection with the construction of the high-energy pile now being built, but in view of the great demand, the production of some especially desirable tracers will be immediately undertaken. The first tracers to become available will be :

$$^{32}_{15}\mathrm{P} \text{ and } ^{131}_{53}\mathrm{I}$$

to be followed by:

$$^{35}_{16}\mathrm{S},\ ^{45}_{20}\mathrm{Ca} \text{ and } ^{59}_{26}\mathrm{Fe}$$

The manufacture of a greater variety of tracers, and in larger quantities, can begin in England only in about a year's time when the high-energy pile will be working; but then the position in that country should be very similar to that in America, i. e. that practically all the tracer material needed by scientific laboratories will be obtainable from the Government. It is intended to send the irradiated material first to a Radiochemical Center instituted by the Government at Amersham (Buckinghamshire) where the separation and concentration of the tracers will be done; in the case of tracers,

however, which have been produced by an (n, γ) reaction and which can therefore not be separated from their isotopes, the aluminium containers in which the irradiation will be effected may also be sent directly from the pile to the research laboratories. While American tracer material is at the present moment not available to industrial laboratories in England, no such restriction is intended for the material produced at Harwell.

Finally it should be mentioned that a third place is also ready to start tracer production, the uranium-heavy water plant in Chalk River in Canada; it is certain that from there too tracer material will be obtainable in the near future, but details have not yet been announced.

REFERENCES.

1. T. P. Kohman, *Science*, July **8** (1947);—*Amer. J. of Physics* **15**, 356 (1947).
2. L. Szilard and T. A. Chalmers, *Nature*, **134**, 462 (1934).
3. J. Weiss, *Nature*, **153**, 748 (1944).
4. See F. Emich, *Lehrbuch der Mikrochemie* (J. F. Bergmann, München, 1926).
5. See A. A. Benedetti-Pichler, *Introduction to the Microtechnique of Inorganic Analysis* (Chapmann and Hall, New York, 1942).
6. See G. T. Seaborg, *Chemical and Engineering News*, **24**, 1192 (1946).
7. F. A. Paneth, *Phys. Zeitsch.* **15**, 924 (1914); *Jahrbuch der Radioaktivität und Elektronik*, **11**, 451 (1915);—K. Horovitz und F. A. Paneth, *Zeitsch. Phys. Chem.*, **89**, 513 (1915);—K. Fajans and F. Richter, *Ber. Deutsch. Chem. Ges.*, **48**, 700 (1915).
8. See O. Hahn, *Applied Radiochemistry* (Cornell University Press, New York, 1936).
9. S. Ruben, M. D. Kamen and W. Z. Hassid, *J. Amer. Chem. Soc.*, **62**, 3443 (1940).
10. R.-R. Wilson and M. D. Kamen, *Phys. Rev.*, **54**, 1031 (1938).
11. E. Gluckauf and J. W. J. Fay, *J. Chem. Soc.*, **1936**, 390.
12. H. Suess, *Zeitsch. Phys. Chem.* (B) **45**, 297 (1940).
13. P. E. Yankwich, G. K. Rollefson and T. H. Norris, *J. Chem. Phys.*, **14**, 131 (1946).
14. P.E. Yankwich, *ibid.* **15**, 374 (1947).
15. W. J. Arrol and R. Glascock, *Nature*, **159**, 810 (1947).
16. R. D. Cramer and G. B. Kistiakowsky, *J. Biol. Chem.*, **137**, 549 (1941);—B. Vennesland, A. K. Solomon, J. M. Buchanan, R. D. Cramer and A. B. Hastings, *ibid.* **142**, 371 (1942).
17. G. W. Kilmer and V. du Vigneaud, *J. Biol. Chem.* **154**, 274 (1944).
18. R. D. Evans et al., *Journ. Clin. Investigation* **25**, 605 (1946).
19. H. A. Barker, S. Ruben and M. D. Kamen, *Proc. Nat. Acad. Sci.*, **26**, 426 (1940).

Discussion du rapport de M. Paneth

M. de Hevesy. — Professor Paneth rightly emphasizes the importance of bombardment of stable atoms by neutrons and deuterons in the preparation of radioactive isotopes. We should not, however, underestimate the role of the heavy ionizing particles. When we are faced with the task of preparing ^{42}K, for example, we bombard potassium chloride with deuterons in the cyclotron or with neutrons in the pile. By these procedures we obtain radiopotassium contaminated by appreciable amounts of stable potassium, which may be disturbing. If we want to measure the permeability of red corpuscles to potassium, for example, and add 1 milligram of labeled potassium chloride to a few millilitres of blood, we create unphysiological conditions since 1 millitre of blood contains only 200 gamma of potassium.

We must furthermore envisage that the specific activity of a short living isotope such as ^{42}K (T = 12 hours) decreases appreciably within a comparatively short time and if we wish to use such a sample of labeled potassium several days after its preparation, its specific activity may be reduced to a very low level. It is therefore of importance to prepare carrier-free ^{42}K which can be done according to the equation $^{39}_{18}A + {}^{4}_{2}He = {}^{42}_{19}K + {}^{1}_{1}H$. *Hamilton*, who introduced this method of preparation of ^{42}K, percolates argon through a spacious gold vessel. Irradiation of argon by energetic α-particles leads to the formation of ^{42}K in the gaseous phase. The radiopotassium formed settles on the walls of the gold vessel and on the surface of the glasswool stopper through which the irradiated gas escapes. The ^{42}K is removed from these surfaces by water.

Carrier free ^{42}K can also be prepared by the reaction :

$$^{45}_{21}Sc + {}^{1}_{0}n = {}^{42}_{19}K + {}^{4}_{2}He$$

This reaction requires the use of scandium which is not easy to obtain.

As to the use of the expression « nuclides » instead of isotopes, one may object that the introduction of new expressions should be as far as possible avoided. Though the word isotope was originally

used in a different sense we use it today to denote what we could call different « editions » of an element. [A classical chemist will consider these different « editions » (except hydrogen and deuterium) to be the same substance, just like the reader of the bible considers different editions of the bible to be the same book. The nuclear scientist considers different isotopes to be very different substances as a book collector considers old and new editions of the bible to be very different books.] Stable isotopes are stable « editions » of an element, radioactive isotopes unstable « editions ». If only one stable isotope exists, the element has one stable « edition » only. The meaning of the statement, for example, that the fission of uranium leads to the production of radioactive isotopes of numerous elements seems to be entirely unambiguous and used in most of the literature. Should a new expression be introduced to denote different « editions » of elements, it should only be done after careful consideration.

We can also make the following comparison:

The word « child » is used both to denote that Peter is a child of Jones and also to characterize Peter as a member of the large family of human offspring. In an analoguous way the word « isotope » is used in most of the literature to denote ^{24}Na as an edition of sodium and also as a member of the large family of atomic species.

M. Joliot. — 1. Je suis tout à fait d'accord avec ce que vient de dire le Professeur Paneth concernant le mauvais emploi du substantif isotope et l'avantage d'employer le mot nuclide pour désigner le noyau particulier. C'est ainsi, par exemple, qu'on ne sera plus tenté de dire « cet élément chimique n'a qu'un isotope ».

2. Il est certain que le cyclotron pourra encore dans de nombreux cas être plus avantageux pour faire la synthèse de certains radio-éléments utilisés comme indicateurs.

3. Dans un autre ordre d'idées, je signale une expérience que je poursuis au laboratoire concernant un exemple de séparation du type Szilard-Chalmers en partant d'un solide. Il suffit de laver avec une solution très faiblement acide une poudre de V_2O_5 irradiée par des neutrons pour séparer le sel de vanadium enrichi par un facteur de l'ordre de 5 en ^{52}V radioactif. La capture du neutron thermique accompagnée de l'émission d'un photon doit modifier l'état d'oxydation du Vanadium et conduire à un oxyde plus soluble que V_2O_5.

4. Enfin je ferai remarquer qu'il me semble logique de faire figurer le nombre de masse à la gauche du symbole chimique et avantageux de le placer en haut. En bas à gauche on place le nombre de charges ou numéro atomique. Souvent on a oublié le numéro atomique de l'élément dont on connait le symbole chimique. En outre, avec cette disposition des nombres de masse et de charge, il est plus aisé de faire la différence de ces deux nombres, qui représente le nombre de neutrons.

A Zürich avant la guerre, lors d'un Congrès de physique, les physiciens de divers pays discutèrent cette question. Les Italiens écrivaient $_2He^4$, les Anglais He_2^4 et les Français ^4_2He; nous n'arrivâmes à aucune conclusion.

M. G. Guében. — A propos des méthodes de séparation des éléments radioactifs à utiliser comme indicateurs, je me permets de rappeler que l'hypothèse de travail qui a conduit à la méthode de Szilard et Chalmers, rappelée par M. le Professeur Paneth, contient aussi l'hypothèse d'une charge électrique possible du radioélément formé. Dès lors on peut songer à le séparer commodément par action d'un champ électrique. C'est du reste ce qu'ont fait MM. Fay et Paneth pour la séparation du brome, de l'iode et du chlore radioactifs formés par irradiation de l'élément gazeux. C'est aussi la méthode mise au point par M. J. Govaerts (Liège) pour la collection rapide et commode du radiophosphore dans l'irradiation du sulfure de carbone par les neutrons. Cette méthode est susceptible de généralisation dans tous les cas où le milieu à irradier est sous forme gazeuse ou sous forme d'un liquide diélectrique.

M. J. Govaerts. — 1. Le Professeur Joliot vient de signaler la possibilité de séparer le radiovanadium en traitant simplement par de l'eau l'oxyde de vanadium irradié. Dans le même ordre d'idées, j'ai pu montrer, il y a quelques années, la possibilité de séparer dans certains cas l'élément radioactif formé en mettant à profit des différences de solubilité. Par exemple, un élément M est irradié sous forme d'un composé MR insoluble, le radical R étant choisi de telle manière que le composé M_1^*R soit soluble. Ainsi j'ai pu séparer notamment le radiosodium obtenu par irradiation de l'hydroxyde d'aluminium en traitant par de l'eau le produit irradié. Ne disposant que d'une source de neutrons constituée par un mélange de Ra et de Be, j'ai dû limiter les exemples aux cas des isotopes radioactifs des métaux alcalins et je n'ai pu appliquer cette méthode aux isotopes radioactifs des

métaux tels que le Cu, Zn, Co, Fe et d'autres. C'est précisément dans ces derniers cas que la méthode serait susceptible d'applications les plus nombreuses.

2. M. le Professeur de Hevesy vient de signaler la nécessité de pouvoir préparer dans certains cas l'élément radioactif sans qu'il soit mélangé avec l'élément stable correspondant.

D'autre part, nous savons que les quantités de substances ajoutées comme entraîneurs peuvent être très faibles et de l'ordre de 10^{-6} g. Des quantités aussi petites sont-elles encore susceptibles de modifier les conditions physiologiques normales?

M. de Hevesy. — If we prepare ^{42}K in the usual way, by bombarding a potassium salt, we obtain ^{42}K contaminated with appreciable amounts of stable potassium. The presence of 10^{-6} g. of stable isotope mentioned by Dr Govaerts will be usually harmless, though the introduction of 1 gamma iodine into the circulation of the mouse, for example, will create unphysiological conditions.

M. Paneth. — I agree with Professor de Hevesy that the meaning of scientific expressions may change in time; frequently the new meaning is better suited to the advanced state of knowledge and should be tacitly accepted, or even sanctioned by a new definition. I am of the opinion, however, that the altered meaning of the word « isotope » does not constitute such a progress because it threatens to deprive us of a good and clear word for expressing the special relationship in which the isotopes of one and the same element stand to each other. If we accept the diluted sense in which the word isotope is frequently used to-day, we shall soon have to invent a new word for the precise old meaning of isotope. Anyone looking at one of the modern tables of isotopes (i. e. nuclides) would be entitled to say that, e. g. ^{131}I and ^{14}C are isotopes, but to avoid misunderstanding he would then have to add that they are not «isotopes» in respect to each other or «isotopes in the old sense», or « isotopes of one and the same chemical element ». I agree, therefore, with M. Kohman that it is preferable to introduce for the new conception a new word, and to stick to the old, but still very necessary, meaning of isotope as defined by Soddy, if we use the old word.

As views on this point obviously vary, I think it would be useful if the question would be taken up by a committee, as international in composition as circumstances permit. Such a committee could,

at the same time, discuss the best method of putting superscripts and subscripts to the symbols of the chemical elements (the confused state of which has just been mentioned by Prof. Joliot), and also the question of the names of the newly discovered chemical elements where it seems that at least in one case (element 61) the issue is becoming controversial.

I believe that Professor Joliot is chairman of one or two committees interested in similar questions; perhaps he would care to take the initiative in arranging such discussions.

Dr. Aten. — 1. With regard to the symbols for isotopes it may be worth while to recall that according to the chemical custom the right hand top corner is reserved for the ionic charge: La^{3+}.

2. Can Professor Paneth tell us, whether the pile now working at Harwell can supply materials with total activities and specific activities sufficiently high for therapeutic uses?

M. Paneth. — I am afraid I am not well enough acquainted with the therapeutic uses of radioactive material to know what degree of activity might be considered as sufficient. On the whole I think it would be wise not to expect much help in this respect from the Harwell Graphite Low-Energy Experimental Pile or, abreviated, « Gleep ». Its main function is to act as a pilot plant for the construction of the full-sized uranium-graphite pile; the testing of material for this pile will have first priority, and the preparation of a few radioactive tracers is being undertaken simultaneously only because the demand for them is so urgent. Any other possible uses of the uranium piles will most likely have to wait till the large pile begins to operate.

M. J. Timmermans. — Je propose à M. le Professeur Joliot de soulever la question de la nouvelle terminologie et des notations dans la chimie nucléaire devant le Conseil International des Unions Scientifiques afin que celui-ci désigne une commission compétente pouvant prendre une décision à ce sujet.

The preparation of organic deuterium compounds

by

M. A. LANGSETH

The number of deuterated organic compounds which have been prepared in isotopically pure state is still comparatively small, in spite of the great importance that the investigation of isotopic species has for the molecular spectroscopy. The reason seems to be the scantiness of suitable procedures for introducing deuterium (hydrogen) in definite positions in organic molecules. This field in organic synthesis calls for further investigation. Even if several methods for substitution of hydrogen for other substituents are known, most of these appear to be of limited value for the preparation of partly deuterated compounds, because of the great mobility of the hydrogen atoms, resulting in unwanted intermolecular or intramolecular hydrogen-deuterium exchanges. Much work has already been devoted to the investigation of exchange reactions, but still much more has to be done before the conditions which govern the rearrangements have been sufficiently revealed to find the proper conditions for introduction of deuterium into definite positions in an organic molecule.

The following review makes no claim of completeness. It states the methods hitherto used, which have led to, or under proper conditions might be expected to result in the formation of pure isotopic species.

I.

HYDROLYSIS OR HYDRATION

Of the several methods of approach which are available for the synthesis of organic compounds containing deuterium the most

direct is hydrolysis or hydration of appropriate carbon compounds. The experimental procedures in these cases are mostly rather simple and often lead to samples of high isotopical purity.

Methane-d_4 [1]) and *acetylene-d_2* [2]) can easily be prepared by the reaction of aluminium carbide, resp. calcium carbide, with deuterium-oxide. In both cases the rate of reaction is found to be distinctly slower than with ordinary water. The replacement of acid hydrogen with deuterium may in some cases advantageously be effected through an intermediate metal compound. **O. Redlich and F. Pordes** prepared *methanol-d_1* [3]) and **H. Erlenmeyer et al.** *ethanol-d_1* [4]) by decomposition of magnesium methylate resp. sodium ethylate with D_2O. Similarly, *pyrrole-N-d* [5]) has been prepared by the reactions :

$$2\ C_4H_4NH + 2\ K \rightarrow 2\ C_4H_4NK + H_2$$

$$C_4H_4NK + D_2O \rightarrow C_4H_4ND + KOD.$$

Even if this synthesis is inconvenient and rather wasteful of D_2O and pyrrole [6]) it offers a certain garantee that only the nitrogen-bound hydrogen is replaced by deuterium.

Acetic acid-d_1 is formed directly in a pure state from the anhydride and D_2O [7]). A very pure sample was prepared by **H. Linschitz, M. E. Hobbs and P. P. Gross** [8]) by hydrolyzing acetyl chloride with D_2O. Dry nitrogen was bubbled through the mixture during the reaction and the subsequent distillations to sweep out DCl and excess of acetyl chloride.

Malonic-d_2 acid-d_2 was first prepared by **C. L. Wilson** [9]) by hydration of carbon suboxide in benzene solution with D_2O. **J.O. Halford and L. C. Anderson** [10]) **and P. Hölemann and K. Clusius** [11]) used the pure carbon suboxide. If the reaction was carried out at low temperature (5°) with an excess of D_2O no polymerization of the suboxide occurred. The excess of D_2O was distilled off in vacuum and the malonic-d_2 acid-d_2 dried at 80°.

The hydration of acetylene-d_2 with a deuterium oxide solution of phosphoric acid in the presence of mercuric sulfate as catalyzer has been used for the preparation of *acetaldehyde-d_4* [12]). The reaction products, acetaldehyde-d_4 and paraldehyde-d_{12}, were isolated by fractional distillation.

II.

REDUCTION PROCESSES

A. Catalytic reduction.

It is a well-know fact that the C-H bond in organic compounds is more or less labilized by catalytic active metals even at moderate temperatures [13]). This means that only completely deuterated compounds can safely be prepared by catalytic reduction processes.

Methane-d_4 has been made in this way from carbon dioxide [14]) and from carbon monoxide [15]) using a nickel catalyst at about 300°. The reactions

$$CO_2 + 4\ D_2 \rightleftarrows CD_4 + 2\ D_2O \text{ and } CO + 3\ D_2 \rightleftarrows CD_4 + D_2O$$

are reversible but the methane-d_4 may under proper conditions easily be isolated in isotopically pure state.

Naturally, unsaturated deuterium compounds can by catalytic reduction be converted into the corresponding saturated compounds. *Cyclohexane-d_{12}* was prepared from benzene-d_6 and deuterium at 180° over Ni [16]).

The catalytic reduction, however, is not applicable for the preparation of partly deuterated organic compounds because of the possibility of simultaneous H-D exchange reactions. **H. S. Taylor** et al. [15]) [17]) have shown that intermolecular exchange occurs between methane, methane-d_4, molecular deuterium, ethane, ethane-d_6 and deuterium oxide on an active nickel surface at least at 100-130°. In fact, they prepared *ethane-d_6-* from ethylene, C_2H_4, by the use of excess of D_2. With a platinum catalyst the exchange takes place even at room temperature [18]).

McLean and Adams [19]) have reported the preparation of *dimethyl succinate-d_4* by addition of deuterium to methyl acetylenedicarboxylate using a platinum oxide catalyst. The authors claim that no H-D exchange in the methyl groups takes place. The ester was hydrolyzed to *succinic-d_4 acid* with water acidified with nitric acid. Finally, phosphorous oxychloride was used for converting the acid into *succinic-d_4 anhydride*.

Similarly, **M. T. Leffler and Roger Adams** [20]) reported the reduction of diethyl fumarate and diethyl maleate with deuterium in the presence of a platinum catalyst. The reaction products appeared to

be identical, indicating no isomerism, corresponding to a *meso* and a *racemic* modification. However, no control was undertaken of the isotopical purity of the expected *succinic-α-d_1-α'-d_1 ester*. A contingent H-D exchange during the reduction has very likely taken place.

A. F. Thompson and N. H. Cromwell [21]) have prepared *benzaldehyde-d_1* and *p-phenylbenzaldehyde-d_1* by reduction of the corresponding acid chlorides with deuterium using a Pd-$BaSO_4$ catalyst. An isotopic analysis showed that a considerable exchange must have taken place even if the rate of this process appeared to be slow compared to the rate of reduction.

B. Reduction with nascent deuterium.

Also replacement of halogen by reduction processes with nascent deuterium causes considerable exchange [22]) and can therefore only be used for the preparation of hydrogen-free deuterium compounds.

Potassium acetate-d_3 has been made from CCl_3COOK by reduction with D_2O and potassium amalgam [23]).

E. Mac Wood and H. C. Urey [14]) have reported the preparation of the partly deuterated methanes : *methane-d_1*, *methane-d_2*, and *methane-d_3* from the corresponding iodides by reduction with deuterium oxide and amalgamated aluminium. The Raman spectra of the samples were taken, but no evidence of exchange seems to have been observed. **H. S. Taylor** et al. [25]), however, reduced CH_2J_2 by slowly addition to $C_2H_5OD + D_2O +$ Zn dust. The resulting gas was far from pure CH_2D_2, containing considerable quantities of CH_3D, as well as smaller amounts of CHD_3 and CH_4 revealed by the infra-red spectrum. A similar method for the preparation of CHD_3, using a mixture of CHJ_3 and $CHCl_3$ resulted in a gas, containing considerable amounts of CH_2D_2, CH_3D and CD_4, though preponderantly CHD_3.

C. Reduction with deuterium iodide.

H. Erlenmeyer and H. Gärtner [22]) have reported experiments on deuteration of cinnamic acid according to the method of **Gabriel**, using DJ and red phosphorous. Isotopic analysis of the reaction product revealed considerable exchange even in the phenyl group.

III.

ADDITION OF DEUTERIUM HALOGENIDE TO CARBON-CARBON DOUBLE AND TRIPLE BONDS

M. de Hemptinne, Jungers and Verhulst [23]) have prepared the different isotopical species of *ethylene dibromide*, *ethyl bromide* and *vinyl bromide* by reactions based on the addition of a DBr (resp. HBr) to ethylene (resp. ethylene-d_4) or acetylene (resp. acetylene-d_2), e. g.:

$$CH_2 = CHBr + DBr \rightarrow CH_2Br\text{–}CHDBr$$
$$CH \equiv CH + 2DBr \rightarrow CHDBr\text{–}CHDBr$$
$$CD = CD + 2DBr \rightarrow CD_2Br\text{–}CD_2Br$$
$$CH_2 \equiv CH_2 + DBr \rightarrow CH_2D\text{–}CH_2Br$$
$$CH_2 = CHD + HBr \nearrow CH_2D\text{–}CH_2Br \quad \searrow CH_3\text{–}CHDBr$$
$$CD_2 = CD_2 + HBr \rightarrow CHD_2\text{-}CH_2Br$$
$$CD_2 = CD_2 + DBr \rightarrow CD_3\text{–}CD_2Br.$$

M. de Hemptinne [24]) has succeeded in identifying the spectra of the different isotopic molecules through a systematic investigation of the Raman spectra of the prepared samples. Since the addition reactions are all reversible a certain H-D exchange is likely to occur during the preparation giving rise to the formation of different isotopic species.

A. Langseth and B. Bak [25]) have prepared $CH_2D.CH_2Br$ from ethylene and DBr, which at 200° was led through a tube containing a $BiBr_3$-catalyst. The Raman spectrum revealed that the product mainly consisted of *α-bromo-β-d_1-ethane* (about 90 %) but was contaminated with higher deuterated ethyl bromides, due to exchange reactions of the type :

$$CH_2 = CH_2 + DBr \rightleftarrows CH_2D\text{–}CH_2Br \rightleftarrows CHD = CH_2 + HBr$$
$$CHD = CH_2 + DBr \rightleftarrows CHD_2\text{-}CH_2Br \text{ or } CHDBr\text{–}CH_2D,$$

and so on.

In this connection two other methods for the synthesis of $CH_2D.CH_2Br$, carried through by **Langseth and Bak**, may be mentioned. In the first diazomethane was decomposed by acidified D_2O :

$$(1)\quad CH_2N_2 + D_2O \xrightarrow{(D^+)} CH_2D.OD + N_2.$$

The $CH_2D.OD$ was transformed into *ethyl-d_1 bromide* through the following reactions:

(2) $CH_2D.OH + HJ \rightarrow CH_2D.J + H_2O$

(3) $CH_2DJ + Mg \rightarrow CH_2D.Mg.J$

(4) $CH_2D.Mg.J + CH_2J_2 \rightarrow CH_2D.CH_2J + MgJ_2$

(5) $2\,CH_2D.CH_2J + 2\,CuBr_2 \rightarrow 2\,CH_2D.CH_2Br + 2\,CuJ + Br_2$.

According to expectations the product contained no higher deuterated compounds. Since it is difficult to prepare absolutely dry CH_2N_2, there was formed in the first stage a little CH_3OH causing a contamination of the final product with CH_3CH_2Br (ca. 10%).

In the second synthesis the following way was chosen :

(1) $CH_2(COOCH_3)_2 + D_2O \xrightarrow{(D^+)} CH_2(COOCH_3)(COOD) + CH_3OD$

(2) $CH_2(COOCH_3)(COOD) \rightarrow CH_2D.COOCH_3 + CO_2$

In order to suppress exchange of the hydrogen atoms of the CH_2-group in $CH_2(COOCH_3)_2$ during reaction (1) a strongly acidified D_2O was used. After decarboxylation the Raman spectrum of the sample was taken. This showed that actually a rather pure $CH_2D.COOCH_3$ was optained as no lines due to $CHD_2.COOCH_3$, $CH_3.COOCH_3$ or $CD_3.COOCH_3$ could be detected.

(3) $CH_2D.COOCH_3 + H_2O \xrightarrow{(H^+)} CH_2D.COOH + CH_3OH$

(4) $2\,CH_2D.COOH + Ag_2O \rightarrow 2\,CH_2D.COOAg + H_2O$

(5) $CH_2D.COOAg + Br_2 \xrightarrow{(\text{in } CCl_4)} CH_2DBr + CO_2 + AgBr$.

The Raman spectrum of the methyl-d_1 bromide prepared in this way showed that a serious exchange reaction had taken place supposedly during (5). The sample was a mixture of CH_3Br,CH_2 DBr, CHD_2Br and even a little CD_3Br could be detected. In spite of this derailment the synthesis was carried through as in the preceding method, resulting finally in a sample containing $CH_2D.CH_2Br$, $CH_3.CH_2Br$, $CHD_2.CH_2Br$ and a little $CD_3.CH_2Br$.

Addition reactions of the type, f. ex.:

$$CD \equiv CD + 2Cl_2 \rightarrow CDCl_2\text{–}CDCl_2$$

may, naturally, be used for the preparation of isotopical pure substances. By the use of a negative catalyst the combination of the gases can be well controlled and the risk of explosions minimized [26].

From sym. tetrachloroethane-d_2, $CDCl_2$-$CDCl_2$, chlorine can easily be split off by treatment with zinc in alcoholic solution, thus forming CDCl=CDCl in good yields. Similarly, splitting off deuterium halogenide (or D_2O) from deuterated alkyl halogenides (or alcohols) may in special cases lead to isotopically pure compounds, e. g.

$$CD_3.CD_2Br \xrightarrow{(KOH)} CD_2{=}CD_2$$

$$CD_3.CD_2OD \xrightarrow{(H_2SO_4)} CD_2{=}CD_2.$$

No exchange between the light solvent, KOH or H_2SO_4 and the *ethylene-d_4* was observed in the Raman spectrum [27]).

IV.

DECARBOXYLATION

Decarboxylation of deuterium acids can only be used for preparation of isotopically pure compounds if the decomposition can be carried through at low temperatures or if the substance to be synthesized is completely deuterated.

Formic-d_1 acid-d_1 [28]) was prepared by the thermal decomposition of anhydrous oxalic acid-d_2 in vacuum at about 180°. Similarly, **C. L. Wilson** [29]) converted malonic-d_2 acid-d_2 (prepared by hydration of carbon suboxide) into *acetic-d_3 acid-d_1* by simple heating to 150°. In this case isotopically pure samples seem to have been obtained.

Erlenmeyer and Lobeck [30]) prepared *benzene-d_6* by the reaction:

$$C_6(COO)_6Ca_3 + 3Ca(OD)_2 \rightarrow C_6D_6 + 6CaCO_3.$$

An isotopic analysis showed that the heavy benzene statistically contained about 0,5 H per molecule. This result is undoubtedly due to the difficulty of exclusing traces of ordinary water absorbed to the glass surface of the apparatus or present in the starting materials. **Morita and Titani** [31]) have reported the synthesis of *benzene-d_1* and **Weldon and Wilson** [32]) of *sym-benzene-d_3* from the corresponding carboxylic acids.

The decarboxylation method, however, is unsuitable for preparing partly deuterated aromatic compounds. **Redlich and Stricks**[33]) found the *benzene-d_2* formed by decomposition of as well phtalic acid-d_2 as of iso-phtalic acid-d_2 with $Ca(OD)_2$ to give identical Raman

spectra. It seems as if a complete intramolecular randomization of the positions of the two deuterium atoms occurs during the rather vigorous decomposition. **Weldon and Wilson** [34]) have confirmed this result in a careful investigation of the decarboxylation method for preparing partly deuterated benzenes. When calcium trimesate was heated with calcium deuteroxide exchange reactions took place before the decarboxylation, and even the deuterated benzene, formed by decomposition, could be shown to exchange with $Ca(OD)_2$. The second exchange could be minimised by working at low pressure, but the product obtained was a complicated mixture of different isotopic species, as shown by the infra-red spectrum.

V.

GRIGNARD REACTION

The Grignard reaction has often been used for the introduction of deuterium in definite positions in organic compounds. The experimental procedure is in most cases the standard method, except for special precautions, necessary to exclude light water during the reaction. By this method several attempts have been made to solve the stereochemical problem, whether a substance such as $\begin{matrix} R_1 \\ R_2 \end{matrix} \!\!>\!\! C \!\!<\!\! \begin{matrix} R_3 \\ R_4 \end{matrix}$, in which two of the groups differ only in their isotopic compositions can give rise to optical isomerism. As the compounds made for this purpose mostly have been investigated for optical activity only, nothing definite can be said about the isotopical purity of the products.

Clusius and Popp [35]) have obtained *methane-d_1* by decomposition of CH_3MgJ with D_2O. The molecular weight of the gas was found to be 17.054 compared to the theoretical 17.047. The isotopical purity seems therefore to be satisfactory.

The Grignard reaction when applied to the synthesis of an aliphatic compound often gives the corresponding olefinic compound, produced by splitting off hydrogen halogenide, as a byproduct, which can be difficult to separate from the saturated deuterium compound. In the case of volatile substances the olefinic compound can be distilled off together with the ether solvent before the Grignard compound is decomposed with D_2O [36]).

The Grignard reaction is most thoroughly investigated as a method for preparing the various deuterated benzenes. **Redlich ans Stricks** [37]) prepared *benzene-d_1* and *o-*, *m-*, and *p-benzene-d_2* from the corresponding bromobenzenes. From the measured Raman spectra can be seen that the samples must have contained considerable amounts of lower deuterated compounds. **Langseth and Klit** [38]) modified the Grignard reaction in order to be able to obtain the higher deuterated benzenes from polyhalogenated benzenes in one operation. The essential feature of the method is that deuterium chloride rather than deuterium oxide is used for decomposition of the Grignard compounds. In this way even *benzene-d_5* could be prepared from 2,3,4,6-tetrabromo-1-iodobenzene, the replacement of halogen by deuterium being effected continuously in a single chemical operation. All the different deuterated benzenes (except C_6D_6) were prepared in this way. The method was found to be quite satisfactory except for the great difficulty of removing the very last traces of water from the ether and from the apparatus, in which the Grignard reaction takes place. From the Raman spectra it could be made abundantly clear that only small amounts of certain lower deuterated benzenes, but never of isomers of the particular compound under consideration contaminated the products.

Weldon and Wilson [39]) have made a very careful study of the Grignard reaction as a method for preparation of isotopically pure *benzene-d_1 and* 1,4-*benzene-d_2*. It was found that when every precaution was taken to exclude moisture, a pure sample of benzene-d_1 could be obtained, whereas the benzene-d_2 prepared in the same way from the 1,4-dimagnesium Grignard complex contained a considerable amount of benzene-d_1. On the other hand, the monomagnesium compound from p-dibromobenzene gave isotopically pure *p-bromobenzene-d_1*, which in turn could be converted into the isotopically pure p-benzene-d_2. The authors therefore conclude that it seems essential to proceed the Grignard reaction in two steps, thus avoiding the formation of the dimagnesium Grignard complex. It is suggested that it is this compound which acquire the light hydrogen from the ether solvent. Further, **Langseth and Klit's** method was reinvestigated in the example of 1,4-benzene-d_2. Here also a certain amount of unwanted light hydrogen appeared in the product, which similarly was explained as due to the formation of some dimagnesium compound during the reaction.

In the referent's opinion, the Grignard reaction, within the limi-

tations of its general applicability, is the best method for introduction of deuterium into definite positions in organic molecules. For the benzene derivatives at least there is no intramolecular exchange, and the usually observed acquirement of hydrogen from sources outside the benzene molecule may be overcome by modification of the procedure without taking the drastic precaution of using heavy ether solvent.

VI.

EXCHANGE REACTIONS

The exchangeability of hydrogen atoms in organic compounds has been the object of numerous investigations [40]) most of which, however, was not undertaken with the purpose of preparing the deuterated compounds. This research has resulted in a fairly extensive knowledge of the general nature of the exchange process. On the basis of this knowledge it is now possible to estimate the possibilities of the reaction for synthetic purposes.

The number of pure deuterium compounds prepared in this way is, however, surprisingly small, considering the apparant simplicity of the procedure. This may chiefly be due to the fact that a comparatively large excess of the rather expensive deuterium oxide is necessary to ensure a sufficient isotopic purity of the deuterocompound.

If the exchange reaction is of the ionic type it reaches rapidly the equilibrium in homogeneous solution provided the implied acids are not excessively weak. In cases of low solubility of the organic compound in water an organic deuteroacid compound as f. ex. C_2H_5-OD may be used as solvent and deuterium donor. The procedure consists in principle simply in successive treatments of the organic compound with the deuterium donor in appropiate doses. The closer this procedure can be approximated to the differential exchange, i. e. the deuterium donor being removed from the reaction mixture at the same rate as it is introduced, the more economical of deuterium material the exchange reaction will be.

Oxalic acid-d_2, $(COOD)_2$, *formic-d_1 acid*, DCOOH (from D.COOD and H_2O)[28]) C_6H_5.CHOD.COOD and C_6H_5.C(CH_3) (OD).COOD [41]) are examples of organic acids prepared in pure state by exchange. In malonic acid all of the four hydrogen atoms can be replaced by exchange with D_2O. The previous mentioned synthesis of

$CD_2(COOD)_2$ from carbon suboxide will, however, probably be a better method. **Hölemann and Clusius** [42]) have used the exchange method to prepare *methylmalonic-d_1 acid-d_2*, $CH_3.CD(COOD)_2$, from which the propionic *α-d_2 acid-d_1*, $CH_3.CD_2.COOD$, was obtained by decarboxylation.

Methylamine-d_2, $CH_3.ND_2$, was prepared from hydrochloride $CH_3.NH_3Cl$, by exchange with deuterium oxide [43]).

Hydrogen bound directly to carbon atoms may often be able to exchange with deuterium oxide, for instance through an intermediate enolization. Numerous examples of exchange processes catalyzed by as well bases as acids are known. **Koizumi and Titani** [44]) have investigated the exchange between D_2O and pyrrole at different pH. They conclude that at $pH \geqq 2$ only one hydrogen exchanges, but that at $pH \leqq 1$ all five hydrogene exchange rapidly. **F. A. Miller** [45]) has applicated this to the preparation of *pyrrole-N-d_1*, *sym-pyrrole-d_4* and *pyrrole-d_5*. The deuterated products were investigated spectroscopically [46]) and found to be isotopically pure. The pyrrole-d_1 obtained by repeated treatment of pyrrole with small portions of D_2O was found to be identical with a sample of pyrrole-N-d_1, prepared from potassium pyrrole. Hence exchange between pyrrole and neutral water involves only the N-hydrogen.

Ingold, Raisin and Wilson [47]) have shown that deuteroacids are able to exchange with the aromatic nucleus of benzene and benzene derivatives. They have discovered the extremely interesting fact that the exchange reaction shows all the characteristics of an electrophilic substitution process, which is facilitated or retarded, and therefore oriented, in just the same way as all other aromatic substitutions of this kind.

For the preparation of *benzene-d_6* an aqueous sulphuric acid-d_2 of about 50 mols % concentration was used as deuterium donor [48]). The exchanges were performed by merely shaking the reagents together at room temperature for 3-4 days. Under these conditions equilibrium in exchange was reached well within the time allowed without practically any sulphonation taking place. In this way very pure samples of C_6D_6 have been prepared.

Further, it was demonstrated [49]) that, in the reaction between aniline hydrochloride and deuterium oxide, the ortho- and para-positions are the only nuclear positions which participate in the exchange. For the preparation of pure 1-,3-,5-*benzene-d_3*, aniline hydrochloride was brought to equilibrium repeatedly with deuterium

oxide, and the amino group in the formed 2,4,6-$C_6H_2D_3.ND_2$ was then replaced by hydrogen by diazotization and treatment with sodium stannite. It could be shown that the nuclear hydrogen atoms are not affected by the deamination process.

Best and Wilson [50]) have prepared 1,4-*dibromobenzene-d_4* by shaking p-dibromobenzene at 107° with successive portions of a strong sulphuric acid-d_2 containing 88.5 mols % D_2SO_4. An isotopic analysis of the final product showed the deuterium content to be 99.5 atoms%. The $C_6D_4Br_2$ was converted by the Grignard method into 1,2,4,5-*benzene-d_4*. The exchange reaction between bromobenzene and sulphuric acid-d_2 was likewise studied. Under the conditions investigated, however, the rate of sulphonation was comparable with the rate at which the last two hydrogen atoms exchanged, and therefore this method could not conveniently be used for the preparation of bromobenzene-d_5. This substance was prepared by bromination of C_6D_6 and then, by the use of Grignard reaction, converted into *benzene-d_5*.

Klit and Langseth [51]) prepared benzene-d_6 from benzene by an exchange reaction with deuterium chloride using $AlCl_3$ as a catalyst. The final product contained ca. 98 atoms % deuterium corresponding to a contamination with 10 % benzene-d_5. The course of the exchange was followed by taking Raman spectra at certain steps which showed that the exchange passed through all of the intermediate deuterium derivatives.

Bowman, Benedict and Taylor [52]) prepared C_6D_6 by exchange between benzene and deuterium oxide using nickel as a catalyst.

The possibilities of the exchange reaction as a method for the synthesis of isotopically pure deuterium compounds are still far from being fully explored. Further investigation may lead to a more or less general experimental procedure which is easier to perform and less wasteful in deuterium material than most of the methods hitherto used.

REFERENCES

1. H. C. Urey and D. Price, *J. Chem. Phys.* **2,** 300 (1934); — cf. R. M. Barrer, *Trans. Farad. Soc.* **32,** 486 (1936).
2. A. McKeller and C. H. A. Bradley, *Phys. Rev.* (2) **46,** 664 (1934); — F. W. Breuer, *J. Am. Chem. Soc.* **58,** 1289 (1936).
3. O. Redlich and F. Pordes, *Monatsh.* **67,** 203 (1936).
4. H. Erlenmeyer, H. Schenkel and A. Epprecht, *Helv.* **20,** 367 (1937).
5. O. Redlich and W. Stricks, *Monatsh.* **68,** 47 (1936); — G. B. Bonino and R. Manzoni-Ansidei, *Atti Accad. Lincei, Cl. sci. fis. mat nat.* **25,** 294 (1937).
6. F. A. Miller, *J. Am. Chem. Soc.* **64,** 1543 (1942).
7. A. Langseth, B. Bak and E. Möller, Unpublished.
8 H. Linschitz, M. E. Hobbs and P. M. Gross, *J. Am. Chem. Soc.* **63,** 3234 (1941); — cf. Engler, *Z. Phys. Chem.* **B, 32,** 471 (1936).
9 C. L. Wilson, *J. Chem. Soc.*, **1935,** 492.
10. J. O. Halford and L. C. Anderson, *J. Am. Chem. Soc.* **58,** 736 (1936).
11. P. Hölemann and K. Clusius, *Z. Phys. Chem.* **B, 35,** 261 (1937).
12. J. E. Zanetti and D. V. Sickman, *J. Am. Chem. Soc.* **57,** 2735 (1935); **58,** 2034 (1936).
13. Farkas, Farkas and Rideal, *Proc. Roy. Soc.* (London) **146A,** 630 (1934);— Horiuti and Polanyi, *Nature* **134,** 377 (1934); — Polanyi, *Trans. Farad. Soc.* **30,** 663 (1934); — Bowman, Benedict and Taylor, *J. Am. Chem. Soc.* **57,** 960 (1935); — Farkas and Farkas, *Trans. Farad. Soc.* **33,** 827 (1937).
14. G. E. MacWood and H. C. Urey, *J. Chem. Phys.* **4,** 402 (1936); — K. Clusius and L. Popp, *Z. Phys. Chem.* **B, 46,** 70 (1940).
15. W. S. Benedict, K. Morikawa, R. B. Barnes and H. S. Taylor, *J. Chem. Phys.* **5,** 1 (1937).
16. A. Langseth and B. Bak, *J. Chem. Phys.* **8,** 404 (1940).
17. K. Morikawa, W. S. Benedict and H. S. Taylor, *J. Am. Chem. Soc.* **58,** 1445, 1795 (1936).
18. A. Farkas, *Trans. Farad. Soc.* **36,** 522 (1940).
19. McLean and R. Adams, *J. Am. Chem. Soc.* **58,** 804 (1936).
20. M. T. Leffler and R. Adams, *J. Am. Chem. Soc.* **58,** 1551 (1936).
21. A. F. Thompson and N. H. Cromwell, *J. Am. Chem. Soc.* **61,** 1374 (1939).
22. H. Erlenmeyer and H. Gärtner, *Helv.* **19,** 145 (1936).
23. Verhulst, Jungers and de Hemptinne, *Naturwetensch. Tijdschr.* **22,** 56 (1940).

24. M. de Hemptinne and C. Velghe, *Physica* **5,** 958 (1938); — M. de Hemptinne, *Trans. Farad. Soc.* **42,** 5 (1946).

25. A. Langseth and B. Bak, *Kgl. Danske Vidsk. Selsk. Medd.* (in press).

26. F. W. Breuer, *J. Am. Chem. Soc.* **58,** 1289 (1936).

27. A. Langseth, unpublished.

28. Heman and William, *J. Chem. Phys.* **8,** 447 (1940).

29. C. L. Wilson, *J. Chem. Soc.* **1935,** 492.

30. H. Erlenmeyer and H. Lobeck, *Helv.* **18,** 1464 (1935); **19,** 336 (1936).

31. N. Morita and T. Titani, *Bull. Chem. Soc. Japan* **10,** 557 (1935).

32. L. H. P. Weldon and C. L. Wilson, *Nature* **137,** 70 (1936).

33. O. Redlich and W. Stricks, *Monatsch.* **68,** 47, 374 (1936).

34. L. H. P. Weldon and C. L. Wilson, *J. Chem. Soc.* **1946,** 244.

35. K. Clusius and L. Popp, *Z. Phys. Chem.* **B, 46,** 72 (1940).

36. A. Langseth and B. Bak, unpublished.

37. O. Redlich and W. Stricks, *Monatsch.* **67,** 213 (1936); **68,** 374 (1937).

38. A. Langseth and A. Klit, *Kgl. Danske Vidensk. Selsk. Math.-Fys. Medd,* **15,** 13 (1937).

39. L. H. P. Weldon and C. L. Wilson, *J. Chem. Soc.* **1946,** 235.

40. A review of exchange reactions, C. K. Ingold and C. L. Wilson, *Z. Elektrochem.* **44,** 62 (1938).

41. H. Erlenmeyer and H. Schenkel, *Helv.* **19,** 1199 (1936).

42. P. Hölemann and K. Clusius, *Ber. Chem. Ges.* **70,** 819 (1937).

43. H. J. Emeleus and H. V. A. Briscoe, *J. Chem. Soc.* **1937,** 127.

44. M. Harada and T. Titani, *Bull. Chem. Soc. Japan* **11,** 465 (1936); — M. Koizumi and Titani, *ibid,,* **12,** 107 (1937); **13,** 85 (1938).

45. F. A. Miller, *J. Am. Chem. Soc.* **64,** 1543 (1942).

46. R. C. Lord, Jr. and F. A. Miller, *J. Chem. Phys.* **10,** 328 (1942).

47. C. K. Ingold, C. R. Raisin and C. L. Wilson, *J. Chem. Soc.* **1936,** 1637.

48. C. K. Ingold, C. R. Raisin and C. L. Wilson, *J. Chem. Soc.* **1936,** 915.

49. C. K. Ingold, C. R. Raisin and C. L. Wilson, *J. Chem. Soc.* **1938,** 28.

50. A. P. Best and C. L. Wilson, *J. Chem. Soc.* **1946,** 239.

51. A. Klit and A. Langseth, *Z. Phys. Chem.* **A, 176,** 65 (1936).

52. P. J. Bowman, W. S. Benedict and H. S. Taylor, *J. Am. Chem. Soc.* **57,** 960 (1935).

Discussion du rapport de M. Langseth

M. J. Govaerts. — C'est avec un très vif intérêt que j'ai suivi l'exposé du Professeur Langseth passant en revue les différentes méthodes qui permettent de préparer les deutéro composés. Il est heureux de constater que malgré certaines difficultés résultant de l'existence des réactions d'échange des atomes d'hydrogène il est possible très souvent de choisir parmi différentes techniques. Les expériences réalisées avec les isotopes radioactifs et lourds du carbone suggèrent une autre possibilité de préparation : les synthèses organiques « in vivo ».

Les expériences étudiant la photosynthèse par la méthode des indicateurs radioactifs montrent que les microorganismes utilisent le Co_2 au cours de leur métabolisme. Ces organismes réalisent ainsi la synthèse de différentes molécules organiques marquées qu'il est possible d'isoler par la suite. Outre le bon rendement, cette technique présente l'avantage de supprimer les manipulations chimiques souvent longues et fastidieuses. Ces méthodes de synthèse « in vivo » doivent pouvoir s'appliquer à la préparation des deutéro composés par administration continue d'eau lourde chez l'animal. Je ne sais pas si des expériences de ce genre ont déjà été tentées mais pour le moment nous essayons de mettre à profit ces réactions de synthèse «in vivo» pour l'obtention de certaines molécules organiques marquées à l'aide du deuterium.

M. Rittenberg. — The utilisation of living organisms for the synthesis of labeled compounds is attended by many technical difficulties. The living cell makes not only, the compound desired but a lot of others. The yield of the desired compound is thus in general rather low. For example a 300 gram rat synthesizes per day only 20 mg of cholesterol. To bring the deuterium concentration of a rat (300 grams) up to 20 atom % D, requires the injection of 40 grams of D_2O. To keep the D concentration of the body fluids at 20% D it is necessary to give the rat 15 cc of 30 % D drinking water per day. Under these conditions, after 30 days, the rat will contain

about 1 gram of cholesterol containing but 10% D. The Pt catalysed exchange reaction will produce such a deutero cholesterol much more simply and cheaper.

The biological synthesis is further complicated by the difficulty one generally meets in the separation of the desired compound from the other labeled compounds which have been formed. Nevertheless in certain cases no method other than the biological one is available. Ten years ago we prepared deutero oleic acid, a compound which can neither be synthesized or prepared by the Pt catalysed exchange reaction, by feeding to mice deutero-stearic acid. Part of this stearic acid was converted « in vivo » to oleic acid. Such synthesis in general give very poor yields.

Dr Langseth has chiefly considered the preparation of isotopically pure deutero-compounds. For most biological compounds the synthetic requirements are much less stringent. Incompletely exchanged compounds are quite useable. For the preparation of such compounds less rigorous experimental conditions are practicable.

M. Ingold. — I should like to develop the point, which is implicit in several remarks contained in Professor Langseth's report, *viz.*, that isotopic exchanges are not to be considered as a separate class of reactions, since they are only special cases of substitution, and have available to them the same mechanisms which have been recognised as applying to substitutions in general. Such mechanisms may be classified according to the way in which the bond electrons become distributed during the displacement of the substituted atom X. We recognise, indeed, the three classes of substitution mechanism termed homolytic, electrophilic and nucleophilic:—

Mechanism		Type of fission	X = H	X = Cl
Homolytic		X . . R	+	+
Heterolytic	Electrophilic	X . . R	+	—
	Nucleophilic	X . . R	—	+

In this scheme, + means that the mechanism is typical, and — means that it is uncommon or non-typical, if not non-existent. Which of these two types of heterolytic substitutions is typical in a given case will depend, amongst other factors, upon whether this displaced atom, when ionic, is most stable as a positive ion or as a negative ion.

As the above scheme shows, hydrogen-substitution is typically either homolytic or electrophilic. Homolytic substitution arises with all types of hydrogen compounds, at high temperatures in the gas-phase (because homolysis has a smaller energy requirement than heterolysis), on the surfaces of metals able to atomise hydrogen (hydrogenation catalysts), in certain thermal decompositions (e. g., decarboxylations of acids and heavy-metal salts of acids), and in a limited number of radical generating reactions in solution (e. g., amalgam reductions under conditions in which ketones might give pinacols). Homolytic substitutions may be recognised through their possessing the kinetic characteristics of chain reactions; for homolytic fission necessarily involves free radicals, and radicals commonly produce other radicals. Such substitutions may also be recognised by the absence of sharply characteristic orientation laws, a circumstance due to the absence of strong polarity in neutral radicals.

Most of these points may be illustrated for the special case of homolytic hydrogen exchange, which takes place, *e. g.*, under conditions of catalytic hydrogenation, or as side-reactions during decarboxylation, or in amalgam reductions. As Professor Langseth has pointed out, there are no orientation laws, and randomisation of the hydrogen is a common feature. Therefore, as he says, the reactions are mainly useful for the preparations of completely deuterated compounds.

Electrophilic hydrogen substitution is typical of unsaturated and aromatic hydrogen compounds,—compounds which contain shells of so called π-electrons. Unlike σ-electrons which are concentrated mainly between the atomic nuclei, π-electrons occupy always an exposed situation, exterior to the nuclei which they shield; and this may be why π-electron compounds are particularly prone to attack by electron seeking reagents, rather than by nucleus-seeking reagents. Electrophilic hydrogen substitution thus requires an electrophilic reagent. It also requires a polar solvent or polar surface, because solvation energy or some other form of electrostatic energy is needed to supply their relatively high energy-demand of heterolysis. I have mentioned that the substituting agent must be constitutionaly electrophilic: what this involves may be illustrated with reference to nitration.

Any compound $Z\text{-}NO_2$ will be a nitrating agent provided Z strongly attracts electrons. Its efficiency as a nitrating agent depends on the strength of this attraction. The limit is reached if Z removes the

shared electrons completely, leaving the bare ion NO_2^+ (which is now well known, both as solid salts and in solution). Below this limit, we can make Z, in turn, a series of groups which, when free and negatively charged, would be anions of acids of decreasing strengths, and thus we may theoretically construct a series of nitrating agents in order of diminishing efficiency, *e. g.*,:

$$NO_2^+ > NO_2.SO_4H > NO_2.NO_3 > NO_2.\overset{+}{O}H_2 > NO_2OH$$

To summarise, electrophilic substitutions can be recognised by the facts that they take place with π-electronic compounds, in polar solvents, and with reagents whose type and relative efficiency can be specified in the manner illustrated. They also take place in accordance with sharply different orientation laws, which are very well worked out for reactions such as nitration. This is because of the essential polarity of the heterolytic processes. All this is just as true for electrophilic hydrogen exchange. In particular, reagents for this exchange reaction—deuterating agents (to pass at once to the form in which hydrogen exchange reagents are usually investigated),—are compounds of the type D-Z, in which Z attracts electrons: they are, in fact, deuteron-doners, *i. e.*, compounds of the class which Brönsted has taught us to classify as *acids*. And the stronger acids they are, the better deuterating agents they will be. The theoretical limit is reached when Z carries the electrons right away, and we are left with a bare deuteron; but this is never realised in practice. Proceeding from this limit, we can construct a series of reagents exactly as before, to represent decreasing relative efficiencies in electrophilic hydrogen exchange:

$$(H^+) > H.SO_4H > H.NO_3 > H.\overset{+}{O}H_2 > H.OH$$

We know that, in fact, aromatic compounds are deuterated by acids, the efficiency of the reagent depending, as illustrated, on the acid strengths. Furthermore, as Professor Langseth has already remarked, we know that the orientation laws, and the variations of reactivity from one aromatic compound to another, are the same for deuteration as they are for nitration.

The above bears on the question of whether the covalent ion H_3O^+ exists in aqueous acid. Some people believe that it does not, because Magat and others have failed to find it in the Raman spectrum. They assert that the acid protons of aqueous acid are not covalently

bound, and that when one of them joins a water molecule, one of the previously bound protons of that molecule simultaneously goes away. I do not accept this conclusion, because, if the protons in aqueous acids were bare, then aqueous acid should be a more powerful deuterating agent than molecular sulphuric acid, and this is most certainly not the case. I think the extra protons must be present as H_3O^+ ions, and that the difficulty of detecting such ions in the Raman spectrum is due to low intensities (which in turn are due to the high polarity and low polarisability of the bonds).

I would like to illustrate these remarks concerning electrophilic deuteration by a simple example. Phenol in aqueous solvents is deuterated instantaneously on the oxygen atom. In alkaline solutions, deuterium also enters the ortho and para positions. This is a time-reaction. Its rate passes through a sharp maximum when one half a molecule of alkali hydroxide is present for every molecule of phenol. The interpretation is as follows. In an alkaline solution of phenol, the four entities present are phenol, phenoxide ion, water and hydroxide ion. Of these, the aromatic compound which is most reactive towards electron-seeking reagents will obviously be the negatively charged phenoxide ion. And the best deuterating agent will be the strongest acid, which is phenol (possessing an OD group as a result of the instantaneous exchange). The rate is thus proportional to the product [OPh] [DOPh] and this will clearly go through a maximum when the amount of added alkali is enough to convert one-half of the originally taken phenol into phenoxide ion.

M. de Hemptinne. — Comme l'a dit Monsieur Langseth, il est parfaitement exact que les résultats obtenus en utilisant une même méthode peuvent dépendre de la façon de procéder des expérimentateurs.

C'est ainsi que, alors que la réaction :

$$CH_2N_2 + D_2O \rightarrow CH_2DOD + N_2$$

est signalée comme donnant du CH_2DOD pur, la même réaction effectuée par J. Beersmans et J.-C. Jungers (*Bul. Soc. Chim. Belg.*, **56**, 72, 1947) a donné un mélange des différents composés deutérés, ce qui en l'occurrence ne déplaisait pas aux auteurs.

De même, dans la préparation des bromures de vinyle, d'éthyle et d'éthylène, il est possible que des phénomènes d'échange puissent

se manifester de façon plus ou moins importante suivant les méthodes employées pour l'adjonction de l'acide bromhydrique à la molécule non saturée. Nous avons effectué cette fixation par réaction photochimique en prenant la précaution d'éliminer le produit formé par condensation à basse température, au fur et à mesure de sa formation. Nous n'avons pas observé d'échange notable.

La préparation des divers produits se fait par étapes successives. Dans le tableau ci-joint les flèches indiquent par quelles étapes nous avons généralement passé pour obtenir les produits désirés. L'arrachement d'une molécule de brome ou d'acide bromhydrique a été fait par les méthodes classiques.

Tableau I et II (v. pp. 262 et 263).

La réaction photochimique a été étudiée en détail par J.-C. Jungers et J. Verhulst (à paraître dans les mémoires de l'Académie Royale de Belgique).

On observe une période d'induction plus ou moins longue après laquelle la réaction s'effectue parfaitement. De légères traces d'oxygène, d'aldehydes ou de cétones sensibilisent la réaction, l'iode par contre l'arrête complètement.

Je voudrais signaler aussi un travail de Beersmans et Jungers (*Bul. Soc. Chim. Belg.*, **56**, I, 1947) dans lequel ces auteurs décrivent un mode opératoire aisé pour l'obtention en laboratoire de CD_3OD par réaction catalytique du CO sur le deutérium à 280° C et à 3,5 atmosphères. Cette réaction peut être utile pour l'obtention du groupe méthyle contenant $_{13}C$ en partant du $_{13}CO$. Par esterification des méthanols, ils ont pu préparer les composés halogénés correspondants (*Bul. Soc. Chim. Belg.*, **56**, 238, 1947).

Enfin, J.-H. Ganeff et J. Jungers ont pu mettre au point un procédé de préparation du chlorure de méthyle par réaction photochimique du Cl_2 sur CH_4.

En réglant convenablement les proportions Cl_2/CH_4 on peut obtenir le CH_3Cl et CH_2Cl_2 en mélange à l'exclusion de $CHCl_3$ et CCl_4.

On peut ainsi, en diminuant le rapport Cl_2/CH_4 produire le chlorure de méthyle, à l'exclusion de tout autre produit. En partant de $_{13}CH_4$ on pourrait ainsi obtenir aisément $_{13}CH_3Cl$ qui peut servir de point de départ pour les réactions ultérieures.

TABLEAU I

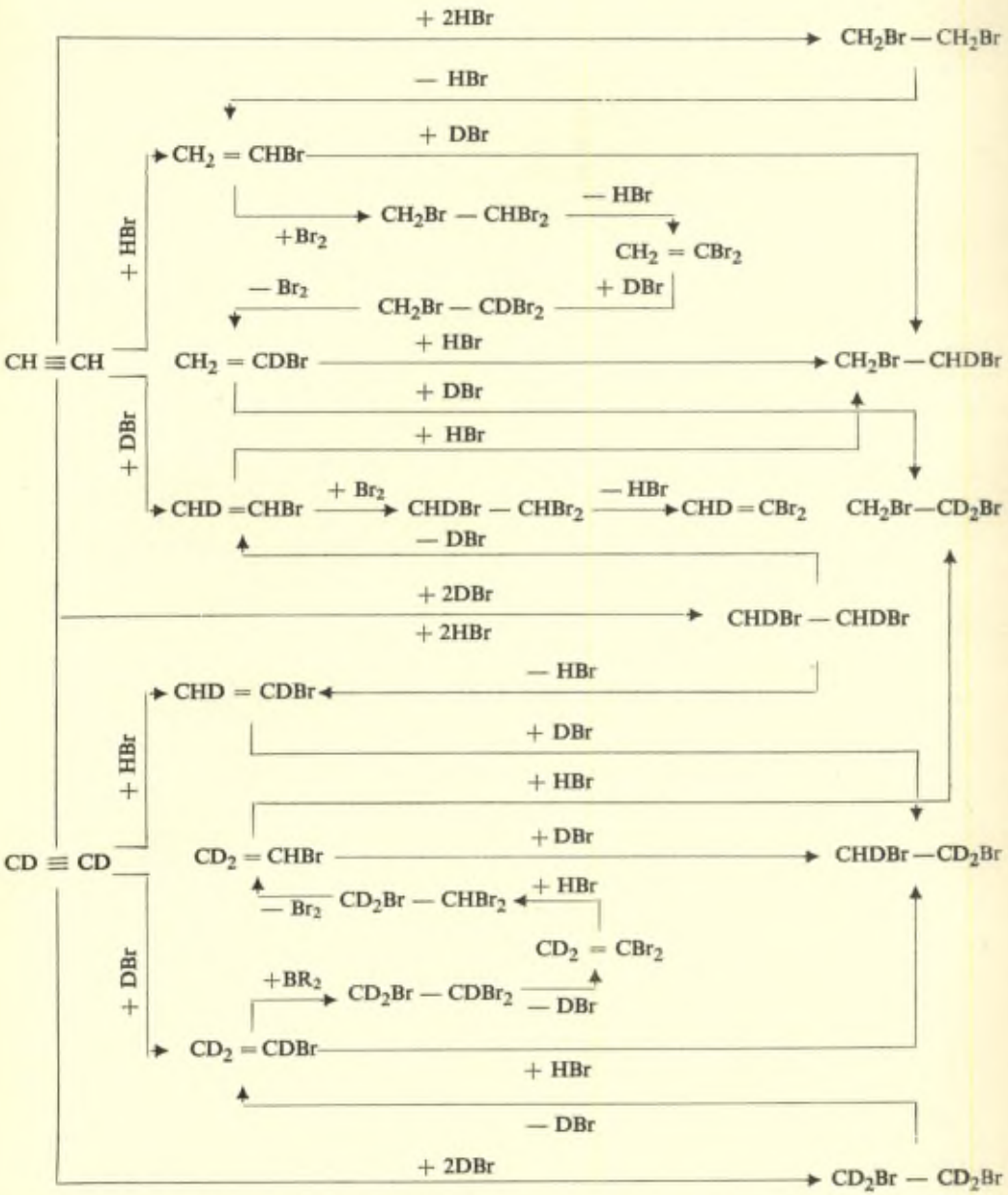
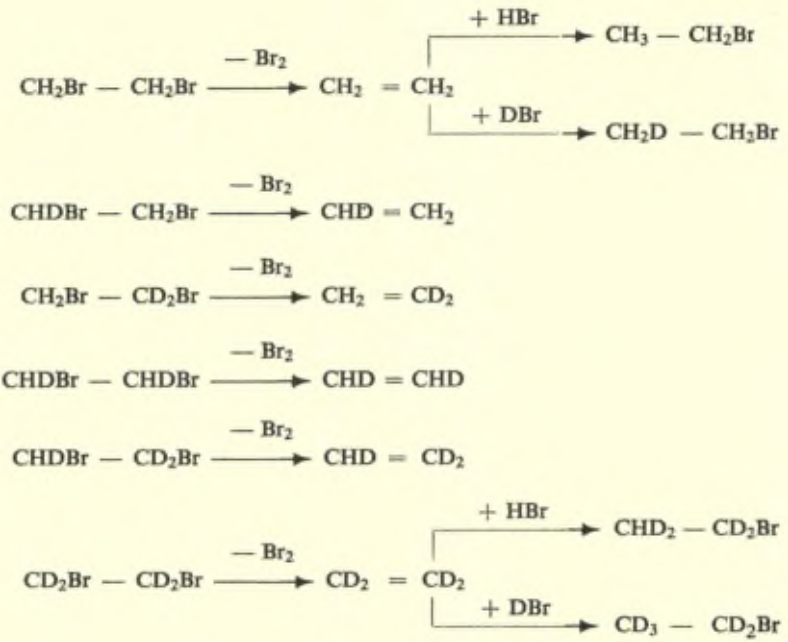


Schéma de préparation des bromures de vinyle et d'éthylène.

TABLEAU II



M. Langseth. — In our experiments we did not observe any exchange between the hydrogen atoms in CH_2N_2 and the deuterium oxide. The reason must be that when the ethereal solution is dropped into the acidified heavy water the reaction $CH_2N_2 + D_2O = CH_2DOD + N_2$ is much faster than the exchange reaction.

M. Rittenberg. — The difficulty just mentioned in the synthesis of CH_2D Br via diazomethane may arise from the fact that diazo methane exchanges its hydrogen atom with any water present. At O° and in the absence of acid the exchange is much faster than the decomposition to methanol. If D_2O is added to an ethereal solution of a diazo compound (CH_2N_2 , CH_3CHN_2 , $\begin{matrix}CH_3\\CH_3\end{matrix}$ > $CH\text{–}CHN_2$) there results the corresponding deutero compound ($R\text{-}CDN_2$). If the diazo compound is decomposed by the addition of a deutero acid, R' COOD, the disubstituted deutero ester results (R^1COOCD_2R). Hydrolysis yields the deutero alcohol. This route supplies a simple procedure for the preparation of small quantities of these alcohols (from 0.1 to 1 mole).

M. Calvin. — While it is certainly true that chemical synthesis is in general to be prefered over biosynthesis, especially by animals and plans, there are many bacteria which accomplish fairly clean syntheses and can be used to incorporate isotopic carbon into a variety of compounds, for example $^*CO_2 \rightarrow {}^*CH_4$ and $^*CO_2 \rightarrow {}^*CH_3$ $^*CO_2H$. It is very doubtful however whether such methods could by used for the preparation of deuterium labelled compounds, labelled in specific positions and certainly not possible for the preparation of isotopically pure compounds for spectroscopy studies such as those described to-day by Dr Langseth.

M. de Hevesy. — Not only bacteria but also mammalia are useful in carrying out biological synthesis.

Such a complicated substance as desoxyribose-nucleic acid is almost hopeless to synthetise, but we can easily obtain for example $_{32}P$ containing desoxyribose-nucleic acid by administering labeled sodium phosphate to the rat and extracting desoxyribose-nucleic acid from the intestinal mucosa or other organs.

M. Karrer. — On nous a raconté que la substitution de l'aniline et du phénol par le deuterium ne produit que des dérivés ortho et para. Les règles de substitution sont très souvent moins exactes, on obtient souvent quelques pour cents des produits meta.

Est-ce que la substitution par le deuterium est plus orientée en ortho o- et para p- ou peut-on observer aussi une petite quantité de l'isomère meta?

M. Ingold. — Orientation may be observed which is not typical for electrophilic substitution of the aromatic compound employed, if either the conditions permit homolytic substitution, or they are such that the entity substituted is not that introduced.

For example, Wibaut has shown that no sharply defined orientation laws apply to the bromination of certain aromatic substances as gasses at high temperatures. Under such conditions bromine molecules are converted with appreciable velocity into bromine atoms and I assume that it is the atoms which attack the aromatic nucleus. The reaction is thus homolytic, and no sharp orientation laws are to be expected.

Again partial meta nitration arises in the nitration of aniline or diméthylaniline in very strongly acid conditions. In this case there

is good reason to believe that the meta substitution product arises, not from the aniline itself, but from the anilinium ion. This should be substituted exclusively in the meta position, but very much more slowly than the aniline molecule is substituted in the ortho and para positions.

The deuteration of aniline has not yet been studied under such strongly acid conditions.

Under less strongly acid conditions aniline deuterates exactly where it nitrates and halogenates, *viz.*, in the ortho and para positions exclusively. As far as I know, phenol deuterates in the ortho and para position only, provided the conditions are not such as would lead to the deuteration of benzene itself.

M. Timmermans. — Monsieur Langseth a attiré l'attention sur l'importance de travailler avec des composés purs où le dérivé hydrogéné ne contamine pas trop le dérivé contenant le Deuterium. Il semble pourtant que dans beaucoup de cas la présence de quelques pourcents de dérivé hydrogéné léger ne gène pas trop l'étude spectroscopique puisque par comparaison on peut éliminer les raies provenant de cette source.

D'autre part dans beaucoup de cas il doit être possible d'extrapoler les propriétés du composé idéalement pur à partir de l'étude de composés souillés de pourcentages croissants d'impuretés.

Cependant il pourrait y avoir des cas où la préparation de composés contenant moins de 1/5.000e du composé correspondant contenant l'hydrogène léger, doive être réalisée et M. Langseth a insisté sur la nécessité de chasser complètement l'eau des appareils et des réactifs intervenant dans les préparations. Il reste alors quelques millièmes d'eau ordinaire contenue dans les échantillons commerciaux d'eau lourde même très pure et je voudrais savoir s'il existe une méthode pratique de préparer soit le Deutérium soit un de ses dérivés courants comme l'eau lourde contenant moins de 1/5.000e d'hydrogène léger.

M. Rittenberg. — One of the sources of dilution of deuterium in the synthesis of deutero compounds is the water absorbed on the glass apparatus employed. We have found that a glass vessel (pyrex) which has been carefully baked and evacuated appears to have a layer of water three molecules deep, over its entire surface. Of course the method employed did not differentiate between absorbed water and OH groups of the silicates.

The most general procedure for the synthesis of deutero-labelled organic compounds is the platinum catalysed exchange reaction. This reaction, though slow at room temperatures, proceeds at a sufficient rate at 125° C to become practical for synthetic purposes. It is effective with quite complicated substances and often is the only method available for their synthesis.

M. Ingold. — I do not know why catalytic hydrogen exchange in aniline and phenol is so sensitive to the solvent, but perhaps it may be that the phenomenon is connected with the way in which these molecules lie down, or refuse to lie down, on the catalytic surface, and with the question of whether or not the hydrogen bearing group gets into the layer of absorbed hydrogen atoms.

This in turn, may depend on how the molecules, which both contain more or less basic groups, are solvated by the acid and neutral solvents which provide the observed contrast.

Application of Labeled Phosphorus

by

G. de Hevesy

I.

CHEMICAL APPLICATIONS

Phosphorus compounds containing radiophosphorus found so far only a restricted application in inorganic and physical chemistry. Interchange studies were carried out between the P atoms of orthophosphate and of hexosemonophosphate (2), glycerophosphate (3), lecithin (4), casein (4), and nucleic acid (5), resp., which all led to a negative result. Nor was interchange found to take place between the phosphorus atoms of the orthophosphate and the phosphorus atoms of the pyrophosphate or metaphosphate in acid or alkaline solution between 20° and 100°, or between those of phosphate and hypophosphite in neutral or acid solution (1, 1_a).

^{32}P was applied in the study of the efficiency of the method of *Woy* (6) and that of *Delory* (7). When precipitating 14 mg labeled P by adding to the solution conc. NH^3, $CaCl^2$ and $MgCO^3$, as described by *Delory*, the filtrate was found to contain 0.2% only of the added ^{32}P. When however a solution containing 40 micrograms of P was precipitated, as much as 36% of the added ^{32}P was found to be present in the filtrate, showing that this method is not suitable to determine very small amounts of phosphorus.

^{32}P found also application in the radiometric titration of phosphorus (*7a*).

II.

BIOCHEMICAL APPLICATIONS

A. Application of Labeled Phosphate in the Study of the Intermediary Reactions of Glycolysis.

Experiments on the cell-free tissue extract were carried out in order to study the intermediary reactions of glycolysis (8, 9, 10). The following equation shows in which way inorganic phosphate participates in the splitting of sugar :

a) 1 glycerolaldehydphosphoric acid + 1 cozymase + 1 adenosinediphosphoric acid + 1 phosphoric acid $\rightleftarrows$ 1 phosphoglyceric acid + 1 dihydro-cozymase + 1 adenosintriphosphoric acid; or, alternately,

b) 2 glycerolaldehydphosphoric acid + 2 cozymase + 1 adenosinemonophosphoric acid (adenylic acid) + 2 phosphoric acid $\rightleftarrows$ 2 phosphoglyceric acid + 2 dihydrocozymase + 1 adenosintriphosphate.

When equilibrium is obtained, the application of the usual chemical methods does not indicate any change to go on in the above mentioned systems. However, when labeling the phosphate by addition of ^{32}P, we can observe a gradual replacement of active phosphate ions by non-active ones, and vice versa. The application of the radioactive indicators permits the splitting of the dynamic equilibrium and its 2 components and the measurement of the velocity of each of the reactions.

Meyerhoff and his associates (8) combined the above reaction with the reaction of reoxydation of dihydro-cozymase.

c) 2 dihydro-cozymase + 2 pyruvic acid $\rightleftarrows$ 2 cozymase + 2 lactic acid, investigating thus the reaction;

d) 2 triosephosphoric acid + 2 pyruvic acid + 1 adenosinemonophosphoric acid + 2 phosphoric acid $\rightleftarrows$ 2 phoshphoglyceric acid + 2 lactic acid + 1 adenosinetriphosphoric acid.

Cozymase acts as a catalyst.

One of the points investigated is the exchangeability of cozymase P.

B. Effect of Cozymase on the Interchange between Inorganic and Creatine Phosphate.

No significant interchange takes place between creatine P and inorganic P in a muscle extract dialysed for 50 hours in the absence of cozymase. If however cozymase is added to the system an appreciable percentage of creatine P interchanges with inorganic P, as seen in table I.

TABLE I

Effect of Cozymase on P Interchange between Creatinephosphoric Acid and Inorganic Phosphate (8).

SYSTEM	Labeled inorganic P in mg.		Creatine phosphoric acid P in mg.	Labeled creatine phosphoric acid P in mg.
	After 0 min.	After 30 min.	After 30 min.	After 30 min.
Muscle extract dialysed for 6 hours	1.15	0.810	0.65	0.315
Muscle extract dialysed for 50 hours without cozymase	1.29	1.25	1.09	0.03
Muscle extract dialysed for 50 hours with 0.4 mg cozymase	1.29	0.91	1.15	0.40

In these experiments, to 1 ml muscle extract, 6 mg. creatine, 0.1 mg. Mg and adenosinetriphosphate containing 0.43 mg labile P were added. The total volume amounted to 2.1 ml. The experiment was carried out at 40° C.

C. Transfer of Phosphate Groups.

That, in several cases, a phosphate group is transferred from one molecule to another without passing through the inorganic stage can be shown by an absence of ^{32}P in the reaction products in spite of the presence of labeled inorganic phosphate. If to muscle extract phosphoglyceric acid, magnesium and labeled inorganic phosphate are added, the reversible reaction

$$3 \text{ phosphoglyceric acid} \rightleftarrows 2 \text{ phosphoglyceric acid}$$

was found to take place without the phosphoglyceric acid becoming active.

In a similar way, the transition of glucose -1- phosphoric acid (Cori ester) into glucose –6– phosphoric acid (Robinson ester) was studied by *Hevesy* and his associates (9) and by *Parnas* (10) in the presence of labeled inorganic P and it was found that the esters did not become active. Furthermore, when adenosinetriphosphoric acid was formed from adenylic acid and phosphoglyceric acid in the presence of labeled phosphate, the phosphoglyceric acid was found to be inactive. In all these cases, a direct transfer of phosphate group from one molecule to another took place without passing through the inorganic stage. A possible explanation of the above results is that, for example, phosphoglyceric acid and adenylic acid together form a molecular complex with the enzyme, the phosphate radical is shifted from one molecule to the other, and then the products, phosphoglyceric acid and adenosinetriphosphate, leave the enzyme surface. The labeled inorganic phosphate present in the solution has then clearly no opportunity to participate in the reactions.

Schlamowitz and Greenberg (10*a*) investigated recently the conversion of glucose-I-phosphate to glucose-6-phosphate under the influence of phosphoglucomutase in a medium containing ^{14}C labeled glucose. As was to be expected from the results stated above, no incorporation of radioactive glucose into the glucose-6-phosphate molecule could be detected. As to the transfer of the phosphate group within the glucose molecule from 1 to the 6 position it is suggested that it possibly takes place through the formation of an intermediate phosphate diester.

When adenylic acid is formed from adenosine in the presence of hexosediphosphate and labeled inorganic phosphate in yeast, one half of the phosphorus atoms were found to be those originally located in the hexosediphosphate molecules, while the other half were originally present as inorganic phosphate (9, 10).

D. Exchangeability of Cozymase P.

If the cozymase molecule splits off in the course of the above-mentioned reaction and is rebuilt under incorporation of other phosphate radicals, in the presence of labeled phosphate the cozymase molecule should become active. Though in a cell-free system containing cozymase, hexosediphosphate, acetylaldehyde, glucose, labeled phosphorus as inorganic phosphate and other ingredients,

each cozymase molecule was shown to participate in the interchange cozymase $\rightleftarrows$ dihydrocozymase 4.5 times during the experiment, the cozymase isolated after the lapse of 75 minutes was found to be inactive (8). *Lindahl* and assoc. (14) found recently that in the yeast cells some interchange between cozymase P and inorganic P does take place (14).

E. Conversion of β-Glycerophosphoric Acid into the α Form.

Radio-phosphorus was applied by *Chargaff* (11) in the investigation of the problem whether the migration of phosphoric acid from the β to the α position of glycerol, which can be effected by acid catalysis or by enzymes, is accompanied by an exchange between the esterified phosphoric acid and the inorganic phosphates of the surrounding medium. To a solution of sodium β-glycerophosphate labeled sodium phosphate and conc. sulphuric acid were added. The barium α-glycerophosphate obtained after the solution was kept boiling and barium hydroxide added was found to be inactive. The same negative result was obtained when the hydrolysis of β-glycerophosphoric acid was effected by kidney phosphatase in the presence of labeled sodium phosphate. Thus during isomerization of β-glycerophosphoric acid to the α form in acid medium the esterified phosphoric acid could at no time have been in equlibrium with the inorganic phosphate present in the solution.

The absence of radioactivity from the residual glycerophosphoric acid isolated in the enzyme experiments, which were stopped before the hydrolysis was complete, demonstrates that under the experimental conditions no appreciable synthesis of the phosphoric acid ester from glycerol and inorganic phosphate took place and that the enzymatic attack dit not labilize the substrate to such an extent as to induce free exchange with the inorganic phosphate of the medium.

F. Conversion of Prothrombin to Thrombin.

It has long been a matter of conjecture whether thrombin represented a compound between prothrombin, calcium and the thromboplastic factor (or the cephalin contained in it), or whether these substances interacted in some different manner. Labeled phosphate

was used to answer this question (12). Radioactive thromboplastin has been isolated from rats to which labeled phosphate was administered. Experiments carried out making use of the radioactive thromboplastin lead to the result that the amount of P-containing compounds transferred to the thrombin, if at all significant, is extremely small.

C. Exchange between Inorganic and Acetyl-bound Phosphate.

By use of radioactive phosphate, the exchange between inorganic and acetyl-bound phosphate with extracts of *Clostridium butylicum* and of *Escherichia coli* was studied by *Lipmann and Tuttle* (13). Reversibility of a reaction of the phosphoroclastic type: (*)
$CH_3CO.COOH + HO.PO_3 \rightleftarrows CH_3COO \sim PO_3(H_2 + CO_2)$ or HCOOH
involves a continuous shift between inorganic and acetyl phosphate and this exchange was explored with labeled phosphate. After the lapse of 3 minutes a 34 % exchange is reached while in analogous experiments with *Escherichia coli* an exchange of similar magnitude is only observed after the lapse of 20 minutes.

The rate of exchange is influenced none or little by the addition of reactants other than acetyl phosphate.

III.

ANIMAL PHYSIOLOGY.

Radiophosphorous found its main application in the field of animal and partly also of plant physiology. Since the first application in 1935 of ^{32}P in the study of the circulation and distribution of labeled phosphorus in the rat (15) many hundreds of investigations were carried out, making use of radiophosphorus as an indicator. The application of radiophosphorus far outdistances the application of all other radioactive isotopes. This is due not only to the

(*) The sign $\sim$ is used by *Lipmann* and associates to distinguish the energy-rich phosphate bond with an average bond energy of 12 kilocalories from the ordinary ester bond with around 3 kilocalories.

convenient mode of production and period of decay of this isotope (T = 14.3 days), together with the low absorbability of the rays emitted by it, but mainly to the important part which phosphorus plays in a very great number of metabolic processes. These include skeleton formation, metabolism of carbohydrates and fats, cell division, and many other processes.

A. Permeability Measurements.

^{32}P was applied in the determination of the phosphate permeability of the capillary wall, the tissue cells, the placenta, the blood-cerebrospinal fluid barrier, the red corpuscles, the yeast and so on.

a) *Capillary Wall*

The great permeability of the capillary wall is seen in table I.

TABLE I

Rate of Disappearance of ^{32}P from the Circulation of a Rabbit Weighing 2.1 kg (16).

Time in Min.	Per Cent of injected ^{32}P present in 1 g plasma	Diluting fluid volume	
		in ml (apparent extra cellular volume)	in per cent of body weight
1.1	0.300	333	15.9
1.9	0.234	427	20.4
3.0	0.187	535	25.5
4.5	0.143	699	33.3
6.8	0.112	892	42.5
10.9	0.081	1230	58.6
16.9	0.060	1670	79.5
25.9	0.046	2180	104
39.0	0.032	3130	149

Due to intrusion into the tissue cells the apparent extracellular volume is already after the lapse of 3 minutes larger than the genuine extracellular volume which makes out 500 ml.

The percentage rate of disappearance of labeled phosphate from the blood plasma is less rapid than that of labeled K, but more rapid than that of labeled Na (16, 17).

Liver and kidney cells are in contrast to muscle cells easily permeable to phosphate (18, 19, 20, 21). Rabbit muscle cells are less permeable than those of the rat (almost 100 micrograms of P penetrate in the course of 2 hours from the extracellular fluid into the cells of 1 g gastrocnemius tissue and *vice versa*) and very much lower figures were found for frog muscle. The difference is partly due to the lower temperature at which the permeability of the frog muscle cells was determined. At 22°, in the course of 2 hours, 1.7 microgram, at 0°, 1/5 that value was found to penetrate into the muscle cells (22).

From the above data the coefficient of penetration of the phosphate ion into the frog muscle at 22° works out to be about 1.8×10^{-5} cm/h (23).

The permeability of the liver cells to phosphate was also determined (24) in experiments of very short duration in which the extracellular phosphate of the organs was removed by perfusion. The specific activity of the inorganic intracellular P of the rabbit liver was found 5 minutes after intravenous injection of labeled phosphate to be 8 to 37 % that of the inorganic plasma P. In interpreting these figures we must take into account that the intruding ^{32}P participates speedily in phosphorylation processes going on in the liver cells while simultaneously inactive inorganic phosphate ions are split off from organic phosphorus compounds. This process is instrumental in lowering the specific activity of the inorganic intracellular liver P. The above figures indicate very high permeability of the liver cells to phosphate.

In experiments with skeletal muscles after the lapse of 15 to 20 minutes the specific activity of the intracellular inorganic muscle P was found to be only 1.5 per cent of the value for the inorganic plasma P. If the extracellular ^{32}P was not removed by perfusion of the tissue, the corresponding figure was 3.5. These results demonstrate the very great share of the extracellular ^{32}P in the total ^{32}P of the muscle tissue in experiments of very short duration.

Muscular work does not influence appreciably the interchange between the phosphate of the muscle cells and that of the plasma, as seen in table II. The experiments were carried out on rats weighing 170—210 g. The rats were swimming and resting alternately for 30 min.

TABLE II

Distribution Coefficient of ^{32}P between Gastrocnemius and Plasma of Equal Weight in the Rat (36).

Time of swimming in hours	Time of the experiment in hours	Distribution coefficient of ^{32}P between gastrocnemius and plasma of equal weight
0	7.1	5.52
0	7.6	4.90
0	7.7	5.54
0	7.1	6.80
2.3	7.1	5.25
3.4	7.5	5.27
3.2	6.8	4.38

Other experimentors found the ^{32}P content of inorganic phosphate in the working muscle to be 18 per cent higher only than in the resting muscle (26).

When measuring (27) the rate of penetration of labeled phosphate in slices of the cardiac muscle of the cat, incubated in bicarbonate Ringer at 37°5, 1.7 microgram of P was found to penetrate per minute into 1 g of tissue, this being somewhat more than the amount found to penetrate into 1 g of gastrocnemius of the rat as mentioned above. The penetration of phosphate into the tissue slices at 20° amounts to only 1/5th to 1/10th of that measured at 37°5.

The remarkable rate at which labelled inorganic P leaves the plasma even as late as a few hours after administration and the rate at which liver inorganic P and kidney inorganic P get labeled are seen in table II*a*. The average specific activity of the liver inorganic P works out to be 2.54, not much differing thus from the specific activity found after the lapse of 2 and 3 hours, resp. (cf. also 138). The finding that the end value of the specific activity in a 2 hours experiment almost coincides with the average value during the experiment facilitates the calculation of the renewal figures in the liver discussed on p. 298. When interpreting the figures obtained for the kidney it must be taken into account that the kidneys contain urine produced from plasma of higher activity than measured at the time when the kidney sample was secured.

TABLE II *a*

Specific Activity of Inorganic P of Plasma, Liver and Kidneys of Adult Rats at different Times after Subcutaneous Administration of ^{32}P (108a)

Time in hours	SPECIFIC ACTIVITY (Per cent of ^{32}P administered present in 1 mg inorg. P)		
	Plasma	Liver	Kidneys
0.5	9.80	2.38	5.19
1	5.43	3.56	3.79
1.5	1.67	3.42	3.05
2	1.92	2.76	2.29
3	1.47	2.59	1.76

b) *Blood Flow into the Liver.*

By applying radiophosphorus as an indicator, the streamline flow of blood into the liver was demonstrated (28).

When labeled phosphate was injected into the splenic vein a several times greater concentration of tagged phosphorus was found in the left side of the liver than in the right side within about 3 seconds after the injection. In contrast, when the same material is injected into a branch of the mesenteric vein, there is a markedly greater accumulation of labeled material on the right side of the liver. When the injection is made in the jugular vein and sufficient time (5 min.) is allowed for complete mixing with the circulating blood, there are no demonstrable differences in the concentration of tagged material in various transverse sections taken from the liver. The results indicate that there is a physiological bilaterality of blood flow in the portal vein which is streamline in nature. Splenic blood is thus largely sent to the left side and mesenteric blood to the right side of the liver.

c) *Placenta and Cerebrospinal Fluid.*

Unlike sodium and water which are supplied to the fetus greatly in excess of the quantities incorporated in growth, inorganic phosphate reaches the fetus from the maternal plasma in an amount only approximately equal to the total phosphorus retained in growth (29). The blood-cerebrospinal fluid barrier is passed at a slower rate by phosphate than by K, Na, Br, Rb or Sr (30).

d) *Red Corpuscles.*

When labeled sodium phosphate of negligible weight is added, for example, to human blood kept at 37°, after the lapse of two hours about 1/3 of the ^{32}P atoms is found in the red corpuscles, indicating a fairly rapid penetration of the phosphate ions through the corpuscle membrane (31, 32, 33). In spite of this fact and the finding that the inorganic phosphorus content of 1 g corpuscles is lower than that of 1 g plasma, the ratio of inorganic ^{32}P:^{31}P is found after 2 hours to be much lower in the corpuscles than in the plasma. This result is due to a very rapid renewal of many of the organic acid-soluble compounds present in the corpuscles (34). In the course of phosphorylating processes incessantly going on in the corpuscles such acid-soluble molecules split off their phosphate content while others get phosphorylated under participation of labeled inorganic phosphate. This process leads in experiments of *restricted duration* to a replacement of much of the inorganic ^{32}P of the corpuscles by ^{31}P previously located in the organic molecules. It is due to this fact that, while from 100 ^{32}P atoms added to the plasma, in the course of 2 hours, about 30 pass into the corpuscles, from 100 ^{32}P atoms present in the corpuscles only about 2 move in the opposite direction.

The fact described makes possible the application of «phosphorus labeled» red corpuscles in the study of the circulation of the red corpuscles and in the determination of the amount of circulating red corpuscles in the total body or in a single organ (35, 37, 38, 39, 40). Labeled red corpuscles found not only application in the study of the red corpuscle volume of the body on a very extensive scale, but also in the determination of the red corpuscle content of single organs (40, 40*a*, 40*b*).

e) *Yeast.*

Into growing yeast cells appreciable amounts of phosphate migrate from the nutritive solution (41, 42, 45). The interchange between the phosphorus atoms of the yeast cells and the nutritive solution is, however, for many yeast types very restricted (43). This can best be shown when yeast is grown in a medium containing ^{32}P, the labeled yeast obtained being washed and shaken for a day with non-labeled nutritive solution at 15°. Only a small percentage of the ^{32}P content of the yeast cells is found to be present in the nutritive solution (44).

A detailed discussion of the application of radiophosphorus in permeability studies is to be found in a monograph by the author, which is in print.

B. Absorption, Excretion and Distribution.

a) *Absorption.*

By measuring the activity of known aliquot parts of the urine and the feces excreted, the daily loss of ^{32}P through both the kidneys and the bowels can be followed. Numerous such determinations were carried out (46), the values found showing great variation for different individuals, much depending also on the diet given. Five milligrams of labeled phosphorus as orthophosphate, pyrophosphate, yellow phosphorus, and red phosphorus, respectively, were given to fasting rats weighing about 200 g. (47). The amount of labeled phosphorus absorbed within 4 hours was found to be 89.3, 84.8, 80.2 and 35.0%, respectively.

b) *Excretion.*

The rates of excretion of ^{32}P administered orally or by intravenous injection to human subjects, determined by *Erf, Tuttle* and *Lawrence* (48), are shown in table III.

TABLE III

Rate of Excretion of ^{32}P in Urine and Feces (48).

Excreted in	Per cent Excreted						Total Excreted
	Days after ^{32}P Administration. (*)						
	1	2	3	4	5	6	
Oral Administration.							
Urine	4.63	1.7	0.89	0.72	0.74	0.45	22.9
Feces	12.93	0.52	0.22	0.02	—	0.06	
Intravenous Administration.							
Urine	37.66	5.03	2.48	1.69	1.74	1.16	50.05
Feces	0.098	0.25	0.24	0.16	0.02	0.05	

* 600 mg $NaHPO_4$ given containing 1500 microcuries ^{32}P.

In the course of 4 days, 6 % of the ^{32}P injected into the veins of human subjects were found by *Govaerts* and *Lambrechts* (49) to be excreted by the kidneys. While many aspects of the absorption and excretion of phosphorus can be investigated without making use of isotopic indicators, this statement does not apply to the problem of the origin of feces phosphorus.

c) *Origin of Feces Phosphorus.*

By using radiophosphorus as an indicator, *Hevesy et al* (50) determined to what extent fecal phosphorus is composed of unabsorbed (exogenous) phosphorus and to what extent of phosphorus derived from the body proper.

Let us assume that all phosphorus present in the food is absorbed into the circulation. Then, all labeled phosphorus found in the feces must originate from the body proper. It is ultimately the plasma inorganic phosphorus which is responsible for the formation of the phosphorus compounds present in the digestive juices; and, therefore, the specific activity (activity per milligram of phosphorus) of the feces phosphorus should, in the case mentioned above, be equal to that of plasma phosphorus. The specific activity of the inorganic plasma phosphorus being equal to that of the urine phosphorus, we shall expect to find the specific activity of the feces phosphorus equal to that of the urine phosphorus. If the above assumption does not hold, and if part of the feces phosphorus consists of unabsorbed, inactive phosphorus originating from the undigested food, the specific activity of the feces phosphorus will be found to be lower than that of the urine phosphorus.

The ratio:

$$\frac{\text{specific activity of feces phosphorus}}{\text{specific activity of urine phosphorus}} \times 100$$

gives the per cent phosphorus of the feces which originates from the body proper. If food phosphorus is, for example, quantitatively absorbed, the above ratio will be 100. The objection which might be raised against the above considerations is that the digestive juices contain not only acid-soluble phosphorus compounds, most of which become labeled within a short time, their activity per milligram being equal to that of the specific activity of the plasma inorganic phosphorus, but also phosphatides and other phosphorus compounds which are renewed and thus labeled at a slow rate. This objection will not be valid, however, if we start to collect the urine and feces samples some time after administration of the labeled phosphorus. After the lapse of a considerable time, most of the phosphorus present in the different compounds of the organs responsible for the production of the digestive juices will be in exchange equilibrium with the plasma phosphorus, and will thus have about the same specific activity.

Twenty-eight days after subcutaneous injection of the labeled sodium phosphate into a female subject the specific activity of urine phosphorus was found to be 8.08, and for fecal phosphorus the value was 1.77. Of the total phosphorus of the feces, 18% was residual phosphorus obtained after removal of acid-soluble phosphorus (mostly calcium phosphate) and traces of phosphatide phosphorus. The specific activities of the different phosphorus fractions varied only to a minor extent. The specific activity of the feces phosphorus is only 22% of the specific activity of the urine phosphorus; the feces phosphorus, therefore, to a large extent must have originated from unabsorbed food which is the only source of non-active phosphorus. From the above figures it follows that 78% of the phosphorus present in the feces of the human subject in question was unabsorbed phosphorus; the rest originated from the body proper. This result is, however, not to be interpreted as an indication that only 22% of the food phosphorus was absorbed. When interpreting the above figures we must take into account that the phosphorus excreted through the kidneys constitutes about twice that lost through the bowels; the sum of both values represents the total phosphorus present in the food, if we assume that the subject is in phosphorus balance. Then we find that only 26% of the total phosphorus of the food was not absorbed into the circulation.

A detailed investigation of the excretion of phosphorus by the bowels was carried out by *Kjerulf-Jenens* (51). In his experiments the average endogenous feces P of human subjects on normal diet varied between 24.8 and 31 % of the total feces P.

Govaerts (52) found the endogenous part of fecal phosphorus to be increased after administration of vitamin D to human subjects. Furthermore, the percentage excretion of ^{32}P both through the kidneys and the bowels was found to be increasing after administration of this vitamin.

The method outlined above can be used to determine what percentage of almost any element present in the feces is of endogenous origin.

d) *Distribution in Organs of the Rat.*

In the early phases of distribution studies, about the same percentage of the administered labeled phosphorus (mostly Na_2HPO_4) is found in the muscles and in the skeleton (see table IV). With

increasing time more labeled phosphate accumulates in the skeleton (see table V).

TABLE IV

Distribution of ^{32}P between Different Organs of a Rat 4 Hours after Subcutaneous Injection of Labeled Phosphate (53).

Organ	^{32}P present, per cent	Specific activity
Bones	22.6	0.020
Muscles	18.7	0.191
Liver	17.6	0.475
Digestive tract	15.9	0.365
Skin	11.1	0.192
Lungs and heart	6.3	0.317
Blood	2.5	0.558
Kidneys	2.4	0.370
Spleen	1.3	0.256
Brain	0.02	0.032

TABLE V

Per cent of Total ^{32}P in Various Organs of the Rat (53).

Organ	Time after killing of the rat						
	Hours			Days			
	0.5	4	10	20	30	50	98
Muscles	18.3	19.4	25.8	28.8	25.2	12.1	3.6
Skeleton	19.1	23.4	43.1	43.1	51.8	76.5	92.0

Guant and associates (54) report the following data (table VI) for the percentage distribution of radiophosphorus 90 hours after subcutaneous injection (averages for 4 rats):

TABLE VI

^{32}P Distribution in the Rat 90 Hours after Isotope Administration (54).

Organ	Per cent ^{32}P
Skeleton	48.30
Incisors	2.14
Molars	0.77
Brain	0.48
Skin	4.63
Muscles	27.38
Liver	4.30
Viscera	4.30
Kidneys	8.86

Distribution data of intraperitoneally administered ^{32}P in the organs of normal rats and of thyroparathyroidectomised or nephrotonomised rats were recently stated by *Tweedy et al.* (54*a*).

e) *Uptake of Radiophosphorus by growing Tissue.*

Tissue grown in a labeled medium is bound to contain labeled compounds. Growing tissue, therefore, contains more ^{32}P than fully grown tissue. Resting ovary of rabbits, for example, weighing 0.160 g., takes up only 5 units of the labeled phosphorus administered, while the corpus luteum, weighing 0.254 g, in the same experiment shows an activity of 100 units (*Bulliard et al.*) (55). Tumor tissues show a high and rapid uptake of ^{32}P similar to rapidly growing normal tissues (56).

When applying ^{32}P as a therapeutic agent in cancer it is important to concentrate a large percentage of the ^{32}P administered in the tumor. In order to determine to what extent ^{32}P accumulates in carcinoma of the breast, osteogenic sarcoma, and lymphosarcoma, *Kenney* and his associates (57), after administering labeled phosphate, removed portions of different tissues and determined the ratio in each instance between the amount of the radioactive isotope measured per kilogram of body weight. This ratio has been designated the « differential absorption ratio » and has been used to compare the amount absorbed by the different tissues both in the same patient

and in different patients. The « differential absorption ratio » of ^{32}P for patients with carcinoma of the breast is seen in table VII.

Metastatic node is seen to concentrate ^{32}P to an appreciable extent and both the primary tumor and the normal node to a higher extent than all other organs investigated, with the exception of the most active organs such as liver, kidneys and intestinal mucosa the differential absorption ratio of which is not stated in the table.

TABLE VII

Differential Absorption Ratio of Radioactive Phosphorus for Patients with Carcinoma of the Breast (57).

^{32}P administered per kg body wt.	Exptl. periods,* days	DIFFERENTIAL ABSORPTION RATIO							
		Primary tumor	Breast tissue	Metastatic node	Normal node	Muscle	Fat	Skin	Blood
5.6	1	1.3	0.2	—	1.8	0.7	0.2	0.3	0.8
4.9	2	0.8	0.4	—	2.4	0.8	0.1	0.3	0.7
2.6	5	1.9	0.3	3.3	2.5	1.2	0.2	0.5	0.5
5.4	5	0.8	0.2	—	1.3	0.9	0.1	0.2	0.3
9.1	5	—	—	4.0	2.8	1.3	0.1	0.4	0.4
5.5	5	1.6	0.3	3.6	1.6	1.0	0.1	0.5	0.1

(*) Days between ^{32}P administration and operation.

Among the animal tumors generally used in cancer research, the Brown-Pearce rabbit carcinoma possesses a rather outstanding capacity for forming metastases in almost any part of the body and, thus, affords good opportunities for the study of the distribution of injected ^{32}P, for example, to metastatic and normal tissues. *Forssberg* and *Jacobsson* (58, cf. also 59) found, in their studies on the distribution of intravenously injected labeled phosphate in animals with transplanted Brown-Pearce carcinoma, that the metastases take up on the average as much ^{32}P as the most active organs, such as liver, kidney, spleen, and suprarenal gland, rather independently of their site in the organism. When, however, the uptake of ^{32}P per gram of wet weight of metastases was compared with the uptake of ^{32}P per gram of less active tissues such as diaphragm, pleura, peritoneum, omentum, mesentery, intestines, testis and lymph nodes, the metastases were found to take up as much as five times more ^{32}P than the corresponding normal tissue. Since bone marrow takes up a similar percentage of ^{32}P per gram of tissue as metastases, the possibility by administering labeled sodium phosphate of destroying or stopping the

development of already formed metastases of the Brown-Pearce tumor without damaging vital parts of the body is very restricted. However the administration of radiocolloids (see below) opens such possibilities. Colloidal chromium phosphate was found by *Jones et al.* (60) to be taken up to a very large extent by the liver and to remain for at least one year without detected decrement. The liver took up 59-81% of the intravenously injected colloid, while 4-15% were taken up by the spleen and 1-7% by the lungs. By administering strongly active chromium phosphate samples a local irradiation can be obtained.

C. Turnover Studies.

The lack of interchange of atoms present in organic binding (hydrogen atoms bound to oxygen or nitrogen being an exception (61)) such as that of carbon atoms in glycogen or phosphorus atoms in lecithin with other carbon or phosphorus atoms, respectively, was found to be of great significance for the application of isotopic indicators in biochemical research. Owing to the absence of such « physical » interchange, the presence of labeled carbon atoms in glycogen molecules, or of labeled phosphorus atoms in lecithin molecules, extracted from the organs is to be interpreted as indicating the synthesis of these molecules in the course of the experiment in which the labeled atoms were administered. We can thus distinguish between « old » and « new » molecules and determine the rates at which molecules of the different compounds are built up in or carried to the different organs.

a) *Turnover of Phosphorus-Containing Carbohydrates.*

1. *Rate of Renewal of Labile Phosphorus-Compounds of Muscle Tissue.* — In the determination of the rate of renewal of creatinephosphoric acid, i. e. the rate of rephosphorylation of creatine molecules in muscle tissue, we compare the radioactivity of 1 mg of creatinephosphoric phosphorus at the end of the experiment with the average activity of 1 mg of inorganic intracellular phosphorus prevailing during the experiment.

If this ratio is found to be 0,2, we can conclude that 20% of the creatinephosphoric acid molecules present were renewed in the

course of the experiment. Quantitative determination of the rate of renewal is not as easy a problem. It is not the activity of inorganic intracellular phosphorus that we dertermine experimentally, but the activity of inorganic tissue phosphorus. Knowing the extracellular space of muscle and assuming the inorganic P content of the extracellular fluid to be identical with that of the plasma and to get in a fairly rapid exchange equilibrium with the inorganic P of the blood plasma, we can calculate from the specific activity of the tissue inorganic P and the plasma inorganic P the specific activity of the intracellular phosphorus (62).

Another way of studying the rate of renewal of phosphate compounds in muscle is the removal of the extracellular phosphate by perfusing the muscle with saline. *Kalckar* and his associates (63) compared, under these circumstances, the specific activity of phosphocreatine phosphorus and of adenylpyrophosphate phosphorus with that of the inorganic phosphorus, which is exclusively of intracellular origin. That in perfused muscle the specific activity of inorganic phosphorus is much lower than in unperfused muscle is seen from table VIII. We must expect that perfusion will not influence the specific activity of the phosphocreatine and 2,3-pyrophosphate phosphorus, since these compounds are located in the cells.

Only twenty minutes after an intravenous injection of labeled phosphate, the creatine phosphorus, both of rabbit's and frog's (at 20° C.) skeletal muscle was found to have a specific activity corresponding to 60% of that of the inorganic phosphorus. The same results were obtained for the labile phosphorus of adenylpyrophosphate, conforming with those of early experiments (64), and indicating that the rate of renewal of the labile phosphorus compounds is a rapid process, in contrast to the rate of penetration of labeled phosphate into the muscle cells.

TABLE VIII

Specific Activity of the Phosphorus Fractions Isolated from the Skeletal Muscle of the Rabbit (63).

Type of injection of ^{32}P	Time after injection min.	Inorganic P		Phosphocreatine P		2.3-pyrophosphate P	
		Unperfused	Perfused	Unperfused	Perfused	Unperfused	Perfused
Intravenous	30	235	100	61	61	58	57
Intraperitoneal . . .	180	160	100	—	100	94	94

Flock and *Bollman* (65, 66), using myosin adenosinetriphosphatase as a tool for differentiating between the two labile groups of ATP (adenosine triphosphate), have found, in experiments lasting one hour, a higher ^{32}P concentration in the terminal phosphate group than in the second phosphate group (table IX). After the lapse of one day, the specific activity of the two labile phosphorus atoms of ATP and that of the creatinephosphorus was found to be but slightly lower than the activity of the inorganic phosphorus. The low rate of renewal of the third ATP phosphorus atom had been previously observed by *Korzybski* and *Parnas* (67).

TABLE IX

Specific Activity of the Three P Atoms of Adenosine Triphosphate (66).

Fraction	Specific Activity
Total muscle tissue inorganic P.	100
Terminal adenosinetriphosphate P . . .	29
Second adenosinetriphosphate P	21.6
Third adenosinetriphosphate P	0.9
Creatine P	24

In the turnover of the labile phosphate of ATP which occurs in the working muscle, the uptake of ^{32}P is increased only slightly above that of the resting muscle even after 180 contractions per minute for one hour. The turnover rate of the labile phosphate compounds is already so high in resting muscles that it is difficult to discover any substantial increase in the turnover during or after muscular contraction. The rate of rejuvenation of adenylpyrophosphate phosphorus and of phosphocreatine amounts to 20-30 micrograms per minute per g of muscle (63).

The rate of renewal of creatinephosphate and ATP molecules in cardiac muscle slices was investigated by *Furchgott* and *Shorr* (68). A quantitative renewal of creatinephosphoric and terminal ATP phosphorus was found to take place within 30 minutes at 37.5° C.

The extent of renewal of creatine and of the labile phosphorus of ATP molecules was found, even at 2°, to be appreciable, amounting to about one third the values found at 37.5°. The rate of renewal in these experiments is calculated from the ratio of the specific activities of creatine phosphorus or of labile ATP phosphorus, and that

of inorganic phosphorus after a prolonged washing of the tissue slices to remove the inorganic extracellular phosphorus. Such a procedure may lead to decomposition of the less active organic phosphorus compounds with formation of inorganic phosphorus and thus to a «dilution» of tissue organic P by less active non-genuine inorganic P. Should this be the case, it will result in too high values for the renewal figures.

As for the labile phosphate split off ATP, it was found that it contained a mixture of the terminal, highly active, and a second, less active fraction. On splitting off the terminal phosphate with an enzymic crayfish muscle suspension free of phosphate, *Furchgott* and *Shorr* obtained, after 30 minutes, a fraction having a specific activity of 100, while only one half of the remaining labile phosphorus of ATP underwent renewal in the course of the experiment.

In turnover studies we often determine the ratio of the specific activities of an organic P fraction and that of the tissue inorganic P. It is much prefered to compare in such experiments the specific activities of the organic P with that of the labile adenosinetriphosphate P (preferably considering the terminal P only). As the adenosinetriphosphate molecule is renewed within the cell and a rapid exchange equilibrium between the labile P of the adenosinetriphosphate and the intracellular inorganic P takes place such a comparison has great advantages above the first mentioned one. We must furthermore consider that during the extraction of the inorganic tissue P less active organic compounds may split off some of their phosphate group which will lead to a lowering of the specific activity value obtained for the inorganic P.

In the boundary between the extracellular and the intracellular phases, a more or less continuous drop in the concentration of the introduced labeled phosphate may take place. If a phosphorus compound is built up inside the phase boundary, it is very difficult to determine the specific activity of the inorganic phosphorus that has been utilized in the synthetic process resulting in the compound. *Sacks* (69, 70) found that, in experiments on fasted animals taking a few hours or less, the specific activity of the glucose-6-phosphate phosphorus was very much higher than that of the phosphocreatine, ATP, and fructose-6-phosphate phosphorus, as seen in tables X and XI. (See also the results obtained by *Kalckar* and his associates (63)). This result suggests that in the synthesis of the labeled glucose-6-phosphate molecules no intercellular inorganic

TABLE X

The Course of Uptake of ^{32}P by Acid-labile Groups of Adenosinetriphosphate, Fructose-6-phosphate, and Glucose-6-phosphate in Resting Muscles of Fasted Cats (69) (*).

2 hours after ^{32}P			4 hours after ^{32}P			24 hours after ^{32}P		
ATP	Fructose-6-phosphate	Glucose-6-phosphate	ATP	Fructose-6-phosphate	Glucose-6-phosphate	ATP	Fructose-6-phosphate	Glucose-6-phosphate
38	65	124	75	31	114	193	132	84
80	84	202	84	14	167	167	122	102
66	27	149	52	19	170	165	121	88
79	37	189	77	25	219	224	150	121
88	102	209	78	60	228	—	—	—
—	—	—	65	56	250	—	—	—
—	—	—	70	45	217	—	—	—
Average value :								
70	63	175	72	36	195	187	131	99

(*) Values are expressed in terms of counts per minute per milligram P, calculated to the basis of 10 counts injected per minute per kilogram body weight.

TABLE XI

Uptake of ^{32}P by Fasting Muscles of Frogs (69) (*).

2 hours after ^{32}P			24 hours after ^{32}P		
ATP	Fructose-6-phosphate	Glucose-6-phosphate	ATP	Fructose-6-phosphate	Glucose-6-phosphate
73	82	348	233	142	134
23	62	152	306	155	141
84	154	435	152	100	99
136	144	472	106	62	65
Average value :					
79	111	352	199	115	109

(*) Values are expressed in terms of counts per minute per milligram P, calculated to the basis of 10^6 counts injected per minute per kilogram body weight, as of the day of measurement.

phosphate, but phosphorus of much higher activity, is utilized, and thus that the synthesis of this compound takes place outside the muscle cells, i. e. inside the extracellular space or possibly inside the phase boundary. The high ^{32}P content of glucose-6-phosphate is never found in fed animals (in the postabsorptive phase), but only

in fasted animals. The incorporation of nonintracellular ^{32}P in glucose-6-phosphate phosphorus is possibly involved in the process of entry of glucose into cells of the fasting animal.

The rate of renewal of hexosemonophosphate was found to be considerably lower than that of pyrophosphate or creatinephosphate (64, 71).

TABLE XII

Effect of Stimulation, Recovery and Glucose Administration on ^{32}P Turnover in Muscles of Cats in Postabsorptive State (73)(*).

Phospho-creatine	ATP	Fructose-6-phosphate	Glucose-6-phosphate	Phospho-creatine	ATP	Fructose-6-phosphate	Glucose-6-phosphate
Resting				Stimulated			
119	98	61	77	99	102	41	54
130	132	76	59	138	125	69	77
170	143	—	47	204	160	105	44
157	150	133	73	104	115	—	76
Average value:							
144	131	90	64	136	126	72	63
Resting				Stimulation and recovery			
146	145	73	58	308	282	100	72
137	140	64	45	207	177	126	73
208	172	110	67	263	263	168	83
124	115	75	55	157	174	128	65
Average value:							
154	143	81	56	234	217	131	73
After glucose							
67	58	55	38	89	85	75	74
131	76	51	37	147	118	41	59
89	87	67	70	90	101	71	75
80	87	67	60	124	108	78	70
46	51	32	47	—	—	—	—
39	56	17	30	—	—	—	—
105	99	—	38	—	—	—	—
75	92	50	94	—	—	—	—
Average value:							
79	76	48	53	113	103	66	70

(*) Values are expressed as counts per minute per milligram P, calculated to the basis of one million counts per minute injected per kilogram body weight. The cats were killed 4 hours after injecting subcutaneously ^{32}P.

Analysis of frog muscle at low temperature shows that the hexosemonophosphate in skeletal muscle attained only a very low concentration of ^{32}P. It appears as if some enzymes converting glycogen and phosphate to hexosemonophosphate were inactive in frog muscle at low temperature (63).

Sacks (73, 74) also studies the effects of prolonged stimulation, recovery, and glucose-administration on the uptake of ^{32}P by the phosphorus compounds of muscles of cats in fasting and postabsorptive state. Prolonged contraction was found to be without effect on either the uptake of ^{32}P by any of the acid-soluble organic phosphorus compounds of muscle (see table XII) or its distribution among them. In recovery from prolonged activity all the organic phosphorus compounds investigated were found to show a higher ^{32}P content, as seen in table XII.

Administration of glucose reduces the turnover rate in postabsorptive state of phosphocreatine, adenosinetriphosphate, and fructose-6-phosphate, but not that of glucose-6-phosphate. In the fasting state, the administration of glucose does not affect the turnover rate of phosphocreatine and adenosinetriphosphate.

2. *Effect of Insulin on Phosphorus Turnover in Muscle.* — In the experiments of *Sacks* (70), five insulin units were employed per kg cat weight. Half an hour after administration of labeled sodium phosphate to a cat by subcutaneous injection, 1.5 g glucose was given per kg ; after the lapse of a further half-hour, insulin was administered. The cat was killed 105 minutes after injection of the tracer phosphate. The result of the experiment, shown in table XIII, indicates that both in resting and stimulated muscles insulin brings about an increase in the ^{32}P content of phosphocreatine, adenosinetriphosphate and fructose-6-phosphate-fractions in the postabsorptive state, but not in the fasting state. Insulin increases the ^{32}P content of glucose-6-phosphate as well. *Sacks* suggests that in the latter case we are presumably concerned with an influx of glucose, in which non-intracellular ^{32}P participates, and in the postabsorptive case with a renewal of the glucose-6-phosphate molecules, intracellular phosphorus participating.

A rather small amount of phosphorylation is observed in relation to the total probable glucose absorption. Two possible interpretations are given by *Sacks* :

1) The absorption of glucose by the muscle fibre does not involve the entry of a phosphate group into the cells, or

2) Glucose-6-phosphate is not involved in the principal mechanism of glucose absorption by the resting muscle (74).

The first interpretation is in accordance with the results obtained at by *Kjerulf-Jensen* and *Lundsgaard* (75) in their study of phosphate exchange between plasma and muscle tissue with artificially perfused hind limb preparations in which labeled phosphate was employed. They found that both before and after addition of insulin the quantity of phosphate exchanging per unit time was slight in proportion to the

TABLE XIII

Effect of Insulin on P-turnover in Muscles of Cats Fed Glucose (70)(*).

State of animals	Phospho-creatine	ATP	Fructose-6-phosphate	Glucose-6-phosphate
Fasting, resting	103	77	66	514
Fasting, resting, given insulin . . .	316	189	138	482
Fasting, stimulated and recovering	119	100	55	606
Fasting, stimulated and recovering given insulin	271	205	203	733
Postabsorptive, resting	75	76	49	52
Postabsorptive, resting, given glucose	187	154	111	104
Postabsorptive, stimulated and recovering	113	103	66	70
Postabsorptive, stimulated and recovering given glucose	203	156	120	116

(*) Values are averages of several results and expressed as counts per minute per milligram P calculated on the basis of one million counts per minute injected per kilogram body weight. Time of experiments : 90 minutes.

simultaneously assimilated quantity of glucose. They therefore concluded that passage of glucose into the muscle cells in the form of hexose phosphate formed from the inorganic phosphate of the plasma was to be regarded as out of the question.

The investigation on liver of *Kaplan* and *Greenberg* (78) showed that the maximum ^{32}P content of the inorganic phosphate of liver tissue of fasting rats reaches its maximum 75 minutes after intraperitoneal injection of labeled phosphate while the maximum activity of the total acid-soluble phosphorus is obtained after the lapse of 110-120 minutes.

The effect of propanediol phosphate on the rate of renewal of the acid-soluble phosphorus fractions in rat liver was investigated by

Lindberg (76). Four twenty day rats were fed a diet containing 100 mg phosphorus as propanediol phosphate. To the rats weighing 55 g labeled sodium phosphate was then injected intraperitoneally. After the lapse of one hour the acid-soluble phosphorus compounds of the liver were extracted. The specific activity of the phosphorus fraction obtained by acid hydrolysis for ten to twenty minutes, the « hexose ester » fraction, was found to be much higher than that of the corresponding fraction in the controls.

In tissue slices of rat kidney and liver the accumulation of organic ^{32}P was found to increase appreciably when sodium fluoride was added to the medium containing labeled phosphate. This result is interpreted to be due to the inhibitory effect of fluoride on the phosphatase causing breakdown of the newly synthesized organic compound. The ^{32}P was found to be present in the phosphoglyceric acid fraction. In the absence of oxygen, accumulation of ^{32}P in the organic fractions was found to be much reduced (77).

3. *Effect of Insulin on the Phosphorus Turnover in Liver.* — Insulin and glucose administrations have a marked influence on acid-soluble ^{32}P content of liver (78). Rats weighing about 200 g were fasted for a period of twelve hours, given the treatment shown in the table, and then injected interperitoneally with tracer doses of labeled sodium phosphate. The results obtained are seen in table XIV.

TABLE XIV

Distribution of Labeled Phosphorus in Acid-soluble Phosphorus of Liver 110 Minutes after Distribution of the Phosphate (78)[1].

Treatment	Total acid soluble	Inorganic	Labile	ATP Non-labile	Alcohol soluble [2]	Residual [3]
Control, fasted 12 hours	177	77.6	11.7	3.3	44.0	28.6
Glucose (300-400 mg intraperitoneally) . . .	204.0	56.6	49.0	12.8	25.4	12.9
Insulin	244.3	92.5	41.5	15.6	26.2	14.5
Glucose and insulin . .	262.0	83.4	64.5	16.2	22.7	20.2

[1] This fraction consists largely of glycerol phosphate, but it contains also some hexosemonophosphates.
[2] All values are in per cent of administered dose × 10 of the labeled P per 100 g fresh liver.
[3] This fraction consists largely of phosphoglyceric acid.

The peak of total phosphorus of ATP in the animals given glucose occured at 110 minutes after the injection of ^{32}P, whereas the peak in

the control group occurred at 200 minutes. The peak in radio activity of non-labile phosphorus of the adenosinetriphosphate was similarly shifted from 250 to 110 minutes. The peaks in the radioactivity of the alcohol and residual fractions were displaced in time by glucose administration from 110 to 210 and 245 minutes, respectively.

Phlorizin, malonate, and fluoride, but not iodoacetate, prevent a rise in the ^{32}P content of liver following administration of glucose. These findings support the hypothesis that the primary action of phlorizin is a blocking of the formation of ATP.

Reduction of food intake causes a decrease in the acid-soluble phosphorus content of liver (79). The effect of insulin may therefore possibly partly or wholly be due not to an increased rate of renewal of acid-soluble phosphorus molecules present in the liver, but to an increase in total ATP and free phosphate content following the administration of insulin.

In experiments on perfused cat liver, a high percentage of the ATP molecules and a minor percentage of the ester phosphorus were found by *Lundsgaard* to be renewed in the course of one hour (80).

Five minutes after intravenous injection of radioactive phosphate, *Kalckar* and his associates (63) found the labile phosphorus of adenylpyrophosphate present in rabbit liver to have a specific activity of 83% of the corresponding value for inorganic intracellular phosphorus. In interpreting this and similar figures, we must take into account that in the course of five minutes the plasma activity declines considerably, very highly active phosphate penetrating the liver cells in an early phase of the experiment. When we calculate the percentage renewal from the ratio of the specific activity of the pyrophosphate phosphorus and that of the inorganic phosphorus at the end of the experiment, we obtain correspondingly too high a figure for the extent of renewal. *Kalckar* and his associates estimate the rate of rejuvenation of pyrophosphate phosphorus as 15-20 microgram per minute per g liver and consider these to be minimum values.

Rapoport and his colleagues (81) found the rate of renewal of labile phosphorus of adenosinetriphosphate of kidneys to be reduced under the action of phlorizin. While in the controls the rate of renewal of labile phosphorus amounted to 70% in thirty minutes, when phlorizin was previously administered by intravenous injection, the value was reduced to 33%.

4. *Experiments in Vitro on Rate of Renewal of Adenosinetriphosphate.* — Experiments in vitro, in which much simpler conditions prevail than in vivo, were used for a calculation of the rate of renewal of the labile phosphorus in ATP. In determining the rate of interchange between free phosphate and pyrophosphate, *Meyerhof* and his associates (82) studied the reaction:

2 triosephosphoric acid + 2 pyruvic acid + 1 adenosinemonophosphoric acid + 2 phosphoric acid $\rightleftarrows$ 2 phosphoglyceric acid + 2 lactic acid + 1 adenosinetriphosphoric acid.

To a solution, kept at 20° C, containing dialyzed muscle extract, phosphoglyceric acid, hexose diphosphate, sodium lactate, pyruvic acid, adenosinetriphosphate (containing 0.327 mg pyrophosphate phosphorus), magnesium, manganese, sodium fluoride, and cozymase, labeled inorganic phosphate (containing 0.395 mg phosphorus) was added. The distribution of labeled atoms between the inorganic phosphate and the pyrophosphate fraction was then determined at different intervals. After the lapse of twenty seconds, as is seen in table XV, almost half of the exchange equilibrium of ^{32}P between thetwo fractions was obtained. The average time of interchange of a pyrophosphate group with a phosphate group was shown to be fifty seconds.

TABLE XV

Distribution between Inorganic and Pyrophosphate P of 0.395 milligram Inorganic P added to an in Vitro Muscle System (82).

Time sec	Inorganic P, mg	Pyrophosphate P, mg	Interchange per cent
0	0.395	0	0
20	0.313	0.084	47
40	0.288	0.120	64
75	0.253	0.135	77
150	0.255	0.144	85
600	0.223	0.159	92
—	0.216	0.179	100

The renewal of phosphorus compounds goes hand in hand with the phosphorylation processes that are closely connected with oxidative steps in the utilization of carbohydrates. Each phosphorylation first involves adenosinephosphate and thus necessitates a renewal of the labile phosphorus of ATP molecules. Assuming two phosphorylations through the terminal group of ATP for every

atom of oxygen, and taking the oxygen consumption to be 1.0 ml per g per hour, *Furchgott* and *Shorr* (68) calculate that, in 1 g of cardiac tissue of the dog, 178.4 micromoles of phosphorylation takes place. This would represent a turnover of phosphorus, from intracellular inorganic P to the terminal group of ATP, of about 92 microgram per g tissue per minute. Assuming a rate of renewal in the resting muscle corresponding to that found by *Meyerhof* and his associates in their experiments in vitro, and taking 1 g muscle to contain 120 microgram terminal ATP phosphorus, the rate of turnover proves to be 144 microgram per g tissue per minute.

5. *Phosphorus Metabolism in Brain.* — *Borell* and *Oerström* (83) investigated the uptake of ^{32}P by different parts of the brain. The phosphorus present in the pineal body, the anterior and posterior lobes of the hypophysis, and the plexus chorioideus was found to interchange with the labeled phosphate administered by intraperitoneal injection at a much more rapid rate than the other parts of the brain; the latter exhibit about the same slow phosphorus interchange as the cerebellum.

Of the ^{32}P accumulated in the course of 40 minutes in the pineal body about 65% are present as acid-soluble phosphorus, 25% as inorganic phosphorus and 10% as non-acid-soluble phosphorus.

6. *Application of Radiophosphorus in Studies on Metabolism of the Sexual Cycle.*

Radioactive phosphorus was used by *Borell, Westmann* and *Oerström* (83*a*) in the study of metabolic processes taking place in the tuber cinereum, adenohypophysis (anterior lobe of pituitary) and ovaries in the different phases of the sexual cycle of the rabbit. Labeled sodium phosphate in 5% glucose solution was injected into the veins of anoestrous and oestrous female rabbits, either before or after mating. To each animal about 0.1 millicurie ^{32}P was administered. After the lapse of 30 minutes the rabbit was killed by decapitation. Mating was found to increase very substantially the uptake of ^{32}P by the tuber cinereum and to a minor extent by the adenohypophysis of the rabbit, as seen in table XV*a*. In the tuber cinereum the increased activity is maintained during the first hour after mating. Subsequently, it decreases to values approaching those found in oestrous animals. In the adenohypophysis the high ^{32}P content was maintained during the whole observation time, viz. until 24 hours after mating, with a peak in the second half-hour after mating.

In contradistinction to the above-mentioned organs, in the

ovaries there is no increase in ^{32}P until 30 minutes after mating. The activity is then maintained at a comparatively high level and reaches another maximum about 9-11 hours after mating, i. e., when ovulation in the rabbit occurs.

TABLE XV[a]

Specific Activity of Total Phosphorus in Different Organs of the Rabbit (83[a]).

Group of rabbits	Tuber cinereum	Adenohypophysis	Ovaries
Anoestrous	2.60	28.20	25.14
Oestrous control	2.98	30.26	28.05
Mating 2 min before decapitation	4.65	38.40	
» 5 »	4.95	43.00	
» 10 »	5.55	46.70	
Injected immediately after mating. .	4.92	45.00	27.00
» 30 min after mating	5.18	51.20	54.40
» 60 min to 24 hrs after mating	3.28	42.50	55.00
Castrated animals	2.78	49.70	

No substantial difference is observed in the ^{32}P uptake by the anoestrous and oestrous rabbits.

That the enhanced uptake of ^{32}P after mating is not due simply to increased permeability of the organs investigated is suggested by the results obtained when comparing the specific activity of an organic phosphorus fraction of an organ with the specific activity of the inorganic phosphorus fraction of the same organ. The organic phosphorus fraction was obtained by acid hydrolysis for 30 minutes of the trichloroacetic acid filtrate secured by extracting the organ. The ratio of the specific activities of the organic and the inorganic phosphorus in the case of tuberum cinereum is very markedly increased (from 0.72 to 3.8) after mating.

7. *Renewal of Mineral Constituents of the Skeleton.* — The renewal rate of the greater part of the skeleton «apatite» was found to be a slow process (165) in contrast to the rate of renewal of phosphate located in the surface layer of the apatite-like crystals containing the mineral constituents of the bone tissue. A review of the application of ^{32}P in the study of phosphorus metabolism in teeth is presented by *Armstrong* (166). A detailed discussion of the application of radiophosphorus in the study of skeleton metabolism is to be found in the monograph of the author published by Interscience Publishers, Inc., which is in print.

b) *Turnover of Phosphatides.*

1. *General Considerations.* — As was shown by early experiments, the presence of labeled phosphatides can be detected in the tissues shortly after administration of labeled phosphate (84, 85, 86). The percentage of the dose administered present in the liver of the rat as phosphatide ^{32}P first increases, then declines after about ten hours, as stated by *Perlman* and associates (86). A similar behaviour is shown by the labeled phosphatide content of the gastrointestinal tract. The labeled phosphatide content of the carcass increases up to 100 hours.

The rapid increase in labeled phosphatide content of the liver indicates rapid turnover. In the early phases of the experiment, the specific activity of cellular phosphate is high and the newly synthesized phosphatide molecules incorporate highly active phosphate. The labeled phosphate molecules in later phases of the experiment are repeatedly renewed with the participation of less active phosphate, and labeled phosphate makes its way in the later phases of the experiment from the liver into the plasma. This leads to a decrease in labeled phosphatide content of the liver. Labeled phosphatides of the liver, furthermore, can enter the circulation, but the amount leaving the liver is only 1% of the phosphatide content of the liver per hour (see p. 318). That the labeled phosphatide content of the muscles increases in the course of the first 100 hours may be interpreted as due partly to the low rate of formation of labeled phosphatide molecules in the muscles and partly to the low rate of penetration of phosphate into the muscle cell, the possibility of intrusion of labeled phosphatides into the muscle cells must be considered as well (cf. p. 274).

If we make the assumption that phosphate ions or phosphorus-containing precursors which attain rapid equilibrium with the phosphate ions are incorporated into the phosphatide molecule, we can determine the rate of renewal of phosphatide molecules by comparing the specific activity of the phosphatide phosphorus at the end of the experiment with the mean specific activity of the intracellular inorganic phosphorus prevailing during the experiment. This calculation involves the further assumption that it is the intracellular, inorganic phosphorus which is incorporated into the phosphatide molecule. The rate of renewal of phosphatides extracted from the liver of the rat is calculated by comparing the specific activities of the phosphatide phosphorus and tissue inorganic phosphorus at the end of the expe-

riment taking 2 hours, the result obtained being shown in table XVI*a* (108*a*). Since the average specific activity of cellular inorganic phosphorus of the liver in experiments taking 2 hours hardly differs from the specific activity of cellular inorganic phosphorus measured at the end of the experiment, and the difference between the specific activities of the intracellular and tissue inorganic phosphorus is in this case almost negligible (88), when computing the data of table XVI*a* the tissue inorganic phosphorus values determined at the end of the experiment taking 2 hours were considered.

In table XVI*a* the specific activity of phosphatide P is compared with the specific activity of the labile phosphorus of adenosine-

TABLE XVI*a*

Renewal of Phosphatides in the Rat Liver (108*a*).

AGE OF RATS	Percentage ratio of specific activity of phosphatide P to that of :			
	Liver ATP 2,3 P	Liver inorganic P	Liver total P	Plasma inorganic P
4 days . . . . . (average of 40 rats).	26.5	—	66.7	23.4
10 days . . . . . (average of 25 rats).	21.9	21.8	64.0	20.6
14 days . . . . . (average of 20 rats).	22.4	23.2	74.2	22.2
30 days . . . . . (average of 10 rats).	21.5	—	62.6	24.8
90 days . . . . . (average of 4 rats).	15.0	15.5	43.0	16.8
540 days . . . . . (average of 3 rats).	—	12.7	58.1	14.0

TABLE XVI*b*

Renewal of Phosphatides in the Rat Kidney (108*a*).

AGE OF RATS (in days)	Percentage ratio of specific activity of phosphatide P to that of :		
	Kidney inorganic P	Kidney total P	Plasma inorganic P
10	14.0	44.0	10.3
14	14.4	37.2	10.6
30	15.2	41.3	13.5
90	12.1	27.9	11.2
540	12.1	37.8	11.6

TABLE XVI*c*

Renewal of Phosphatides in the Rat Spleen (108*a*)

AGE OF RATS (in days)	Percentage ratio of specific activity of phosphatide P to that of :		
	Spleen inorganic P	Spleen total P	Plasma inorganic P
4	4.8	13.7	4.9
10	5.2	17.0	4.8
14	5.0	18.3	—
30	3.9	18.5	3.4
90	4.1	12.8	2.1
90	3.5	14.8	2.5
540	3.4	16.0	1.6

triphosphate (ATP, 2,3 P). The comparison of the specific activity of phosphatide and other P fractions with the specific activity of ATP, 2,3 P is much to be prefered to the comparison with the specific activity of inorganic P. In contrast to the inorganic P which is partly of extracellular and partly of intracellular origin, ATP phosphorus is of cellular origin only, and as its 2,3 P comes rapidly in exchange equilibrium with intracellular inorganic P the specific activity of ATP, 2,3 phosphorus is almost identical with the specific activity of the intracellular inorganic P of the tissue. Furthermore, during the extraction of the acid-soluble P some organic phosphorus compounds of low specific activity might be split off from the inorganic phosphorus. Such a process will lead to a lowering of the specific activity of the genuine inorganic P of the tissue and will thus lead to renewal rate figures which are too low.

As seen in tables XVI*a*, *b* and *c*, the percentage renewal of the liver phosphatides of adult rats is per hour about 8, thus very appreciable while a 3 per cent renewal only is reported by *Bollman and Flock* (96*a*). The corresponding figure for the kidney phosphatides is about 6. When comparing these figures with the data obtained for the spleen it must be taken into consideration that contrary to that of the liver and kidneys the intracellular inorganic P of the spleen of adult rats does not get into rapid exchange equilibrium with the plasma inorganic P. Due to this fact, the average value of the specific activity of the inorganic P of the spleen is during the experiment lower than its end value and correspondingly the renewal

figures concluded from the ratio of the specific activities at the end of the experiment recorded in table XVI are too low.

The data of table XVI*a* demonstrate the phosphatide turnover in the rat liver during growth, the turnover rate in the case of an adult rat making out only 60% of the turnover rate observed in a 4 day old animal. The figures obtained for the turnover in the kidney and the spleen do not show such pronounced differences.

The last column of table XVI*c* demonstrates the decreasing permeability of the spleen for inorganic P with age, which was already previously observed (88). The percentage ratio of the specific activity of phosphatide P to that of the inorganic P of the spleen (ratio obtained for the 4 day and 540 day old rat = 1.4) declines with age much slower than the percentage ratio of the specific activity of the phosphatide P to that of the plasma inorganic P (corresponding ratio = 3). This difference is due to an increase in the ratio specific activity of plasma inorganic P/ specific activity of spleen inorganic P and thus to a decrease in the phosphate permeability with age.

To obtain renewal figures that are certainly correct, we should know the specific activity of the immediate phosphorus-containing precursor of the phosphatide molecule, should that not be identical with inorganic P, and insofar as different paths of phosphatide formation exist, each involving a phosphorus-containing precursor, we should know the specific activity of all these precursors. Furthermore, if the site of formation is inside the cell, this site need not necessarily involve the whole cell, but only part thereof. In the latter case, it is the specific activity of the immediate precursor in this part of the cell, not that of the whole cell that is to be considered when calculating the rate of renewal. The figures given in table XVI*a* represent, however, probably fairly correct renewal rate figures.

In the calculation of the renewal figures given in table XVI, the possibility of repeated renewal of the phosphatide molecule was disregarded. When a high percentage of the molecule is renewed, such a procedure is no longer permissible. It is therefore of importance to carry out turnover experiments of short duration.

A calculation of the rate of turnover has been made by *Zilversmith* and associates (89). Application of this calculation to the rate of formation of labeled compounds is still lacking. The result was, however, applied in the calculation of the turnover rate and turnover time of labeled phosphatides introduced into the circulation (90, cf also 121).

The knowledge of exact turnover figures is of interest when comparing, for example, the amount of fat metalbolized with the amount of phosphatides turned over. In many cases, however, we can draw conclusions merely by comparing the specific activity of the phosphatides present in an organ under varying conditions. Such a procedure is applied in the study of the effect of ingested fat on the rate of renewal of phosphatides, of the effect of choline, cholesterol, and other substances on the turnover of liver phosphatides, of the site of formation of the yolk. embryo, and milk phosphatides, and so on.

A comparison of turnover rate of phosphatides and of other compounds, not only in different organs but even in different parts of the cells, is a problem of great importance, solved so far only in a special case : the rate of renewal of cytoplasm phosphatides has been compared with the rate of renewal of nuclear phosphatides, as described on page 341.

Entenman et al. (105) compared the specific activities of various lobes of liver. The values were found to be uniform : for the left main lobe, the right middle lobe, the left middle lobe and the right main lobe, the values 0.014, 0.015 and 0.014 respectively, were obtained.

2. *Effect of Ingested Fat on Rate of Renewal.* — Some time ago *Artom* and colleagues (84) showed that feeding of oil promotes the turnover of phosphatides.

The feeding of oil had the greatest effect on phosphatide metabolism in intestinal mucosa. A marked effect was also found in liver and kidney, but not in other organs of the rat. The increased phosphatide activity may be due either to the formation of additional phosphatide originating from the ingestion of oil, or to an accelerated rate of replacement of the nonactive phosphatide molecules by newly formed active molecules, or to both of these effects.

The effect of ingested fat on the activity of tissue phosphatides has also been investigated by *Perlman* and associates (86). A marked effect of ingested oil on the formation of labeled phosphatides was also found to take place in the stomach and large intestine, but the amount of active phosphatides formed per gram of stomach and large intestine was much smaller than that formed per gram of small intestine. This shows that the major part of the phosphatide turnover taking place in the digestive tract may be ascribed to the small intestine. The same applies to the digestive tract of the bird (111). It was also found that removal of tissue very active in phosphatide

formation (i. e., the gastrointestinal tract and, to a minor extent, the kidneys) does not markedly influence new formation of phosphatides in the liver.

The turnover rate of phosphatide P in the small intestine is accelerated when choline, and especially when choline + oil, is administered to rats kept on a stock diet *(Artom and Cornatzer)* (122*a*). The effect of administration of choline, resp. choline and oil, is still more pronounced when the rats were previously kept on a low fat, low choline diet. In the former case administration of choline + oil leads to an additional phosphatide formation (determined by chemical analysis) making out 30% of the initially present amount, while the ^{32}P content of the liver phosphatides increased with 69%, in the last mentioned case the corresponding figures are 45% and 87% respectively.

TABLE XVI*d*

Formation of Labeled Phosphatides at 6 hour Intervals in Small Intestine of Rats on Low Fat, Low Choline Diet (26) and on Stock Diet (122*a*).

^{32}P injected intraperitoneally 5 minutes after administration of choline, etc.

Total ^{32}P content of phosphatides in relative radioactive units				Phosphatide P content of small intestine of 100 g rats in mg				Specific activity of phosphatide P			
Administered by stomach tube				Administered by stomach tube				Administered by stomach tube			
H_2O	Choline	Oil + Choline	Oil + H_2O	H_2O	Choline	Oil + Choline	Oil + H_2O	H_2O	Choline	Oil + Choline	Oil + H_2O
Rats on Diet (26) for 7 days :											
123	162	181	193	2.67	3.31	3.12	3.27	46	49	58	59
149	169	226	140	2.37	2.11	2.83	2.15	63	80	80	65
125	116	308	122	2.36	1.97	3.11	2.35	53	59	99	52
120	184	229	125	2.31	3.07	3.42	2.78	52	60	67	45
120	183	209	168	2.14	2.32	2.43	3.05	56	79	86	55
88	171	204	133	2.05	2.11	3.23	2.77	43	62	63	48
Average :											
121	164	226	147	2.32	2.48	3.02	2.73	52	65	76	54
Stock Diet for 7 days											
117	145	233	187	2.25	2.79	3.76	2.79	52	52	62	67
121	123	177	161	2.37	2.67	2.95	2.98	51	46	60	54
100	135	163	127	2.17	2.60	3.13	2.36	46	52	52	58
Average											
113	134	191	158	2.26	2.69	3.28	2.71	50	50	58	60

Administration of choline + oil leads thus not only to an increase in the phosphatide content of the intestinal mucosa but produces simultaneously an enhanced rejuvenation of the phosphatide molecules present, which is more marked in animals kept on low fat, low choline diet than in those kept on stock diet, as seen in tabe XVI*d*.

That the rate of renewal of phosphatides in liver is accelerated when the fat content of the circulation is increased was also shown in experiments on perfused cat liver (92). With normal blood, 1.5% of liver phosphatide phosphorus was found to be replaced by active inorganic phosphorus added to the blood as sodium phosphate in the course of 2.5 hours, while with lipemic blood 2.7% was renewed. Thus, the effect of ingested fat on the rate of renewal of phosphatides is very pronounced in those organs which, like the intestinal mucosa and the liver, play a predominant part in fat metabolism.

Schmidt-Nielsen (93) found that, one hour after ^{32}P was administered by intramuscular injection to a rat, the specific activity of phosphatide phosphorus extracted from the intestine was four times larger, after feeding 2.5 g peanut oil by stomach tube, than the specific activity of phosphatide phosphorus of the resting intestine. The increased ^{32}P content cannot be ascribed to the general increase in cell activity, because intestinal loops absorbing glucose did not synthesize phosphatides at a rapid rate but at the same low rate as nonabsorbing intestine. No increase was found in the total amount of phosphatides (about forty micromoles phosphatide phosphorus per gram intestine) present in the intestine during fat absorption. The newly formed phosphatide molecules must therefore either be transported away or split up again near the place of formation. Presumably the latter process takes mainly place. Poisoning with phlorizin does not decrease the rate of formation of phosphatides.

In a recent investigation *Zilversmit* et al. (92a) determined the rate of renewal of phosphatides in the mucosa of the duodenum, jejunum and ileum of fasting and of fat-fed dogs. Similar values were obtained. From this result the conclusion is drawn that fat can pass through the small intestine without involving phosphatides as intermediates.

The total amont of ^{32}P incorporated in the phosphatides of normal and rachitic rats was determined by *Dols* and associates (94). Rachitic rats were found to contain less total ^{32}P but more phosphatide ^{32}P than the controls investigated. The phosphatide phosphorus content

of normal rats weighing 35-40 g was found to be 11% of their total phosphorus content.

3. *Relative Speed of formation of Various Phosphatides.* — *Chargaff* et al. (95) compared the specific activity of the lecithin phosphorus and cephalin phosphorus extracted from 250-300 g rats at 19 to 43 hours after oral administration of labeled sodium phosphate. The rate of turnover of cephalin was found to be somewhat higher, as seen in table XVII.

TABLE XVII

Relative Speed of Formation of Lecithin and Cephalin in Rats (95).

Rat No.	Time Hours	Phosphatide	Weight	Relative speed of formation
1	19	Lecithin	1063.0	100
		Cephalin	353.0	113
2	43	Lecithin	935.3	140
		Cephalin	258.0	151

Comparison of specific activities of lecithin and cepahlin phosphorus extracted 24 hours after oral administration of labeled sodium phosphate indicates that lecithin extracted from the intestinal tract and the liver is somewhat more active than the cephalin while the opposite behaviour is shown by lecithin and cephalin of the brain. In normal rat liver, in experiments that lasted 24 hours, sowewhat greater renewal figures were obtained for lecithin than for cephalin. *Chargaff* et al. found the cephalin-lecithin ratio to be 0.8, *Platt and Porter* (95*a*)—to be 0.7, in experiments taking 6 hours.

The renewal figures (see table XVII*a*) were found by *Hahn and Tyrén* (96) to be somewhat greater for lecithin; they found a great difference between the turnover rate of phosphatides in the liver of rats and rabbits.

TABLE XVII*a*

Renewal of Lecithin and Cephalin in Rat and Rabbit Liver (96) (*)

FRACTION	Per cent renewal	
	Rat liver	Rabbit liver
Inorganic P	100	100
Lecithin P	20.4	6.3
Cephalin P	18.6	6.0

(*) Radiophosphorus administered four hours prior to killing the animal.

Artom and his colleagues (112) conducted experiments in which olive oil and labeled sodium phosphate were administered to rats 9 hours before they were killed, and report the ratio to be about 0.6.

In rat carcinosarcoma the specific activity of lecithin "256", reaches a peak after 30 hours; cephalin attains its peak after 40 hours (97). The rate of renewal of sphingomyelin in liver is slower than that of the other phosphatide fractions.

In kidney about the same rate of renewal is found for all the phosphatide fractions. The ^{32}P of sphingomyelin of rat organs, except in brain and muscles, reaches a maximum eight days after administration. At the end of eight days, the specific activity of liver is 0.027 and that of the intestinal mucosa 0.010, while for skeletal muscle the specific activity is 0.3312, and for brain 0.3008 is found (98).

In experiments taking 24 hours, brain cephalin was found to be more active than the brain lecithin (101).

4. *Effect of Lipotropic Substances on Phosphatide Turnover in Liver.* — The fact that the rate of formation of new phosphatide molecules in the liver is accelerated by the administration of choline was established by *Perlman* and *Chaikoff* (102). Rats were fed for three days on a diet high in fat and low in protein; on the fourth day, half of each group were given 3 mg of labeled phosphate and 30 mg of choline chloride, simultaneously. The remaining half was given the labeled phosphate only. All animals were killed 4 hours after the administration of ^{32}P. While the phosphatides in the liver of the controls were found to contain 2.23% of the ^{32}P administered, the liver phosphatides of the choline-treated rats contained 2.92%. Increased formation of labeled phosphatides was found to appear approximately 1 hour after choline ingestion, and its effect had disappeared about 10 to 12 hours later.

Combined feeding of choline and cholesterol clearly promotes the renewal of phosphatide molecules in the liver and additional formation of phosphatide molecules as well. *Friedländer* and colleagues (106) observed that the additional amounts of radiophosphatides formed under the influence of choline do not long remain in the liver. They pass into plasma and increase the specific activity of plasma phosphatides. A single feeding of 300 mg choline chloride per kg of body weight increases markedly the phosphatide turnover in the plasma. While after 12 hours the effect of choline is most pronounced, after the lapse of 96 hours

the specific activity of the plasma phosphatides shows almost the same value as found in the controls.

The specific activity value of the liver phosphatides was found to increase during the first 6 hours of the experiments.

Artom and *Cornatzer* (122*a*) have shown that the increase in the turnover rate of liver phosphatides after administration of choline occurs only in animals maintained on a low fat diet, but that it is markedly increased by simultaneous administration of a large amount of fat, as seen in table XVII*b*.

TABLE XVII*b*.

Formation of Labeled Phosphatides at 6 Hour Intervals in the Liver of Rats on Low Fat, Low Choline Diet (16) and on Stock Diet.

^{32}P injected intraperitoneally 5 minutes after administration of choline, etc. (122*a*).

Total ^{32}P content of phosphatides in relative radioactive units				Phosphatide P content of the liver of 100 g rats in mg				Specific activity of phosphatide P			
Administered by stomach tube											
H_2O	Choline	Oil + Choline	Oil + H_2O	H_2O	Choline	Oil + Choline	Oil + H_2O	H_2O	Choline	Oil + Choline	Oil + H_2O
Rats on low protein, low fat diet for 7 days											
373	480	603	366	3.76	4.17	4.66	3.90	99	117	130	93
Stock diet for 7 days											
384	402	487	408	4.97	5.36	5.36	5.45	76	76	93	75

The formation of labeled phosphatides is due partly to additional formation of phosphatides molecules during the experiment and partly to replacement of old molecules by new ones (renewal).

The administration of water does not lead to additional formation of phosphatides in the liver. The radioactivity of 373 (see table XVII*b*) is thus due to a replacement of old (non-labeled) molecules by new (labeled ones). If simultaneously an additional amount, corresponding to the amount renewed, would be formed after

administration of choline + oil, we should find a total activity in the liver phosphatides about twice as high as the figure (373) stated above. The administration of choline + oil leads to a 62% increase in the ^{32}P content of the phosphatides; as the phosphatide content of the liver was found by chemical analysis to increase with 24 %, less than half of the increased ^{32}P content of the liver phosphatides is due to additional formation of liver phosphatides under the effect of choline + oil, while the other half is due to the replacement of non-active molecules by active ones. Some renewal of the additional phosphatide molecules takes place as well, but labeled phosphorus being replaced by other labeled atoms, the phosphatide activity will only change if the activity of inorganic P atoms, which replace the phosphatide P atoms, change in the course of the experiment. In experiments taking not more than 3 hours such change hardly occurs, in experiments taking 6 hours the specific activity of the inorganic P decreases in the last phase of the experiment, the renewal of the additional phosphatide P leads thus to the formation of a less active product than formed initially.

In the rats kept on stock diet the administration of choline + oil results in an increase of the activity of the phosphatides by 26% only. The phosphatide content of the liver increased in these experiments by 8%, thus about 1/3 of the formation of labeled phosphatide molecules is due to additional phosphatide formation and about 2/3 to replacement of old molecules by new ones. Hence in animals kept on low protein, low choline diet, the increase in phosphatide content of the liver is enhanced by the administration of choline + oil; moreover the rate of renewal is also more pronouncedly accelerated in the first mentioned case.

Entenman et al. (105) found, in comparing the effect of choline on the specific activity of choline containing and choline not-containing phosphatide phosphorus that ingested choline does not increase the specific activity of choline not-containing phosphatide phosphorus of the liver. On the contrary, administered choline depresses its specific activity.

Platt and *Porter* (95*a*) found recently that the administration under similar conditions of ethanolamine, which is lipotropic, causes a rise in the rate of phosphatide turnover as well, the increase in this case being confined to the cephalin fraction, while the administration of choline leads to an increase in the lecithin turnover

only. They interpret their results to indicate the rise of phosphatide turnover in the liver, due to the administration of choline, to be a mass effect. Their results are seen in table XVII*c* and *d*.

They investigated also the effect of fasting on the phosphatide turnover. After 22 hours fasting, in experiments taking 6 hours, a decrease in the rate of phosphatide turnover was observed as indicated by the figures of table XVII*e*, The effect of fasting on the phosphatide turnover much depends on the time of the experiment (cf. p. 311).

TABLE XVII*c* (95*a*)

Influence ot the Choline on the Rate of Formation of Phosphatides in the Liver of Rats.

(Five rats received the diet as usual, while the other had in addition 50 mg choline mixed with the diet. Six hours later the animals were killed.)

	SPECIFIC ACTIVITY	
Group	Lecithin	Cephalin
Control	0.36	0.24
Choline	0.44	0.26

TABLE XVII*d* (95*a*)

Influence of Ethanolamine on the Rate of Formation of Phosphatides in the Liver of the Rat.

(24 rats were given high diet for six days and injected radio-phosphate on the seventh, but 8 rats received 50 mg choline and 8 rats received 50 mg ethanolamine. Six hours later the animals were killed.)

	SPECIFIC ACTIVITY		
Group	Total phosphatides	Lecithin	Cephalin
1. Control	0.43	—	—
Choline fed . . .	0.67	—	—
Ethanolamine fed.	0.70	—	—
2. Control	0.39	0.44	0.35
Choline fed . . .	0.52	0.57	0.43
Ethanolamine fed.	0.69	0.57	0.77

TABLE XVII*e* (95*a*)

Effect of 22 hours Fasting on Phosphatide Turnover.
(Time of experiment 6 hours.)

	SPECIFIC ACTIVITY OF FED RATS		SPECIFIC ACTIVITY OF FASTED RATS	
	Lecithin	Cephalin	Lecithin	Cephalin
Liver	0.42	0.29	0.31	0.21
Kidney	0.36	0.16	0.15	0.10
Small intestine. .	0.18	0.12	0.13	0.07

The effects of a single dose of methionine, cystine, and cysteine upon the phosphatide activity of the liver of rats fed a high fat and low protein diet were also investigated (104). The amino acids were fed by stomach tube, simultaneously with injection of the labeled phosphate. The livers were analyzed eight hours after the injection. An increase of about 30% was observed in the rate of renewal of liver phosphatides of rats given the amino acids. Amino acids differ in their capacity for stimulating phosphatide activity in liver. Glycine, alanine, serine, tyrosine, proline, glutamic acid, and asparagine were found to increase the rate of renewal of liver phosphatides. A negative result was obtained with taurine, creatine, dihydroxyethyl, sulfoxide, and sarcosine.

Choline phosphate inhibits turnover of phosphatides in liver. The inhibition appears to be limited to the noncholine phosphatide fraction. Choline phosphate as a unit is probably not utilized in the synthesis of phosphatides (107).

Horning and Eckstein (120*a*) demonstrated the lipotropic action of menthionine and choline 8 hours after the oral administration of these methylated products to young male rats that had previously ingested a lipogenic diet for periods varying from 10 days to 4 weeks. While the ^{32}P content of the liver phosphatides increased and a slight increase in the concentration of the liver phosphatides took place as well, these increases were not always accompanied by a fall in liver lipid content and may not be related to lipotropic action.

The answering of the question if and to what extent phosphatide turnover is a step in fat oxidation or transport encounters appre-

ciable difficulties. *Bollman and Flock* (121*a*) calculate that in 100 g of liver each hour 3.5 mg of phosphatide are renewed. This figure accounts for enough phosphatide turnover in the liver and plasma to metabolize or transfer fat equivalent to only 3% of the caloric needs of the animals and indicates that phosphatide formation is probably not an obligatory step in fat oxidation or transfer.

5. *Turnover in Kidney.* — The specific activity of phosphatides extracted from rabbit and rat kidney is lower than that of phosphatides from the small intestine and liver at early intervals after administration of radioactive phosphate. After the lapse of six hours the same result was obtained in experiments with dogs, but after eighteen hours the specific activity of the kidney phosphatide remained lower than that of the liver and about equal to that of the small intestine. At 98 hours the specific activities of the phosphatides in all three tissues were roughly the same (108). In 90 day old rats, 2 hours after subcutaneous injection of ^{32}P extracted from the kidneys, the specific activity of phosphatide P was found (108*a*) to make out 13% of the specific activity of the inorganic P. The specific activity of the kidney phosphatides was thus almost as high as the specific activity of liver phosphatides (percentage ratio of the specific activities of phosphatide P and inorganic P=15). Acidosis induced by ingestion of ammonium chloride increases the turnover of kidney phosphatides, according to *Weissberger* (109). Administration of phlorizin did not influence the turnover rate of phosphatides in kidneys of the rat (110).

6. *Turnover in Muscles.* — ^{32}P slowly appears as phosphatide phosphorus in skeletal muscle. This may be related to the slow rate of entry of labeled phosphate or of labeled phosphatides into muscle cells. The specific activity of phosphatides extracted from cardiac muscle, into which the phosphate penetrates at a higher rate than into skeletal muscle, is found to be higher. *Artom* (112) injected radioactive phosphate into rats and cats in which the femoral and sciatic nerves of one leg had previously been cut. In the denervated muscle newly formed phosphatides and, to a smaller extent, total phosphatides were found to be increased. The specific activity values of phosphatide phosphorus showed a gradient in the following order: liver, plasma, denervated muscle, intact muscle. The same gradient for the specific activities of the phosphatides was obtained in an experiment after introduction of a labeled emulsion

of radioactive phosphatides. These results are interpreted by *Artom* to indicate that the labeled phosphatides synthesized by the liver and released into the plasma penetrate the muscle cells, larger amounts probably being deposited in the denervated muscles. That from the labeled phosphatides injected into the veins of the rabbit resp. dog only 2.5 and 4.2% resp., were recovered in the muscles, (cf. p. 318) is not necessarily in variance with these results. It is quite possible that the labeled phosphatides present in the muscle tissue are partly built up in the muscle cells and partly penetrate from the plasma into the cells.

The turnover rate of phosphatides is found to be increased in muscles of rats maintained on a diet deficient in fat (114). In the fasting mouse, all phosphatide fractions, with the exception of α-cephalin which remained constant, showed a large increase in the rate of renewal and reached a maximum on the second day of fasting (87).

7. *Turnover in Brain.* — The brain is the organ in which both rate of penetration of labeled phosphate and incorporation of ^{32}P into phosphatides is found to be lower than in any other organ (142).

If and to what extent labeled phosphatides migrate from plasma into brain cells is not known. That labeled phosphatide molecules can be built up in brain tissue follows from experiments carried out with brain tissue slices described on page 314.

The maximum ^{32}P content (0.06% of the labeled phosphate administered per g tissue) was observed in adult rat brain 200 hours after administration. In young brain, maximum content was observed only after 300 hours (115).

As the tissue formed in an organism, given a labeled substance, will necessarily become labeled and, furthermore, as there is usually in the growing organism intensified enzyme action which leads to an accelerated rate of renewal of tissue compounds, we should expect a rapid formation of labeled phosphatides to take place in brains of growing rats. Rats of very different ages (including newly born rats) were studied by *Changus* et al. (115) and *Fries* et al. (116). The highest rate of formation of labeled phosphatides was found to take place at birth. Though this general characteristic was shared by all the parts investigated, the phosphatide activity was by no means uniform throughout the nervous system; striking

differences were encountered in the formation of labeled phosphatides in forebrain, cerebellum, medulla, and spinal cord. An abrupt change in the rate of renewal of brain phosphatides occurs in the central nervous system of the rat during its growth from 30 to 50 g. As growth proceeds beyond 50 g, the activity of brain phosphatides decreases throughout the central nervous system, but at a much lower rate than observed between birth and the age when the weight of 50 g is attained. The spinal cord in the 200 g rat possesses an activity of 20% of that of the 50 g rat, whereas in the 300 g rat, the cord retains 15% of the activity found in the 50 g animal. Forebrain, cerebellum and medulla also lose activity as the animal grows from 50 to 300 g, but the rate of decline in activity is lower than that occurring in spinal cord. By the time a weight of 200 or 300 g is reached, the relative activities of phosphatides in forebrain, cerebellum and medulla are as great as those of spinal cord, or even greater.

8. *Adrenal Glands and Phosphatide Formation.* — The ability of the adrenalectomized animal to synthesize new phosphatide molecules was established. The rate of formation of labeled phosphatide molecules in liver and small intestine of the rat is not influenced by complete removal of both adrenal glands (108).

9. *Turnover in Neoplastic Tissue.* — If the phosphatid emolecules of carcinomatous tissue were replaced at a high rate by labeled molecules, and such molecules were given off by the tumor to the circulation shortly after administration of the labeled phosphate, the presence of carcinomatous tissue could possibly be diagnosed by determination of the activity of plasma phosphatides. The facts, however, that the phosphatide turnover of neoplastic tissue is much slower than the turnover in liver, and that phosphatide molecules in the circulation were to a very large extent built up in the liver, frustrate this possibility.

No appreciable difference is found in labeled phosphatide formation of spontaneous and transplanted tumors (117). In both cases, it is appreciably larger than that occurring in muscles, but smaller than that found in liver. Thus, the formation of active phosphatide molecules in carcinomatous tissue is neither extremely pronounced nor very low.

A detailed investigation of phosphatide metabolism in mammary carcinoma, lymphoma, lymphosarcoma, and sarcoma (180), was

carried out by *Jones, Chaikoff* and *Lawrence* (119). Four tumors were transplanted into mice. They differed with respect to cell type and rate of growth. Two of the tumors produced metastates in distant parts, whereas the two other remained entirely localized at the place of inoculation. Uniform phosphatide activity was not found in the several types of tumor examined. The activity of lymphoma was only about one-third of that found in mammary carcinoma or lymphosarcoma. Cell type is apparently not a decisive factor in determining extent of phosphatide turnover, which is independent of the host.

The maximum deposition of phosphatide ^{32}P in neoplastic tissues may last from 10 to 50 hours.

In carcinosarcoma "256" the turnover of lecithin is somewhat more rapid than that of cephalin. The mode of behaviour of the sphingomyelin fraction was found to be similar to that of cephalin, but unlike that of the lecithin fraction of the same tumor (97). The specific activity of sphingomyelin phosphorus increases to a maximum at 48 hours after feeding labeled phosphate. Cephalin shows the same behaviour in contrast to lecithin (99).

10. *Turnover in Blood.* — Shortly after administration of labeled phosphate, tagged phosphatides penetrate from the liver into the circulation. By 40 hours 0.5 to 0.1% of the administered ^{32}P has been incorporated into phosphatides in the total plasma of the dog (128).

TABLE XVIII

Specific Activity of Inorganic and Phosphatide Phosphorus of Rabbit Plasma.

Time	Relative specific activity		Time	Relative specific activity	
	Inorganic P	Phosphatide P		Inorganic P	Phosphatide P
4 hours	100	0.53	45 hours	100	22.0
16 hours	100	3.8	55 hours	100	27.5
25 hours	100	8.1	9 days	100	81.6
37 hours	100	15.0			

In experiments in which labeled inorganic phosphorus in rabbit plasma was kept at a constant level (142), the phosphatide phospho-

rus of the plasma showed the specific activities recorded in table XVIII. To what extent labeled phosphatide molecules are built up in the plasma can be investigated only by experiments in vitro. In such an experiment (122), lasting 4.5 hours, the specific activity of the phosphatide phosphorus was found to be less than 0.1% that of the inorganic phosphorus.

11. *Phosphatide Formation in Tissue Slices.* — The question of whether a tissue can synthesize phosphatides independently, or whether it acquires phosphatides from the plasma only after their formation by a more active tissue, was answered by *Fries* et al. (123) in the following manner: A sciatic nerve of a dog stripped free of all adipose and connective tissue and weighing 300 mg was placed in 5 ml of Ringer solution containing radioactive phosphate. For control purposes the adipose-connective tissue surrounding the nerve was treated in a similar way. Conversion of radiophosphate from the Ringer solution into radiophosphatide by the nerve was found to be considerable, as is seen in table XIX. These experiments show that the nerve process, separated from the nerve cell body, can form phosphatides from inorganic phosphate.

TABLE XIX

Formation of Radioactive Phosphatide by Dog Sciatic Nerves (123) (*).

Time Interval hr.	Nerve	Adipose-connective tissue
0	0.006	0.008
0	0	0
4	0.63	0.036
4	0.72	0.026
4	0.44	—

(*) All values are expressed as per cent of labeled phosphorus of the Ringer solution incorporated into phosphatide per gram wet tissue.

Similar values were obtained for the formation of radioactive phosphatide in brain slices of young and old rats. In the course of 4 hours 0.70 to 0.85% of the labeled phosphorus of Ringer solution was incorporated in brain slices (per gram wet tissue); in brain homogenate lower values (0.20 to 0.22) were obtained.

This type of phosphorylation can only be detected by making use of labeled phosphate. The usual methods of chemical analysis fail to detect the synthesis of the small percentage of new phosphatide molecules, as the formation of these goes hand in hand with the autolysis of a comparatively large percentage of phosphatide molecules present at the start of the experiment. The amount of phosphatide found in brain homogenate after 4 hours is 10—15% less than that present at zero time.

Conversion of labeled, inorganic phosphorus into phosphatide phosphorus by surviving brain slices is greatly increased (up to about five times) by the addition of hexose, galactose, glucose, mannose, and fructose to the bicarbonate Ringer solution containing labeled phosphate. This increase in formation of labeled phosphatide could be due either to an increased rate of penetration of labeled phosphate into the site of synthesis of phosphatides, or to an enhanced rate of formation of labeled phosphatide molecules. That the presence of hexose accelerates the rate of formation of labeled phosphatide molecules is shown by the following experiment. Brain slices, after being kept in a bicarbonate Ringer solution containing ^{32}P, are washed and placed in a bicarbonate Ringer solution for two hours. While in the glucose-free Ringer solution hardly any further formation of labeled phosphatides takes place, a threefold increase of labeled phosphatide content is observed when the Ringer solution contains glucose (126).

It is well known that the oxygen consumption of brain preparations remains nearly constant for long periods when the preparations are placed in a Ringer medium containing glucose, whereas in a glucose-free medium the oxygen uptake decreases rapidly. Presumably the formation of labeled phosphatide molecules is promoted by increased oxygen consumption which provides increased oxidative energy for formation of phosphatide or of a phosphorus-containing phosphatide precursor. The stimulatory effect of the hexose upon formation of radiophosphatide does not occur under anaerobic conditions. The stimulation is abolished when the tissue organization is disrupted by homogenization. Addition of pentoses fails to increase the yield of radiophosphatides (125).

In both liver and kidney slices, phosphatide formation is greatly impaired in the absence of oxygen, as is seen in table XX.

TABLE XX

Effect of Anaerobic Conditions on Phosphatide Formation (125*a*).

Rat No.	Period of incubation, hr.	Per cent added ^{32}P recovered as phosphatide per g tissue				Inhibition %
		Control oxygen present		Anaerobic conditions		
		Wet weight	Dry weight	Wet weight	Dry weight	
Liver						
1	4	9.2	40	0.60	2.6	93
2	4	6.5	29	0.53	2.3	92
3	2	3.2	14	0.44	1.9	86
4	2	3.8	17	0.21	0.92	94
Kidney						
5	4	4·7	26	0.058	0.32	99
6	4	3.4	19	0.41	2.3	88
7	2	3.3	18	0.17	0.95	95
8	2	3.5	20	0.80	4.5	77

Phosphatide formation in surviving liver and kidney slices is extremely sensitive to the presence of sodium cyanide. Concentrations as low as 0.001 M sodium cyanide inhibit the formation in liver slices to the extent of about 97% (126*a*).

Azide and hydrogen sulfide were found to have an effect similar to that shown by cyanide on formation of labeled phosphatides in tissue slices. Carbon monoxide was furthermore shown to have an inhibitory effect, which is more pronounced in the dark than in the presence of strong light.

Homogenized liver tissue completely loses its ability to incorporate phosphate into the phosphatide molecule (126). Much less phosphatide formation is shown by homogenates of kidney and brain than by slices of these tissues (123).

It might be tempting to interpret the formation of labeled phosphatides in tissue slices as due to partial reversibility of hydrolysis of phosphatides. Even though the tendency of the reaction is far in the direction of decomposition, the reverse reaction may occur to a slight degree even during the early period of forward reaction. The results obtained by *Chaikoff* and associates with respiratory inhibitors exclude, however, the possibility that the formation of labeled phosphatides is due to a reversal of the decomposition of phosphatides proceeding in the tissue slices. In surviving rat kidney and liver slices much more radioactive organic phosphorus is found when the uptake of ^{32}P is investigated in the presence of fluoride (77).

12. *Path of Conversion of Inorganic Phosphate to Phosphatide.* — The path of conversion of inorganic phosphate to phosphatide is not known. Glycerophosphate, diglycerides, neutral fat, choline phosphate, phosphoproteins, or other compounds may be involved as intermediates. That aminoethylphosphoric acid can be excluded from this group of substances follows from the work of *Chargaff* and *Keston* (101). Experiments in which 80 mg of labeled disodium aminoethyl phosphate was administered to adult rats by subcutaneous injection showed that the body was unable to utilize aminoethylphosphoric acid as such for the synthesis of cephalin. 28% of the ^{32}P administered as aminoethyl phosphoric acid was found to be excreted through the kidneys in the course of eight hours in these experiments.

13. *Rate of Interchange of Plasma Phosphatides with Tissue Phosphatides.* — The rate of interchange of plasma phosphatides with tissue phosphatides was determined in experiments in which part of the plasma of a rabbit was replaced by an equal volume of plasma from another rabbit containing labeled phosphatides (121); similar experiments were also carried out with chicks (121) and with dogs (90). In another investigation an emulsion of phosphatides prepared from rat liver was introduced into the circulation of a rat (164). In the course of 10 hours a substantial part of the labeled phosphatide molecules was found to have left the plasma and was detected in different organs, especially in the liver. Some results obtained in experiments with rabbits and dogs (90) are seen in tables XXI*a* and XXI*b* respectively.

TABLE XXI*a*

Labeled Phosphatides Found in the Organs of a Rabbit 4 Hours after Replacement of 12 ml of the Rabbit's Plasma by Plasma of another Rabbit Containing Labeled Phosphatides (121).

ORGAN	WEIGHT	Percentage of the labeled phosphatides injected into the vein, present in the blood free organ.
Liver	62 g	28.9 %
Kidneys	9 g	0.88 %
Muscles	910 g	2.5 %
Heart	5 g	0.21 %
Spleen	1.2 g	0.06 %
Small intestine mucosa	46 g	1.1 %
Lungs	10 g	1.0 %
Brain	6 g	0.05 %

TABLE XXI*b*

Distribution of Phosphatides in Tissues of the Dog at End of 5 Hours (90).

TISSUE	Injected dose per whole organ %	Labeled phosphatide P per whole organ mg.	Organ phosphatide supplied by plasma per hr. %
Plasma	53.0	18.5(*)	—
Liver	11.1	173	1.09
Kidney	1.13	28.6	0.67
Small intestine	2.44	68	0.61
Spleen	0.35	8.5	0.71
Red Corpuscles	1.15	—	—
Muscle	4.2	—	—

(*) Milligrams phosphatide P per 100 milliliters.

The fact that 76-83% of the injected labeled phosphatides can be accounted for in the seven tissues of the dog examined suggests that the breakdown of phosphatides in the animal tissue cannot be a rapid process.

That, beside being the principal tissue in the body concerned with the synthesis and supply of plasma phosphatides, the liver is also mainly responsible for the removal of phosphatide molecules from

the plasma is also shown by *Entenman* and his associates (105*a*). When comparing the rates at which the injected labeled plasma phosphatides disappeared from the plasma of the normal and the liverless dogs, the rate of disappearence from the plasma of the normal dog was found to be six to ten times larger than the rate of disappearance from the liverless dog. The specific activities of the plasma phosphatide phosphorus of dogs that had received intravenously radioactive plasma phosphatides did not decrease significantly after the liver had been excluded from the circulation. In contrast to this result the labeled phosphatides disappeared at a normal rate from the plasma when the gastrointestinal tract was removed. While in normal dogs weighing from 7 to 18 kg the plasma phosphatides are completely turned over in 6 to 10 hours, by depriving these dogs of their livers the time required for complete turnover was prolonged from 33 to 160 hours.

14. *Passage of Phosphatides into Lymphatic Channels.* — As mentioned above, in the course of few hours a very appreciable part of the phosphatide molecules originally present in the plasma is found to be replaced mainly by phosphatide molecules previously present in the liver. *Reinhardt* et al. (128) found that part of the labeled phosphatide molecules injected into plasma reached lymphatic channels and were recovered in the thoracic duct lymph. It is not established through which tissues phosphatides migrate, although liver presumably plays an important part in this process.

15. *Study of Lecithinemia.* — About two hours after administration of a meal containing fat, the fat content and phosphatide content of the blood begin to rise. A maximum is reached after four hours (129). The increase in lecithin content of the plasma could be due to lecithin synthesized in the intestinal mucosa and absorbed into the circulation or to mobilization of phosphatides synthesized in the liver or other organs.

The following experiment (130) shows that at least a large part of the phosphatide excess found in lipemic blood is not due to phosphatide molecules taken up from the intestine. At the start of an experiment 150 mg labeled phosphorus as sodium phosphate was administered to a fasting dog weighing 7 kg, and another 150 mg. was given after the lapse of two hours. Simultaneouslty with the second sodium phosphate dose, 50 g olive oil was fed. Six hours after the start of the experiment a pronounced lipemia was found

to have taken place, and a rise of the blood phosphatide phosphorus amounting to 2.5 mg per cent was observed in this lipemic state. Of these 2.5 mg. per 100 ml, however, only 0.048 mg per 100 ml was labeled phosphatide phosphorus. The rest was nonlabeled phosphatide phosphorus mobilized by the liver or by other organs.

When interpreting these figures, it must be considered that the intestinal tract of the fasting dog contained endogenous phosphorus which « diluted » the labeled phosphorus fed to the dog and, furthermore, that an exchange of plasma phosphatides with organ phosphatides takes place which will result in the replacement of a part of the labeled blood phosphatides by unlabeled organ phosphatides. But it was found (cf. p. 318) that in the course of two hours less than half of the plasma phosphatides was replaced by organ phosphatides; thus, renewal of labeled blood phosphatides during the experiment cannot explain the large difference found between the increment of total blood phosphatides and the increment of labeled blood phosphatides, the ratio of wich was as high as 31. Nor can the effect of the « dilution » by intestinal phosphate of the labeled phosphate fed to the animal explain more than part of the above mentioned difference.

16. *Origin of Phosphatides and other Phosphorus Compounds of Yolk.* — A hen laying daily incorporates about 1.5 g of phosphatide in the yolk, which corresponds to about 10% of the yolk weight. This percentage is much more than the amount contained in the daily food of the hen. Furthermore, it was found by different investigators that the fact that a fowl was raised on diets containing phosphorus in inorganic form only did not unfavorably influence its egg-laying capacity. It is, therefore, wholly or mainly in the organs of the hen that the synthesis of the phosphatide molecules of the yolk takes place. For the purpose of securing information regarding the organ in which the yolk phosphatide is primary synthesized, experiments were carried out with ^{32}P as an indicator (131).

Labeled sodium phosphate was administered to laying hens, the eggs laid were collected, and the specific activities of the phosphatide phosphorus extracted from the yolks were determined. In other experiments, the hen was killed and the specific activities of the phosphatide phosphorus of the yolks, removed from the ovary, liver, intestinal mucose, and plasma were compared. The following

figures show the results obtained for the specific activity of phosphatide phosphorus extracted from different organs of a hen 5 hours after administration of labeled sodium phosphate (131):—

Organ	Relative specific activity
Liver	100
Plasma	79
Ovary	7.2
Yolk	9.2
Intestinal mucose	18

Ovary phosphatides are only slightly active; plasma phosphatides show a marked activity and liver phosphatides show the greatest activity.

Thus, the gradient in flow of labeled phosphatides is directed from plasma to ovary. The explanation suggests itself that yolk phosphatides are supplied by plasma phosphatides and that the role of the ovary, in supplying egg phosphatides, is to remove phosphatides from plasma and to incorporate them into yolk. Nature endowed plasma of birds actively engaged in egg laying with a much higher phosphatide content (about 25 mg per cent) than is found in plasma of other animals or in plasma of male birds and immature females, obviously in order to facilitate passage of the large amounts of phosphatides which the plasma of the laying bird has to carry into the ovary. The total phosphatide content of the plasma of the laying hen in question amounted to 15 mg. The hen, laying daily, incorporated about 50 mg phosphatide phosphorus into the yolk within 24 hours (nearly four times the phosphatide content of the plasma). The phosphatide content of the plasma was thus almost wholly renewed in the course of the 5 hours of the experiment.

The above figures suggest that phosphatide molecules of the yolk are mainly synthesized in the liver and are passed on by the plasma to their destination. The liver contained 34 mg of phosphatide phosphorus and, since in the course of five hours some 9 mg. of phosphatide phosphorus was carried into the ovary, about one-fourth of the liver phosphatides must have been renewed within 5 hours to supply the phosphatides incorporated into the yolks, a figure compatible with the results obtained in the investigation of the rate of renewal of liver phosphatides (see page 298). The fact that

the yolks show but a small activity is due to the dilution of the strongly active phosphatides incorporated during the last 5 hours by the large amounts of nonlabeled phosphatides already present in the yolk.

Within the yolk, no new formation of phosphatides (no formation of labeled phosphatides) takes place. This is shown by the fact that, if the labeled phosphate is administered after the egg has left the ovary, no active phosphatides are found in the yolk, as distinct from active inorganic phosphate which penetrates from the circulation into the egg during every phase of its formation.

The same fact is borne out by experiments in vitro in which eggs were placed for one day in a solution containing active phosphate. 99.4 % of the activity found in the eggs was located in the shell, 0.4 % in the white, and 0.2 % in the yolk. The phosphatides extracted from the yolk were found to be inactive.

The egg enters the oviduct about 15 minutes after ovulation, passes through the funnel in 18 minutes, spends 3 hours in traversing the magnum or albumin-secreting portion of the oviduct, 1 hour in the isthmus, and the remainder of the period (usually 20-24 hours) in the uterus. Thus, the egg spends about one day outside the ovary before being laid. When labeled phosphate was administered to a hen 5 hours before laying, the ovum was certainly no longer in the ovary. This egg (see table XXII) did not contain active phosphatides, as was to be expected. Active non-phosphatide (mainly inorganic) phosphorus was found in the yolk, however, and the phosphorus of the white and the shell also showed very high activity.

TABLE XXII

Labeled Phosphorus Content of Eggs (131).

Time between administration of active P and egg laying	Per cent labeled P administered found in			
	Shell	Albumin	Total yolk	Yolk lecithin
5 hours	0.24	0.0015	0.0014	0.0000
1.0 day	0.052	0.032	0.109	0.014
3.0 days	0.036	0.030	0.42	0.17
4.5 »	0.026	0.027	0.95	0.34
6.5 »	0.022	0.020	0.85	0.35

Shell deposition begins practically as soon as the egg reaches the uterus and presumably continues until oviposition. During the time the egg is in the uterus, approximately 5 g of calcium carbonate, containing a small amount of phosphate (3-4 mg phorphorus), is deposited in the shell membranes as the egg shell. This phosphate is secreted from a plasma containing highly active phosphate shortly after the administration of ^{32}P, and the shell phosphate secreted shortly after administration of labeled phosphate is bound to be highly active. In the course of the next few days, the activity of the plasma phosphate decreases and the shell of the eggs layed after a day or more is found to be less and less active, as seen in table XXII.

Lorenz, Perlman and *Chaikoff* (132) showed that the amount of ^{32}P deposited in phosphatides and other compounds in the yolk could be accounted for by an integral function of the two variables, yolk growth rate and ^{32}P availability, during the corresponding period of new formation. These experiments also showed a marked dissimilarity in the deposition of phosphorus in shell and in albumin. Those shells that were being actively formed at the time of injection showed a high ^{32}P content (up to 0.28% of the amount injected), whereas eggs that entered the uterus several hours later contained much smaller amounts of ^{32}P in their shells. Albumin protein is secreted while the egg is in the magnum and its deposition is completed about 22 hours before oviposition. Eggs laid during the 24 to 30 hour interval entered the magnum at a time when plasma radiophosphate was at its maximum. The ^{32}P content in the albumin of these eggs did not exceed 0.05%, whereas eggs laid between 45 and 75 hours contained 2-4 times this amount. The delayed deposition of ^{32}P in the albumin (see also 131) suggests that a synthetic process precedes the deposition of phosphorus-containing compounds. Egg albumin is known to contain slight amounts of phosphorus and it is not unlikely that the delay is due to a comparatively slow rate of incorporation of phosphorus into the albumin.

Chaikoff and associates (133) determined what percentage of ^{32}P administered was turned into phosphatide phosphorus in the laying and the nonlaying bird. The results are shown in table XXIII.

TABLE XXIII

Per cent ^{32}P Administered as Phosphate Found in Phosphatides of Laying and Nonlaying Birds (133).

Organ	Laying bird		Nonlaying bird	
	6 hours	12 hours	6 hours	12 hours
Entire bird	3.62	4.55	3.25	4.57
Gastrointestinal tract	10	10	23	15
Muscle + bone + blood . .	32	36	27	35
Ovary + oviduct + yolks	11 (*)	20	0.4	0.2
Liver	44	29	47	44

(*) 10 % in yolk, and only 1% in ovary and oviduct.

Table XXIII shows that nearly one half the amount of active phosphatides of the bird is located in the liver, although the phosphatide content of the liver may be estimated to represent but 5% of that of the bird.

17. *Effect of Diethylstilbestrol on Turnover of Phosphatides.* — *Flock* and *Bollman* (161) investigated phosphatide turnover following administration of diethylstilbestrol to cocks. When radioactive sodium phosphate was given intraperitoneally to cocks, labeled phosphatides appeared in the plasma in 2 hours and increased at a uniform rate for 12 hours. Six hours after the administration of ^{32}P the specific activity of the phosphatide of plasma was similar in cocks which received diethylstilbestrol and in untreated birds. The concentration of phosphatides was, however, greater in plasma of treated than in plasma of untreated birds, and the total ^{32}P content was correspondingly greater.

Calculations based on the ^{32}P content of liver phosphatides and the amount of ^{32}P content of phosphatides of plasma indicate that 1.51 mg of phosphatide phosphorus entered each 100 ml of plasma every hour in the untreated birds. Similar calculations showed an average of 5.0 mg entering each hour in birds which had received diethylstilbestrol.

In experiments in which surviving liver slices obtained from 1) normal birds, and 2) birds that had received a single injection of 10-15 mg of diethylstilbestrol were compared; a significant increase in the in vitro formation of the stilbestrol-treated bird was observed by *Taurog* and associates (162).

18. *Origin of Plasma Phosphatides.* — Strong additional evidence that plasma phosphatides, which in turn supply yolk phosphatides, are mainly derived from liver, is provided in investigations on formation of labeled phosphatides in the hepatectomized dog by *Fishler* et al. (124) and by *Entenman* et al.

The observation that radiophosphatides are found in the kidney and small intestine of the hepatectomized dog leaves no doubt that the liver is not the only site of phosphatide formation in the animal body. The recoveries of phosphatide ^{32}P per gram kidney phosphatide or per gram small intestine phosphatide in the liverless dog do not differ significantly from those found in the intact dog, the specific activities of kidney and intestinal mucose phosphorus being, after the lapse of five hours, 65 and 41%, respectively, of that of liver phosphatide phosphorus. Nevertheless, only small amounts of phosphatide ^{32}P were recovered from the plasma of the hepatectomized dog as late as 6 hours after excision of the liver.

Six hours after injection of labeled phosphate, the values for phosphatide ^{32}P per gram tissue phosphatide were about 100 times greater in kidney than in plasma. If a transfer to plasma from kidney and small intestine occurs, it must be, in contradistinction to transfer to plasma from liver, a slow process; a similar result was arrived at also in experiments in which plasma containing labeled phosphatides was injected into the circulation (see page 318). In these experiments only a small percentage of the injected phosphatides was found to be present in the kidneys and the intestinal mucosa. These results, and also those obtained in the study of the origin of yolk phosphatides (see p. 321) and in the study of the origin of the phosphatide increament in the plasma, after administration of a meal containing fat, (p. 319) strongly support the conclusion that plasma phosphatides are derived mainly from liver.

19. *Turnover of Vitellin.* — In an investigation carried out by *Chargaff* (134), the ^{32}P content of the phosphorus of « free » lecithin and cephalin, of the « combined » phosphatides accompanying the vitellin fraction, and of the vitellin fraction were investigated. While about 50% of the phosphatides present in yolk, the « free » phosphatides, can be extracted with ether, the remainder, the « combined » phosphatides, are present as a constituent of the lipide-protein complex, lipovitellin, contained in hen-egg yolk.

Phosphorus compounds isolated from yolks of eggs laid in the course of eight days following intramuscular injection of radioactive sodium phosphate were examined individually. The specific activities of the phosphorus extracted from the fractions, and hence the rate of formation of « free » lecithin and cephalin and of the « combined » phosphatides accompanying the vitellin fraction,were found to be equal. Vitellin phosphorus, however, exhibited a considerably higher specific activity in the first five or six days of the experiment than the phosphatide phosphorus. The maximum specific activity was obtained for all fractions after the lapse of six days.

The higher activity of vitellin can be explained by assuming that phosphatides were formed at a slower rate than vitellin and, correspondingly, that vitellin was formed in the first part of the experiment with the participation of more highly active phosphorus than were the phosphatides. If this explanation is correct, we should expect the vitellin phosphorus in the later phases of the experiment to be less active than the phosphatide phosphorus. This is actually found to be the case : after the lapse of eight days the vitellin phosphorus was found to be somewhat less active than the phosphatide phosphorus.

An additional reason for the higher activity of vitellin phosphorus in the first phase of the experiment may be sought in a greater dilution of the active phosphatides by the non-active (old) phosphatides present in the organism. Substantial amounts of phosphatides are present in the organism, and part of these interchange with the plasma phosphatides. The latter will consequently be responsible for diluting the active phosphatides. On the other hand, vitellin is present only in small amounts in the hen, being readily detectable only in the blood of laying hens.

20. *Origin of Phosphatides in Chick Embryo and Rabbit Foetus.* — Only about two-thirds of the phosphatides in the egg are hydrolyzed during its incubation. Considering the large store of phosphatides in yolk (even shortly before the egg is hatched), we should expect the embryo to avail itself of this store when it needs phosphatides to build up its nervous system and other organs containing these substances. This point may be checked by introducing a small amount of labeled sodium phosphate, dissolved in 0.1 ml of physiological sodium chloride solution, into the white of the egg before incubation, and determining whether and to what extent the phos-

phatides of the yolk and the embryo become labeled (163). If none of the phosphatides are labeled, we may conclude that the phosphatide molecules in the embryo are not newly synthesized from inorganic phosphate present there. If, on the other hand, the yolk phosphatide remains unlabeled while that of the embryo becomes radioactive, we may conclude that the phosphatide molecules in the embryo have not come from the yolk, but have been built up in the embryo with the participation of labeled inorganic phosphorus. As seen in table XXIV, phosphatides extracted from the embryo invariably showed a high specific activity, while those from the yolk were barely active. The slight activity of yolk phosphatide, which increases with age of the embryo, is possibly due to an influx from the embryo into the yolk of a small amount of labeled phosphatides or of the enzymes responsible for resynthesis of phosphatides. The behaviour of yolk phosphatides during incubation also illustrates the point discussed above, namely, that labeled phosphatides found in the yolk must have been deposited as such and that phosphatides once incorporated into yolk cannot become labeled.

TABLE XXIV

Relative Specific Activity of Phosphatides extracted from Embryo and Residual Yolk (163).

Time of incubation days	Phosphatides extracted	Specific activity
6	Yolk	0.032
	Embryo	100
11	Yolk	0.10
	Embryo	100
18	Yolk	9.92
	Embryo	100

The specific activities of inorganic phosphorus, hexosemonophosphate phosphorus, creatine phosphorus, phosphatide phosphorus, and residual phosphorus extracted from the embryo had the same value, showing that the inorganic phosphorus atom reaching the embryo has the same chance of entering a phosphatide or other molecule.

Recently the formation of labeled phosphatides in the foetal

liver was compared with the formation in the maternal liver of the rabbit (163*a*, cf. also 162*a*).

From table XXIV*a* it is evident that the activity of the phosphatides extracted from the foetal liver and foetal placenta was much higher than the activity of the maternal plasma phosphatides 4 hours after the injection of the mother with labeled phosphate. The low specific activity of the foetal plasma phosphatides shows that the highly active phosphatides in the foetal liver have been synthesized in that organ, and have not been transported from the foetal placenta.

TABLE XXIV*a*

Radioactivity of Tissue Inorganic P and of Phosphatide P relative to that of Maternal Plasma Phosphatide P (163*a*). (Rabbit 138 : 3.16 kg, 26th day pregnancy, 4 hours after intravenous injection of a single ^{32}P dose as Na_2HPO_4.)

SOURCE	Specific activity in relative units	Activity of liver phosphatide P as % of activity of tissue inorganic P
Maternal plasma phosphatide P. . .	100	—
Maternal liver inorganic P at the end of experiment	2570	—
Maternal liver phosphatide P	405	15.76
Foetal liver inorganic P at the end of experiment	1098	—
Foetal liver phosphatide P	221	20.10
Foetal placenta phosphatide P . . .	328	—
Foetal plasma phosphatide P	39.6	—
Foetal carcass (without liver) phosphatide P	45.3	—

That phosphatide synthesis is taking place in all foetal tissues was also shown by experiments in which the foetuses were injected directly in utero with disodium hydrogen phosphate labeled with ^{32}P. After opening the abdomen, the labeled phosphate was injected with a fine needle through the uterine wall into the back of the foetus. The abdominal incision was then closed. The experiments lasted 1-3 hours; the results of two of them are shown in table XXIV*b*. By this technique it was possible to obtain high activities in the foet-

uses and very low activities in the mother. In one experiment, for example, the inorganic phosphorus in 0.1 ml of foetal plasma gave 500 counts/min. and the same volume of maternal plasma, withdrawn at the same time, only 5 counts/min. above background.

TABLE XXIV*b*

Relative Activities of Foetal Phosphatides 3 Hours and 3 hrs 10 min. after Injection of Labeled Na_2HPO_4 into Rabbit Foetuses 25 days old (163*a*).
Rabbits 151 and 152.

Source of phosphatides	Specific activity of phosphatides	
	3 hours after injection	3 hrs. 10 min. after injection
Maternal plasma	Inactive(*)	Inactive(*)
Foetal liver	100	100
Foetal viscera (intestines, kidneys, lungs and heart)	—	39.1
Foetal carcass (without liver)	17	—
Foetal brain	3.34	3.84

(*) That is, no activity could be detected in phosphatides extracted from 15 ml of plasma.

That labeled inorganic phosphate passes promptly the placenta was shown by *Wilde* and assoc. (159*a*). They measured in guinea pigs the changes in rate of placental transfer of inorganic phosphate from the 31st day of pregnancy until term. The transfer rate per unit weight of placenta was found to increase to about 10 times during this period. Inorganic phosphate reaches the foetus from the maternal plasma in an amount approximately equal to the total phosphorus retained in growth.

Presumably a transfer of phosphatides from the mother to the foetus takes place as well, but the rate of transfer cannot compete with the rate of formation of phosphatides in such active organs as the liver or the foetus. But labeled maternal red corpuscles cannot pass the placenta in a normal human organism. In a single case, which is considered to be pathological, some transfer was observed (158*a*). In these experiments, blood samples of the mother were labeled with ^{32}P and reinjected into the maternal circulation.

21. *Effects of Vitamin D Phosphorus Metabolism in the Chick Embryo.* — The technique described above (163) was used in the study

of the utilization of inorganic phosphorus in the chick embryo. Eggs were injected by *Branson* et al. (152*a*) with 0.1 ml of an isotonic solution of NaH_2PO_4 with an activity of 0.12 microcuries. The eggs were sealed with sterile paraffin, marked, and placed in an incubator. Thirteen day embryos proved convenient for analyses with a relatively high (40%) survival rate.

The viable embryos were cleaned of adhering membranes and dropped into liquid air. They were ground to a powder in a chilled mortar and the powder was extracted with cold trichloroacetic acid. The acid extract was filtered into cold, concentrated sodium hydroxide. The resulting solution was divided into three parts. From one aliquot we determined the average acid-soluble phosphorus compound;

TABLE XXIVc

FRACTIONS	Activity: $\frac{\text{Average counts/mg of phosphorus}}{\text{Counts from 0.1 ml of original } NaH_2PO_4 \text{ solution}} \times 10^3$	
	Group receiving ^{32}P and vitamin D	Group receiving ^{32}P only
Average acid soluble	0.530	0.633
Inorganic P	6.03	5.69
Adenosine P + inorganic P	8.12	2.18
Creatine P + inorganic P	4.15	3.32
Phosphatide P	2.33	0.825
Residual P (nucleoprotein).	3.02	0.285

the second aliquot was precipitated with 25% barium acetate at pH 6.5, the precipitate was washed with dilute barium acetate, centrifuged, and a part dissolved in cold nitric acid. The solution was treated with ammonium molybdate reagent. This precipitate consisted of the inorganic phosphorus. The remainder of the barium acetate precipitate was washed and the phosphorus determined. This fraction consisted of inorganic phosphorus plus adenosine phosphorus. The third aliquot was hydrolyzed with normal hydrochloric acid and 0.1 normal ammonium molybdate for 30 minutes at 40° C. The phosphorus released from the organic compounds was precipitated. This fraction consisted of inorganic phosphorus plus phosphocreatine. The residue from the acid extractions was treated with an alcohol-ether mixture. The filtrate con-

tained the phosphatide phosphorus, and the residue gave the so-called residual phosphorus containing mainly nucleoprotein phosphorus.

The data of table XXIV*c* reveal that the group of 6 embryos receiving the ^{32}P and vitamin D shows a higher specific activity for all fractions, except inorganic P and the average acid-soluble P, than the group of 2 embryos which received only ^{32}P. These results support the assumption that vitamin D accelerates the over-all metabolism of inorganic phosphorus in the developing chick embryo. The effect is most marked in the adenosine, phosphatide and residual phosphorus fractions.

22. *Origin of Milk Phosphatides.* — The origin of phosphatides in goat milk (135, 136) was investigated along similar lines to studies of the origin of yolk phosphatides. Phosphatides extracted from milk a few hours after subcutaneous injection of labeled phosphate were found to be much more active than those present in the plasma. Thus, phosphatide molecules of the milk cannot have originated in the plasma, but must have been built up mainly in the mammary gland. The specific activity of the phosphatide phosphorus extracted from the gland was, in fact, found to be higher than that of the corresponding products from the plasma and the milk, as is seen in table XXV.

TABLE XXV

Activity of Phosphatide Phosphorus of Milk and Organs of a Goat (135)(*).

FRACTION	Relative specific activity per mg phosphatide P
Milk	1
Plasma	0.02
Milk gland	1.4
Liver	1
Kidneys	1.2

(*) 4-5 hours after administration of labeled sodium phosphate.

Phosphatides are renewed in the milk gland at a still higher rate than in the liver. Investigation of the activity of different milk fractions by *Aten* (135) proved that no mixing occurred in the milk while stored in the udder. Moreover it was found that, a few hours after the start of the experiment, the specific activity of the phosphorus of casein and of acid-soluble organic phosphorus compounds was but

slightly lower than that of inorganic phosphate of the milk. This fact makes it seem very probable that these substances are formed in the milk gland from inorganic phosphate. The phosphorus atom is found to require 0.5 to 2.5 hours more to enter milk casein than to enter milk phosphate.

23. *Turnover of Phosphatides in Cell Nuclei, Effect of Roentgen Rays on Phosphatide Turnover.* — A comparison of turnover rates of phosphatides in sarcoma (137) shows that no pronounced difference is found between rates of renewal of phosphatides in nuclei and in tissue, and correspondingly in cytoplasm. In nuclei of liver, however, the rate of renewal of phosphatides clearly lags behind the rapid rate of turnover of these compounds in cytoplasm. To what extent failure to detect appreciable differences between turnover of phosphatides in nuclei and in tissue of sarcoma is due to a relatively rapid interchange between phosphatide molecules of cytoplasm and those of nuclei is not yet elucidated.

The effect of Roentgen rays on the turnover rate of phosphatides present both in tissue and in nuclei was investigated as well.

The rate of turnover of phosphatides in liver nuclei is markedly diminished by the action of Roentgen rays; that of the cytoplasm is also diminished though to a somewhat lesser extent.

c) *Turnover of Nucleic Acids.*

1. *Turnover of Desoxyribonucleic Acid.* — Desoxyribonucleic acid is wholly or mainly confined to cell nuclei. Since the desoxyribonucleic acid content of cell nuclei increases and decreases in the various stages of mitosis, we can expect an appreciable turnover of nucleic acid to take place in growing tissue, and in such organs as well which secrete products containing desoxyribonucleic acid. To the latter belong, among others, thymus, spleen, and bone marrow.

Liver and kidney of mature animals show, as is to be expected, a very low rate of renewal of their desoxyribonucleic acid content. The percentage ratio of activity of 1 mg of desoxyribonucleic acid phosphorus and 1 mg of inorganic phosphorus in experiments taking two hours is found to be about 0.1 mg both in liver (138, also 140) and in kidneys. The corresponding ratio is very much larger in the case of spleen and intestinal mucosa, as seen in table XXVI. The figures represent the average of numerous values obtained.

TABLE XXVI

Specific Activities of Desoxyribonucleic-acid Phosphorus and Inorganic Phosphorus Two Hours after Labeled Phosphate Administration to Mature Rat (138).

ORGAN	Percentage ratio of specific activities of desoxyribonucleic acid P and inorganic P
Liver	0.14
Spleen	2.50
Kidneys	0.16
Intestinal mucosa	4.80

It can be assumed that much of the turnover of desoxyribonucleic acid takes place in dividing or secreting cells and it is quite possible that such cells are more permeable to phosphate than the average cell of the tissue in question. Such an enhanced permeability would not much influence the renewal figures obtained for liver desoxyribonucleic acid, as within a short time the specific activity of inorganic phosphorus of liver reaches the value in plasma. In the case of organs which show a restricted cell permeability, as for example in brain, testes, or muscle in experiments taking two hours, tissue inorganic phosphorus has, however, an appreciably lower specific activity than plasma inorganic phosphorus. In the case of these organs, it is possible that desoxyribonucleic acid is synthesized from inorganic phosphorus having a higher specific activity than the inorganic phosphorus extracted from tissue; thus the renewal values arrived at are too high. The correct renewal figures, however, cannot be lower than the figures obtained by comparing the specific activity of desoxyribonucleic-acid phosphorus of the organ with that of the inorganic phosphorus of the plasma. The latter figures are given in table XXVII. It is advisable in these, and also in other

TABLE XXVII

Specific Activities of Desoxyribonucleic Acid of Some Organs and that of Inorganic Phosphorus Plasma (138).

ORGAN	Percentage ratio of specific activities of 1 mg desoxyribonucleic acid P and of 1 mg plasma inorganic P
Kidney	0.15
Liver	0.20
Spleen	1.79
Intestinal mucosa	2.1

turnover experiments, to determine both the ratio of the specific activity of the phosphorus of the organic compound in question to the specific activity of tissue inorganic phosphorus and the ratio of the former to the specific activity of plasma inorganic phosphorus. If the formation of the organic compound is preceded by an organic precursor, the ratio of the specific activities of the phosphorus of the final product and the precursor should be determined as well.

Experiments in which the specific activity of desoxyribonucleic acid of the liver was investigated several days after administration of labeled phosphate (143) are discussed on page 337. *Andreasen* and *Ottesen* (141) determined the percentage ratio of the injected amount of ^{32}P in 1 mg desoxyribonucleic acid and 1 mg inorganic plasma phosphate of lymphoid organs in experiments taking 3 hours in infant, young, mature and old rats. Their results indicate a decrease with age of the animal in rate of formation of labeled desoxyribonucleic acid in thymus, lymph nodes, and spleen, the decrease being partly due to a decreasing rate of penetration of the radioactive tracer into the tissues. This rate of penetration for lymph nodes and spleen was found to be appreciably lower than for thymus.

In organs of growing rats, beside renewal of nucleic acid molecules, an appreciable additional formation of nucleic acid takes place, since the nucleic acid content of the organs increases with increasing weight. That the percentage ratio of labeled, and thus newly formed, nucleic acid molecules is much larger in 3.5-day old than in mature rats is seen by comparing the figures of tables XXVII and XXVIII.

TABLE XXVIII

Specific Activities of Nucleic Acid P and Inorganic P 2 Hours after Administration of Labeled Phosphate to 3.5-day old Rats (138).

ORGAN	Percentage ratio of specific activities of desoxyribonucleic acid P and free P
Liver	1.96
Spleen	9.76

The percentage increase in total desoxyribonucleic acid content of the liver of the 3.5-day old rat amounts, in the course of two hours, to 0.9 %. About half of the newly formed (labeled) desoxyribo, nucleic acid molecules present are due to increase in nucleic acid

content of liver, while the other half are due to renewal of old molecules. A similar result was obtained in the investigation of formation of labeled nucleic acid in Jensen sarcoma. While in the course of two hours the increment in nucleic acid content of the sarcoma is found to average 1.5%, labeled nucleic acid molecules formed during this time amount to about twice this value (144, 148).

After administration of labeled phosphate to mice, the ^{32}P content of nucleoproteins (containing both desoxyribose and ribose compounds) of normal livers and of livers in which cancer was produced by feeding azo dyes was compared. Tumorous livers, in which a rapid formation of new cells takes place, were found to exhibit an increased uptake compared with the controls (152). In sarcoma slices incubated four hours at 37° C. in plasma containing labeled phosphate, about 0.1 % of the desoxyribonucleic acid molecules is found to have been renewed (146). In red corpuscles of the hen no renewal of appreciable amounts of desoxyribonucleic acid present takes place (147).

In earlier investigations of the turnover of nucleic, acid-soluble and phosphatide components of tissue were extracted with trichloroacetic acid and with ether-alcohol, and the activity of the residual part was determined. Such residues contain, beside thymonucleic acid, ribonucleic acid and possibly also phosphoproteins. Since the rate of renewal of ribonucleic acid is much larger than that of thymonucleic acid, no conclusion about the value for thymonucleic acid can be drawn from these experiments.

2. *Effect of Roentgen Rays on Turnover of Desoxyribonucleic Acid.* — In view of the importance of desoxyribonucleic acid in cell division, the effect of Roentgen rays on the turnover of desoxyri-

TABLE XXIX

Effect of Irradiation of Jensen Rat Sarcoma on Formation of Desoxyribonucleic Acid (144).

Dosage, r.	Time between irradiation and injection	Time between injection and killing of rat, in hours	Ratio of newly formed nucleic acid in controls and in irradiated sarcoma
750 — 1500	several minutes	0.5	3.2
335 — 1500	»	1	2.4
450 — 1500	»	2	2.2
1500	»	4 — 6	2.8
1230 — 1500	3 to 7 days	2	1.7

bonucleic acid phosphorus was investigated (138, 144,145, 146, 148).

In experiments with rats having two inoculated sarcoma, one sarcoma was irradiated while the other was effectively shielded. A reduction of labeled nucleic acid formation was found to take place in both; the reduction was smaller in the shielded than in the irradiated sarcoma (148).

Roentgen radiation blocks the turnover of desoxyribonucleic acid molecules not only in growing tissue, but also in normal organs of adult animals, as is seen in table XXX.

TABLE XXX

Ratio of Newly Formed Desoxyribonucleic Acid Molecules present before and after Irradiation in the Organs of Adult Rats (138) (*).

ORGAN	Ratio of newly formed nucleic acid in the organs of controls and of irradiated rats
Liver	3.3
Spleen	2.4
Intestinal mucosa	2.3

(*) Dose applied :-1.480-3.000 r. Rat irradiated in toto. ^{32}P injected after irradiation. Rat killed two hours after administration of the ^{32}P.

The effect of irradiation on the formation of nucleic acid molecules in organs of normal adult rats is thus similar in magnitude to the effect of Roentgen rays on the growing Jensen sarcoma, listed in table XXIX.

Roentgen rays were found to block the formation of nucleic acid in organs of rapidly growing rats also. The percentage inhibition of the desoxyribonucleic acid turnover was found to be similar to that in normal organs of adult animals and in Jensen sarcoma.

The ratio of newly formed desoxyribonucleic acid in organs of controls and in irradiated rats was found to be 2.3 in experiments taking two hours, in which the 3- to 4-day old rats were irradiated with 2,000-2,250 r previous to administration of labeled phosphate.

3. *Rate of Renewal of Ribonucleic Acid.*— In contradistinction to desoxyribonucleic acid—the main constituent of cell nuclei—ribonucleic acid which, according to *Brachet* (149) and *Caspersson* (150), is to a large extent found in cytoplasm, is renewed at a fairly rapid rate in the organs. *Hammarsten* and *Hevesy* (139) found, for the percentage ratio of the specific activities of desoxyribonucleic acid phosphorus and inorganic phosphorus, and for that of ribonucleic

TABLE XXXI

Rate of Renewal of Ribonucleic and Desoxyribonucleic Acid in Organs of Rat Two Hours After Administration of Labeled Phosphate (139).

Organ	Nucleic Acid	Percentage ratio of specific activity of nucleic acid P to that of inorganic P of organ	Ratio of specific activity of ribonucleic acid P to that of desoxyribonucleic acid P
Liver.....	Ribose	3.45	33
	Desoxyribose	0.105	
Spleen	Ribose	6.6	3
	Desoxyribose	2.2	
Intestine ...	Ribose	6.1	2
	Desoxyribose	2.8	—

acid phosphorus and inorganic phosphorus, two hours after subcutaneous administration of labeled sodium phosphate, the values recorded in table XXXI.

In a recent investigation (139*a*) the ratio between the specific activity of the ribonucleic acid P and the specific activity of the desoxyribonucleic acid P of the Jensen sarcoma was found to be 2.4 in experiments taking 2 hours.

Brues and his associates (143) determined the specific activity of both the ribonucleic acid and the desoxyribonucleic acid of resting and regenerating liver and also of hepatoma. In these experiments the labeled phosphate was administered several days before the rats were killed.

TABLE XXXII

Specific Activities of Nucleic Acid Phosphorus (143).

Organ	Time after injection days	Nucleic acid, per cent of specific activity of inorganic P		Ratio
		Ribose	Desoxyribose	
Resting liver	3	54.9	10.6	5.2
	8	123	20.8	5.9
Regenerating liver	3	230	180	1.3
	13	314	576	0.5
Hepatoma	3	171	64	2.7

The results obtained, seen in table XXXII, show that the rate of renewal of ribonucleic acid is higher than that of desoxyribonucleic acid and that the turnover in regenerating liver and hepatoma is more

rapid than in resting liver. That the ratio of the specific activities of ribonucleic and desoxyribonucleic acid is found, in these experiments, to be appreciably smaller than in the experiments of *Hammersten* and al., may be due, at least in part, to the much longer duration of the former experiments.

Kohlman and *Rusch* (152) administered labeled phosphate to rats and mice and determined the ^{32}P content of nucleoproteins (containing both desoxyribose and ribose compounds) of normal liver and of liver in which cancer was produced by feeding azo dyes. The tumorous liver, in which a rapid formation of new cells takes place, was found to have a 45% increase in uptake of ^{32}P compared with the normal liver.

4. *Specific Activity of Nucleic acid Phosphorus of the whole Rat.* — *Hammersten* et al. (139) determined the specific activity of both total desoxyribonucleic acid phosphorus and total ribonucleic-acid phosphorus extracted from a rat weighing 194 g. The activity of labeled sodium phosphate administered amounted to 8.1 microcuries per 100 g animal weight. The time of the experiment was two hours. The results of this experiment are shown in table XXXIII. As shown in the table, the specific activity of the average nucleic acid phosphorus of the rat is almost identical with the value for ribo- and desoxyribonucleic acids, respectively, extracted from the intestine.

TABLE XXXIII

Specific Activities of Nucleic Acid Phosphorus of whole Rat, Liver, Spleen and Intestinal Mucosa (139).

Sample	Specific activity (whole rat ribonucleic-acid P = 100)		
	Ribonucleic	Desoxyribonucleic	Inorganic P
Total Rat	100	60	—
Liver	164	4.4	5,100
Spleen	292	63	2,850
Intestine	112	63	2,770

Andreasen and *Ottesen* (141) attempted to estimate lymphocyte production in different lymphoid organs from the rate of turnover of desoxyribonucleic acid in these organs. They concluded that the thymus must be the most important lymphocytopoietic organ except in old age.

d) *Phosphorus Turnover in Cell Nuclei.*

Marshak (153) has isolated nuclei from liver and lymphoma tissue of rats weighing 150 g and has shown that most ^{32}P taken up by the nuclei is present in the residue obtained after extraction with trichloroacetic acid and an ether-alcohol mixture.

^{32}P was found to accumulate in nuclei of rapidly growing tumor to a much greater extent than in fully grown liver. This difference in accumulation of ^{32}P in nucleic of liver and of lymphoma is to be expected in view of the facts that most of the phosphorus of nuclei is present as desoxyribonucleic acid phosphorus, and that turnover of this compound is very low in nuclei of fully grown liver (138 and 140) but is appreciable in nuclei of rapidly growing tumor tissue or even in nuclei of rapidly growing normal tissue.

Marshak and *Walker* (155) injected labeled chromatin into the blood of rats weighing 55 g after partial hepatectomy and found that the liver nuclei retained much more ^{32}P than after injection of labeled inorganic phosphate of the same activity. This is another example of the enhanced uptake of phosphorus compounds foreign to the plasma liver. If the labeled chromatin splits off inorganic labeled phosphate by enzyme action in the liver, we would expect enhanced formation of labeled compounds in the nuclei corresponding to the high ^{32}P level maintained in the liver.

That labeled desoxyribonucleic acid intravenously injected into rats weighing 100-200 g rapidly splits off labeled phosphate in liver was shown by *Ahlström* and co-workers (156).

In experiments in which liver slices were incubated in bicarbonate-Ringer solution containing labeled desoxyribonucleic acid, the phosphate group of more than two-thirds of the nucleic acid added was found to be split off in the course of four hours, four-fifths of the total acid-soluble ^{32}P being present in the phosphate fraction.

Not only nucleic acid, but also other organic phosphorus compounds introduced into the circulation give off inorganic phosphate. Choline phosphate appears for example within a short time as inorganic phosphate in the circulating blood of the rat following intraperitoneal injection of the ester (107). Triphenyl phosphite was found to be decomposed readily after being injected intraperitoneally (157). Phosphatides which are normal constituents of plasma are not decomposed in the circulation at an appreciable rate.

e) *Studies of Virus Reproduction.*

When mosaic-diseased Turkish tobacco plants were fed a nutrient solution containing radioactive phosphorus in the form of disodium phosphate over a period of several weeks, about 30% of the phosphorus taken up by the plants was isolated by *Stanley* (158) in the form of purified tobacco mosaic virus (cf. also *Born* and associates (159). The tobacco mosaic virus growing, in contrast to the plant, at a rapid rate is bound to take up a large percentage of the labeled phosphate which reaches the plant. While an organ or a substance grown in a labeled medium becomes labeled throughout, parts of a nature organism became labeled only by interchange, the rate of which varies greatly with the organ and the compound in question.

The virus containing radioactive phosphorus, to a large extent present as a constituent of the ribonucleic acid molecule, was rubbed into the lower leaves of Turkish tobacco plants. After twelve days the lower inoculated and the upper uninoculated leaves were investigated, with the result shown in table XXXIV.

TABLE XXXIV

Distribution of ^{32}P in Turkish Tobacco Plants Twelve Days After Inoculation of Lower Leaves with 58 Mg Labeled Tobacco Mosaic Virus (158).

FRACTION	Relative activity
Virus isolated from inoculated leaves	8.3
All material of inoculated leaves except virus	33.4
Virus isolated from uninoculated leaves.	5.8
All material of uninoculated leaves except virus . . .	52.5

Since, in *Stanley's* experiments (as seen in table XXXIV) most of the radioactivity was found associated with nonvirus components in both inoculated and uninoculated portions of the plants, it was impossible to determine whether or not the small amount of radioactive virus found in the uninoculated portions resulted from movement of the inoculated virus. In view of these results it is exceedingly difficult to distinguish between ^{32}P taken up by the plant in the form of virus and that taken up in the form of virus disintegration products.

7. *Renewal Probability of Different Molecules of a Compound.* — The problem whether all phosphatide molecules present in the liver

cells, or all creatinephosphoric acid molecules present in the cells of the gastrocnemius, for example, have the same chance of being renewed under a given interval, is one of great importance. If we wish for example to answer the question if phosphatide formation is an obligatory step in fat oxidation, we compare the amount of fat oxidised in the liver with the amount of phosphatides turned over during the same interval. The last mentioned magnitude is calculated by comparing the specific activities of the liver phosphatide P and intracellular inorganic P. In carrying out this calculation we assume that all phosphatide molecules have the same chance of renewal. Now let us assume that 1% of the phosphatide molecules present is turned over at a preferential rate and this turnover is a step in fat oxidation. If the privileged phosphatide molecules are renewed for example at a rate at which adenosinetriphosphate molecules are rejuvenated (cf. p. 294), they will experience an about 100-fold renewal in the course of two hours, thus the renewal of 1% of phosphatide molecules present will be responsible for 100 mg phosphatide P being turned over in 100 mg liver tissue in the course of 2 hours, while the experiments with rats described on p. 298 indicate a corresponding amount of about 20 mg only. The presence or absence of a small amount of such privileged phosphatide molecules will not be indicated by the results of the turnover experiments taking few hours. If we find in the absence of privileged molecules a 20% renewal in the course of 2 hours, in the presence of 1% privileged molecules we should find 20.8% thus almost the same value. Only experiments of very short duration may reveal the existence of such privileged molecules.

Phosphatide molecules present in the cytoplasma of the liver were found to be turned over at a more rapid rate than those located in the nuclei (137, 137*a*). Furthermore, metaphosphate (156*a*) isolated from the trichloroacetic acid extract of yeast was found to show a very slow renewal rate, while another fraction of metaphosphate, possibly associated with a proteinaceous material of yeast, was found to be rejuvenated at a very appreciable rate. Whether the two metaphosphate fractions have the same composition or not (many varieties of metaphosphate exist) is not yet known.

8. *Use of Radioactive Isotopes in Immunological Investigations.* — Radiophosphorus was applied for the analysis of specific precipitates formed by the union of antigen and antibody and for the

determination of the antigen-antibody ratio in these precipitates. The antisera applied in the experiments of *Bursnell* and associates (160*a*), obtained by immunisation of rabbits, are not specific for phosphorylated proteins. They have however been applied to determine whether or not radioactivity determinations can readily be carried out on specific precipitates containing radiophosphorus. Radiophosphorus could easily be detected in the serological precipitates. For example, 0.4 ml of the antiserum was added to 1.0 ml of antigen solution, and the mixture was kept at 37° for 3 hours. The precipitate was then centrifuged, washed with 0.9% sodium chloride, dissolved in a small volume of 0.1 N sodium hydroxide, and the solution dropped evenly on to a layer of kaolin on a brass ring holder, and dried. Radiophosphorus determinations were then made on this precipitate. In other immunological investigations ovovitellin containing radiophosphorus was used, obtained from the eggs of hens which had received injections of radiophosphorus (as inorganic phosphate). In these experiments radioactivity determinations were carried out on the serological precipitates to determine the amount of antigen in these precipitates. The results of a typical experiment of this type are given in table XXXIV*a*.

TABLE XXXIV*a*

Radio-Phosphorus Content of Serological Precipitates with Vitellin (160*a*).

Antigen : Radiovitellin (in 10 % sodium chloride).
Antiserum : Antivitellin rabbit serum.
Precipitin tests : A mixture of 2.0 ml of antigen, 0.20 ml of antiserum and 0.20 ml of 20% sodium chloride, kept at 37° for 3 hours.

Conc. of antigen (% vitellin)	Radiophosphorus in the precipitate	
	g	As % of the radiophosphorus in the antigen used
2.3	0.0	0
0.37	31.2	19.9
0.13	17.8	34.1
0.042	6.3	36.2
0.014	2.1	36.2
0.0046	0.7	37.6

9. *Dynamic State of Body Constituents.* — Perhaps the most remarkable result obtained in the study of the application of labeled phosphorus and of other radioactive and stable [see *Schoenheimer* (160)] indicators is the discovery of the dynamic state of the body constituents. The molecules constituting the plant or animal organism are incessantly renewed. In the course of this renewal, not only the atoms and molecules taken up with the food participate, but atoms and molecules located in one organ or in one type of molecule will soon be found in another organ or in another type of molecule present in the same or in another organ.

A phosphate radical taken up with the food may first participate in the phosphorylation of glucose in the intestinal mucosa, soon afterwards pass into the circulation as inorganic phosphate, enter a red corpuscle, become incorporated with an adenosinetriphosphoric acid molecule, participate in a glycolytic process going on in the corpuscle, return to the circulation, penetrate the liver cells, participate in the formation of a phosphatide molecule, and after a short interval enter the circulation in this form, penetrate the marrow, and leave this organ after some time as a constituent of a lymphocyte. We may meet the phosphate radical again as a constituent of the plasma, from which it may find its way into the skeleton. Being incorporated in the uppermost molecular layer of the skeleton, it will have a good chance of being replaced by other phosphate radicals of the plasma or the lymph, but it may also have the good fortune to find a more or less lasting abode in the skeleton. This will be the case when it becomes embedded in a newly formed apatite-like bone crystallite.

D. Plant Physiology.

In early experiments (167) it was shown that phosphorus atoms present in the plant interchange with labeled phosphorus atoms taken up from the nutritive solution. 66 per cent of the P present in the leaves of the sunflower, for example, is replaced by labeled P in the course of 9 days, as seen in table XXXV, while the corresponding figure for the stem is 86.

In these experiments, just as in that on the maize plant, a very considerable migration of the phosphorus atoms takes place, and the probability of a labeled phosphorus atom of being found in a performed leaf is not very different from the probability of its being

TABLE XXXV

Replacement of P Atoms in the Sunflower by Atoms of the Nutritive Solution (167).

Time in days	Part of the plant	Total P (mg)	Labeled P (per cent)	Unlabeled P (mg)
0	Lower leaf Upper leaf Stem	0.980	0	0.980
4	Lower leaf	0.848	43.6	0.478
	Upper leaf	0.840	51.5	0.407
	Stem	0.361	63.7	0.131
9	Lower leaf	0.742	66.9	0.246
	Upper leaf	2.838	65.6	0.976
	Stem	0.844	85.6	0.122

found in a leaf grown in a labeled solution. From the above results it follows that the greater part of the phosphorus atoms are not fixed in position in the leaves, but can migrate. Since more than one half of the phosphorus atoms present in the plant is in an organic state we can also conclude that a renewal of a large part of the molecules of the organic phosphorus compounds present in the leaves took place in the course of the experiment.

Some experiments were also conducted with seeds (168). Two different series of experiments were conducted, one with maize seeds, the other with those of the pea. In both cases, the seeds were germinated until rootlets 2-3 cm long were formed. Then the seeds were placed in small flasks with the rootlets dipping into a nutritive solution containing labeled phosphorus. In the case of the maize seeds (table XXXVI), the germ and endosperm were removed after the lapse of 4-14 days and analysed separately. While the germ was found to contain an appreciable amount of labeled phosphorus taken up from the nutritive solution, the endosperm did not contain the slightest trace; this shows that no exchange takes place between the phosphorus atoms of the germ and the endosperm. In the case of the pea, there is no such marked distinction between germ and endosperm, the cotyledons occupying most of the space inside the seed, so that the labeled phosphorus atoms are taken up by the different parts of the seed. The concentration of the labeled phosphorus in the leaves and rootlets was here considerably higher than in the rest of the seed (cotyledons), as is seen from table XXXVI.

TABLE XXXVI

Replacement of P of Maize Seeds by Labeled P Atoms of a Nutritive Solution (168).

Time of experiment (days)	Part of the plant	Total P (mg)	Labeled P (mg)
3.9	Rootlet + leaf + scutellum . .	0.511	0.0134
	Endosperm	0.098	0.0001
7.0	Rootlet + leaf + scutellum . .	0.778	0.0275
	Endosperm	0.068	0
13.8	Rootlet + leaf + scutellum . .	1.008	0.0576
	Endosperm	0.016	0

TABLE XXXVII

Replacement of P of Pea Seeds by Labeled P Atoms of a Nutritive Solution (168).

Time of experiment (days)	Part of the plant	Total P (mg)	Labeled P (mg)
3.9	Rootlet + leaf.	0.167	0.0118
	Cotyledon.	0.281	0.0013
7.0	Rootlet + leaf.	0.445	0.0411
	Cotyledon.	0.314	0.0033
13.8	Rootlet + leaf.	0.481	0.0508
	Cotyledon.	0.151	0.0050

In other experiments 5 minutes after labeled phosphate was added to a culture solution containing 25 cm high corn seedlings, activity could be detected in the leaves (169). The rate of propagation of radiophosphorus was determined by placing a bean seedling grown on in a medium, free of phosphate, into a culture solution containing radio-phosphate. ^{32}P was found to move with a velocity of about 10 cm per hour from the roots into the leaves (170).

Wheat seedlings were found to take up more ^{32}P when exposed to light than in the dark. In nitrogen only slightly less than in air, atmosphere of carbon dioxide decreased the uptake of labeled phosphate. The final distribution, in general, was quite uniform between the leaves as well as along each leaf except the tip, the activity of which remained low (171*a*).

A technique of injection was employed to isolate ^{32}P in a bean leaf, and a method of « counting » was developed, whereby direction and amount of movement from the leaf could be detected. The results show; 1) The p. c. of ^{32}P migration and distribution throughout the plant varied throughout the day; 2) the initial direction of migration from the leaf is predominantly downward, 3) during the evening hours 4% of the migrating phosphorus reaches the root system; 4) upward migration showed a maximum towards noon, but the amount was small. These results suggest the existence of a mechanism whereby a daily periodic « circulation » of phosphorus may take place within the plant (171).

In experiments with tomato plants after 40 minutes newly absorbed P was detected in the leaves and tips of plants over 6 feet tall. Fully ripe tomato fruit attached to the vine continued to absorb small but measurable amounts of P. The absorption was limited to the pulp, whereas in the green fruit it was marked in both the seed and the pulp. Under conditions of a restored P supply, the fruit, ripe as well as green, was drawing on the P present in the leaves. With a liberal external supply both leaves and fruit showed a gain in P (172).

The granular protoplasma of *Nitella* was found to take up to 11 times as much labeled phosphate from a 0.01 ml Na_2HPO_4 solution as does the hyaline protoplasma (173).

By use of radioactive phosphorus and by separation of the bark from the wood in some sections of the stem, it was possible to prove through the use of willow and geranium plants that the main if not the entire amount of salts moves upward in the wood and it is only owing to the contact of the bark with the wood that salts are transmitted laterally to the bark. This evidence supports strongly the prevalent hypothesis of plant physiologists that the xylem and not the phloem is the path of rapid upward movement of salts (174).

To determine the phosphate fixation by soils, measurements were made of the uptake by test-plants (tomatoes, sudangrass and radishes) of ^{32}P added to soils. These values were then compared with the uptake by plants grown in water and sand cultures, to give a measure of the absolute fixation of the soils (175).

^{32}P was applied in the study of the depth of penetration of phosphate in different soils (175*a*).

To investigate if the P content of several pollen contribute to the formation of the seed the following experiment was carried out (177),

Spiring aspen branches were placed in a solution containing radioactive phosphate. By this procedure labeled pollen was obtained. Female aspen branches were fertilized with the labeled pollen and the radioactivity of the seeds obtained was determined. ^{32}P of 8 seeds were found to participate in the formation of 1 seed.

REFERENCES

A

1. Wilson, J, N., *J. Am. Chem. Soc.*, **60**, 2697 (1938).
1*a* Hull, D. E., *J. Am. Chem. Soc.*, **63**, 1269 (1941).
2. Hevesy, G. and A. H. W. Aten, *Danske Vidensk, Selsk. Biol. Medd.*, **14**, No. 5 (1939).
3. Perrier, C. and E. Segré, *Ricerca sci.*, **9**, 638 (1938).
4. Aten, A. H. W., *Diss., Utrecht* (1939).
5. Hahn, L. and G. Hevesy, *Nature*, **145**, 549 (1940).
6. Ferla, F., *Ann. Chim. applicata*, **28**, 331 (1938).
7. Ahlström, L., personal communication.
7*a* Lange, A., *J. physiol. Chem.*, **45**, 639 (1941).

B

8. Meyerhof, O., Ohlmeyer, P., Gentner, W. and Maier-Leibnitz, H., *Biochem. Z.*, **298**, 396 (1938).
9. Hevesy, G., Baranowsky, T., Gutke, A. G., Ostern. P. and Parnas, J. K., *Ac. Biol. Exp.* (Warsaw), **12**, 34 (1938).
10. Parnas, J. K., *Enzymologia*, **5**, 170 (1938). — *Soc. Chim. Biol.*, **21**, 1059 (1939)
10*a* Schlamowitz, M. and Greenberg, D. M., *J. Biol. Chem.*, **171**, 293 (1947),
11. Chargaff, E., *J. Biol. Chem.*, **144**, 455 (1942).
12. Chargaff, E., Ziff, M. and Cohn, S. S., *J. Biol. Chem.*, **135**, 351 (1940).
13. Lippmann, F., and Tuttle, L. C., *J. Biol. Chem.*, **158**, 505 (1945).
14. Lindahl, P. E., Malm. M., Strindberg, B. and Lagergren, B. M., *Nature*, **158**, 746 (1946).
15. Chiewitz, O. and Hevesy, G., *Nature*, **136**, 754 (1935).
16. Hahn, L. and Hevesy, G., *Acta Physiol. Scand.*, **1**, 347 (1941).
17. Greenberg, D. M., Aird, R. B., Boelter, M. D., Wesley, W., Campbell, W., Cohn, W. E., Murayama, M. M., *Amer. J. Physiol.*, **140**, 147 (1943/44).
18. Hevesy, G. and Rebbe, O., *Nature*, **141**, 1097 (1938).
19. Hevesy, G., *J. Chem. Soc.*, 1213 (1939).
20. Lundsgaard, E., *Scand. Arch. Physiol.*, **80**, 291 (1938).
21. Sachs, J., *Amer. J. Biol.*, **129**, 227 (1940).
22. Hevesy, G. and Rebbe, O., *Acta Physiol. Scand.*, **2**, 171 (1940).
23. Krogh, A., *Proc. Roy. Soc., B.*, **133**, 195 (1945).

24. Kalckar, H. M., Dehlinger, J. and Mehler, A., *J. Biol. Chem.*, **154**, 275 (1944).
25. Kalckar, H. M., *Am. Rev. Biochem.*, XIV, 283 (1945).
26. Flock, E. V. and Bollman, J. L., *J. Biol. Chem.*, **152**, 371 (1944).
27. Furchgott, R. F. and Shorr, E., *J. Biol. Chem.*, **151**, 65 (1943).
28. Hahn. P. F., Donald, W. D., and Grier, R. C., jur., *Am. J. Physiol.*, **143**, 105 (1945).
29. Wilde, W. S., Cowie, D. B. and Flexner, L. B., *Amer. J. Physiol.*, **147**, 360 (1946).
30. Greenberg, D. M., Bird, R.-B., Boelter, M. D. D., Campbell, Wesley, N, Cohn, W.-E. and Murayama, M.-M., *J. Biol. Chem.*, **140**, XIVIII (1941.) — *Amer. J. Physiol.*, **140**, 147 (1943/44).
31. Hahn, L. and Hevesy, G., *Acta physiol. Scand.*, **3**, 193 (1942).
32. Bayard, P., *Bull. Soc. Roy. Sci.*, Liège, **11**, 621 (1942).
33. Levi, H., *Sv. Vet. Akad. Ark. f. Kemi, Mineral., Geol.*, **21A**, No. 5 (1945).
34. Hevesy, G. and Aten, A. H. W., *Kgl. Danske Vidensk. Selsk. Biol. Medd.* XIV, 5 (1939).
35. Hahn, L. and Hevesy, G., *Acta Physiol. Scand.*, 3, 193 (1942).
36. Hevesy, G. and Rebbe, O., not previously published.
37. Hevesy, G. and Zerahn, K., *Acta Physiol. Scand.*, **4**, 376 (1942).
38. Hevesy, G., Köster, K. H., Warburg, E. and Zerahn, K., *Acta Med. Scand.*, V-VI, 561 (1944).
39. Nylin, G. and Malm, M., *Cardiologica*, VII, 153 (1943).
40. Nylin, G., *Sv. Vet. Akad. Ark. f. Kemi, Mineral., Geol.*, **20A**, No. 17 (1945).
40*a* Nylin, G., *Am. Heart J.*, **34**, 174 (1947).
40*b* Nylin, G., *Am. J. Physiol.*, **149**, 180 (1947).
41. Lawrence, J. H., Erf, L. A. and Tuttle, L. W., *J. Appl. Physiol.*, **12**, 333 (1941).
42. Mullins, L. J., Cell, J., *Comp. Physiol.*, **14**, 403 (1939). — *Biol. Bull. Woods Hole*, **83**, 82 (1942).
43. Hevesy, G. Linderström-Lang, K. and Olsen, C., *Nature*, **137**, 66 (1936).
44. Hevesy, G. and Zerahn, K., *Acta Radiol.* **27**, 316 (1946).
45. Malm, M., *Ueber die Permeabilität der Hefezellen*, Upsala 1947.
46. Cf. Hevesy, G., *Ann. Rev. Biochem.*, **9**, 641 (1940).
Hamilton, J. G., *J. Appl. Physics*, **12**, 440 (1941).
47. Hevesy, G., *Enzymologia*, **5**, 167 (1938/39).
48. Erf, F. A., Tuttle, L. W. and Lawrence, J. H., *Ann. Intern. Med.*, **15**, 367, No. 3 (1941).
49. Govaerts, J. and Lambrechts, A., *Bull. Soc. Roy. Sci.*, Liège, **11**, 138 (1942).
50. Hevesy, G., Hahn, L. and Rebbe, O., *Kgl. Danske Vidensk. Selsk. Biol. Medd.* XIV, 3 (1939).
51. Kjerulf-Jensen, L. K., *Acta Physiol. Scand.*, **3**, 193 (1942).
52. Govaerts, J. and Lambrechts, A., *Bull. Soc. Roy. Sci.*, Liège, **11**, No. 3, 361 (1942).
53. Hevesy, G., *J. Chem. Soc.*, 1213 (1939).
54. Gaunt, W. E., Griffith, D. H. and Irving, J. I., *J. Physiol.*, **110**, 372 (1941-1942).
54*a* Tweedy, W. R., Chilcote, M. E. and Patras, M. C., *J. Biol. Chem.*, **168**, 597 (1947).

55. Bulliard, H., Grundland, J. and Moussa, A., *C. R.*, **207**, 745 (1938).
56. Jones, H. B., Chaikoff, I. L. and Lawrence, J. H., *Amer. J. Cancer*, **40**, 243 (1939).
57. Kenney, J. M., Marinelli, L. D. and Graever, L. F., *Amer. J. Roentg. Rad. Therapy*, **41**, 20 (1941).
58. Forssberg, A. and Jacobsson, F., *Acta Radiol.*, **26**, 232 (1945).
59. Forssberg, A., *Acta Radiol.*, **27**, 88 (1946).
60. Jones, H. B., Wrobel, Ch. J. and Lyons W. R., *J, Clin. Inv.*, **23**, 783 (1944).
61. Bonhoeffer, K. Z., *Elektrochem.*, **40**, 4069 (1943).
62. Hevesy, G. and Hahn, L., *Kgl. Danske Vidensk. Selsk. Biol. Medd.*, XIV, 5 (1940).
63. Kalckar, H. M., *J. Biol. Chem.*, **154**, 267 (1944).—Kalckar, H. M., Dehlinger, J. and Melher, A., *ibid.* **154**, 275 (1944).
64. Hevesy, G., *Enzymologia*, **3**, 138 (1938/1939).—Hevesy, G., Hahn, L. and Rebbe, O., *Kgl. Danske Vidensk. Selsk. Biol. Medd.*, XVI, 8 (1941).
65. Flock, E. V., and Bollman, J. L., *J. Biol. Chem.*, **150**, 151 (1944).
66. Flock, E. V. and Bollman, J. L., *ibid.*, **152**, 371 (1944).
67. Korzybski, T. and Parnas, J. K., *Bull. soc. chim. biol.*, **21**, 713 (1939).
68. Furchgott, R. F. and Shorr, E., *J. Biol. Chem.*, **151**, 65 (1943).
69. Sacks, J., *Am. J. Physiol.*, **142**, 145 (1944).
70. Sacks, J., *ibid.*, **143**, 157 (1945).
71. Sacks, J., *ibid.*, **129**, 227 (1940).
72. Sacks, J. and Altschuber E. H., *ibid.*, **137**, 750 (1942).
73. Sacks J., *ibid.*, **142**, 621 (1944).
74. Sacks, J., *Science*, **98**, 388 (1943).
75. Kjerulf-Jensen, K. and Lundsgaard, E., *Acta Physiol. Scand.*, **3**, 209 (1943).
76. Lindberg, O., *Sv. Vet. Akad. Ark. f. Kemi, Mineral., Geol., B.*, **21**, nº 3 (1945).
77. Kaplan, N. O., Memelsdorf, J. and Dodge, E., *J. Biol. Chem.*, **160**, 631 (1945).
78. Kaplan, N. O. and Greenberg, D.-M., *ibid.*, **150**, 479 (1943).—**156**, 511, 525 (1944).
79. Nelson, N., Rapoport, S., Guest, G. M. and Mirsky, I. A., *J. Biol. Chem.*, **144**, 291 (1942).
80. Lundsgaard, E., *Skand. Arch. Physiol.*, **80**, 291 (1938).
81. Rapoport, S., Nelson, N., Guest, G. M. and Mirsky, I. A., *Science*, **93**, 88 (1941).
82. Meyerhof, O., Ohlmeyer, P., Gentner, W. and Maier-Leibnitz, H., *Biochem. Z.*, **298**, 396 (1938).
83. Borell, U. and Oerström, A., *Acta Physiol. Scand.*, **10**, 231 (1945). — *Ibid.*, in press.
83*a* Borell U., Westman, A., Oerström, A., *Gynæcologia*, **123**, 186 (1947).
84. Artom, C., Sarzan, G., Perrier, C., Santangelo, M. and Segré, E., *Arch. Intern. Physiol.*, **45**, 32 (1937). — **47**, 245 (1938).
84*a* Artom, C. and Cornatzer, W. E., *J. Biol. Chem.*, **111**.
85. Hahn, L. and Hevesy, G., *Skand. Arch. Physiol.*, **77**, 148 (1937).
86. Perlman, J., Ruben, S. and Chaikoff, I. L., *J. Biol. Chem.*, **122**, 169 (1937).
87. Hodge, H. C., Mac Lachlan, P.-L., Bloor, W. R., Welch, E. A. and Levy, S. R., *Federation Proc.*, **2**, 63 (1943).

88. Ahlström, L., v. Euler, H. and Hevesy, G., *Sv. Vet. Akad. Ark. f. Kemi*, **19A**, Nr. 9 (1944).
89. Zilversmit, D. B., Entenman, C. and Fishler, M. C., *J. Gen. Physiol.*, **25**, 327 (1943).
90. Zilversmit, D. B., Entenman, C., Fishler, M. C. and Chaikoff, I. L., *ibid.*, **26**, 333 (1943).
91. Entenman, C., Rubens, S., Perlman, I., Lorenz, F. W. and Chaikoff, I. L., *J. Biol. Chem.*, **124**, 795 (1938).
92. Hahn, L. and Hevesy, G., *Biochem. J.*, **32**, 342 (1938).
92*a* Zilversmit, D. B., Chaikoff, I. L. and Entenman, C., *J. Biol. Chem.*, **172**, 637 (1948).
93. Schmidt-Nielsen, K., *Acta Physiol. Scand.* Suppl. XXXVII (1946).
94. Dols, M. J. L., Jansen, B. C. P., Sizoo, G. J. and Barendregt, F., *Proc. Acad. Sci.* Amsterdam, **41**, 997 (1938). — **42**, 2 (1939).
95. Chargaff, E., Olson, K. B. and Partington, P.-B., *J. Biol. Chem.*, **134**, 505 (1940).
95*a* Platt, A. P. and Porter R. R., *Nature*, **160**, 905 (1947).
96. Hahn, L. and Tyrén, H., *Sv. Vet. Akad. Ark. f. Kemi, Mineral., Geol.*, **A21**, N° 11 (1945).
96*a* Bollman, J. L. and Flock, E. V., *J. Lab. Clin. Med.*, **31**, 478 (1946).
97. Haven, F. L., *J. Natl. Cancer. Inst.*, **1**, 205 (1940).
98. Hunter, F. E., *Proc. Soc. Exptl. Biol. Med.*, **46**, 287 (1941).
99. Hunter, F. E. and Levy, S. R., *J. Biol. Chem.*, **145**, 577 (1942).
100. Haven, F. L. and Levy, S. R., *ibid.*, **136**, 301 (1940). — **141**, 417 (1941).
101. Chargaff, E. and Keston, A. S., *J. Biol. Chem.*, **134**, 575 (1940).
102. Perlman, I. and Chaikoff, I. L., *J. Biol. Chem.*, **128**, 735 (1939).
103. Patterson, J. M., Keevil, N. B., and Mac Henry, E. W., *J. Biol. Chem.*, **153**, 489 (1944).
104. Perlman, I., Stillman, N. and Chaikoff, I. L., *ibid.*, **133**, 641 (1940). — **135**, 359 (1940).
105. Entenman, C., Chaikoff, I. L. and Friedlander, H. O., *ibid.*, **162**, 111 (1946).
105*a* Entenman, C., Chaikoff, I. L. and Zilversmit, D. B., *ibid.*, **166**, 15 (1946).
106. Friedlander, H. D., Chaikoff, I. L. and Entenman, C., *J. Biol. Chem.*, **158**, 231 (1945).
107. Riley, R. F., *ibid.*, **153**, 535 (1944). — *J. Am. Chem. Soc.*, **66**, 512 (1944).
108. Chaikoff, I. L., *Physiol. Rev.*, **22**, 291 (1942).
108*a* Hevesy, G., *not formerly communicated.*
109. Weissberger, L. H., *J. Biol. Chem.*, **132**, 219 (1940).
110. Weissberger, L. H., *ibid.*, **139**, 543, (1941).
111. Entenman, C., Ruben, S., Perlman, I., Lorenz, F. W. and Chaikoff, I. L., *ibid.*, **124**, 795 (1938).
112. Artom, C., *J. Biol. Chem.*, **139**, 953 (1941).
113. Friedlander, H. D., Perlman, I. and Chaikoff, I. L., *Am. J. Physiol.*, **132**, 24 (1941).
114. Hevesy, G. and Smedley-Maclean, I., *Biochem. J.*, **34**, 903 (1940).
115. Changus, G. W., Chaikoff, I. L. and Ruben, S., *J. Biol. Chem.*, **126**, 493 (1938).
116. Fries, B. A., Changus, G. W. and Chaikoff, I. L., *J. Biol. Chem.*, **132**, 23 (1940).

117. Hevesy, G., *Acta contra Cancrum*, **4**, 175 (1939).
118. Jones, H. B. and Chaikoff, I. L., *J. Biol. Chem.*, **128**, 631 (1939). — **133**, 319 (1940).
119. Jones H. B. Chaikoff, I. L. and Lawrence, J. H. *Am. J. Cancer*, **40**, 235 (1940).
120. Jones, H. B., Chaikoff, I. L. and Lawrence, J. H., *J. Biol. Chem.*, **133**, 319 (1940).
120*a* Horning, M -J. and Eckstein, H. C., *J. Biol. Chem.*, **166**, 711 (1946).
121. Hevesy, G. and Hahn, L., *Kgl. Danske Vidensk. Selsk. Biol. Medd.* XV, 6 (1940).
122. Hahn, L. and Hevesy, G., *Compt. rend. trav. lab. Carlsberg, Ser. Chim.*, **22**, 190 (1937).
122*a* Artom, C. and Cornatzer, W. E., *J. Biol. Chem.*, **171**, 779 (1947).
123. Fries, B. A., Schachner, H. and Chaikoff, I. L., *J. Biol. Chem.*, **144**, 59 (1942).
124. Fishler, M. C., Entenman, C., Montgomery, M. L. and Chaikoff, I. L., *J. Biol. Chem.*, **150**, 47 (1943).
124*a* Zilversmit, D. B.
125. Taurog, A., Entenman, C., Fries, B, A. and Chaikoff,I. L., *J. Biol. Chem.*, **155**, 1944 (1942).
125*a* Fishler, M. C., Taurog, A., Perlman, I. and Chaikoff, I. L., *J. Biol. Chem.*, **141**, 809 (1941).
126. Schachner, H., Fries, B. A. and Chaikoff, I. L., *J. Biol. Chem.*, **146**, 95 (1942).
126*a* Taurog, A., Chaikoff, I. L. and Perlman, I., *ibid.*, **145**, 281 (1942).
127. Entenman, C., Chaikoff, I. L. and Zilversmit, D. B., *J. Biol. Chem.*, **166**, 15 (1946).
128. Reinhardt, W. O., Fishler, M. C. and Chaikoff, I. L., *J. Biol. Chem.*, **152**, 79, (1944).
129. Bloor, W. R., *J. Biol. Chem.*, **23**, 317 (1915).
130. Hevesy, G. and Lundsgaard, E., *Nature*, **140**, 275 (1937).
131. Hevesy, C. and Hahn, L., *Kgl. Danske Vidensk. Selsk. Biol. Medd.* XIV, n° 2 (1938).
132. Lorenz, F. W., Perlman, I. and Chaikoff, I. L., *Am. J. Physiol.*, **138**, 318 (1942/1943).
133. Entenman, C., Ruben, S., Perlman, I., Lorenz, F. W. and Chaikoff, I.-L., *J. Biol. Chem.*, **124**, 795 (1938).
134. Chargaff, E., *ibid.*, **142**, 505 (1942).
135. Aten, A H. W., Jr. and Hevesy, G., *Nature*, 142, **111** (1938).
136. Aten, A. H. W., Jr., *Isotopes and Formation of Milk and Egg*, Dissertation, Utrecht (1939).
137. Hevesy, G., *Nature*, **157**, 368 (1946).
137*a* Hevesy, G., *Sv. Vet. Akad. Arkiv f. Kemi, Mineral., Geol.*, **24A**, n° 26 (1947).
138. Ahlström, L., v. Euler, H. and Hevesy, G., *Sv. Vet. Akad. Ark. f. Kemi*, **19A**, n° 9 (1944).
139. Hammarsten, E. and Hevesy, G., *Acta Physiol. Scand.*, **11**, 289 (1946).
139*a* v. Euler, H., Hevesy, G. and Solodowska, W., *Sv .Vet. Akad. Ark. f. Kemi*, 26A, 4 (1948).

140. Hevesy, G. and Ottesen, J., *Acta physiol. Scand.*, **5**, 237 (1943).—Cf. also Hahn, L. and Hevesy, G., *Nature*, **145**, 549 (1940).
141. Andreasen, E. and Ottesen, J., *Acta Physiol. Scand.*, **5**, 258 (1946).
142. Hevesy, G. and Hahn, L., *Kgl. Danske Vidensk. Selsk. Biol. Medd.*, XV, 5 (1940).
143. Brues, A. M., Tracy, L. M. and Cohn,W. E., *J. Biol. Chem.*, **155**, 619 (1944), cf. Brues, A. M., Tracy, L. M. and Cohn, W. E., *Science*, **95**, 558 (1942).
144. v. Euler, H. and Hevesy, G., *Kgl. Danske Vidensk. Selsk. Biol. Medd.*, XVII, 8 (1942).— *Sv. Vet. Akad. Ark. f. Kemi. Mineral., Geol.*, **17a**, nº 30 (1944).
145. Ahlström, L., v. Euler, H., Hevesy, G. and Zerahn, K., *Sv. Vet. Akad. Ark. f. Kemi.*, **23A**, nº 1 (1946).
146. Ahlström, L., v. Euler, H. and Hevesy, G., *Sv. Vet. Akad. Ark. f. Kemi*, **21A**, nº 6 (1945).
147. Hevesy, G. and Ottesen, J., *Nature*, **156**, 534 (1945).
148. Ahlström, L., v. Euler, H. and Hevesy, G., *Sv. Vet. Akad. Ark. f. Kemi*, **19A**, nº 13 (1945).
149. Brachet, J., *Compt. rend. soc. biol.*, **135**, 88 (1940).
150. Caspersson, T., *Skand. Arch. Physiol.*, Suppl. No. 8, 73 (1936).— *Chromosoma*, 1, 562 (1940).—Capersson, T. and Schultz, J., *Nature*, **142**, 294 (1938).—143, 602 (1939).
151. Cohen, W. E. and Brues, A. M., *J. Gen. Physiol.*, **28**, 459 (1945).
152. Kohman, T. P. and Rusch, H. P., *J. Exp. Biol. Med.*, **46**, 403 (1941).
152*a* Branson, H., Banks, H. W. and Dodson, L. B., *Science*, **106**, 637 (1947).
153. Marshak, A., *Science*, **92**, 460 (1940).
154. Marshak, A., *J. Gen. Physiol.*, **35**, 275 (1941).
155. Marshak, A. and Walker, A. C., *Am. J. Physiol.*, **143**, 251 (1945).
156. Ahlström, L., v. Euler, H., Hevesy, G. and Zerahn, K., *Sv. Vet. Akad. Ark. f. Kemi*, **23A**, nº 7 (1946).
156*a* Juni, E., Kamen, M.-D., Spiegelman, S. and Wiame, J.-M., *Nature*, **160**, 717 (1947).
157. Aird, R. B., Cohn, W. E. and Weiss, S., *Proc. Soc. Exp. Biol. Med.*, **45**, 306 (1940).
158. Stanley, *J. Gen. Physiol.*, **25**, 881 (1942).
158*a* Naeslund, J. and Nylin, G., *Acta Med. Scand.*, suppl. CLXX, 390 (1946).
159. Born, H. J., Lang, A., Schramm, G. and Zimmer, K. G., *Naturwissenschaften*, **29**, 222 (1941).
159*a* Wilde, W. S., Cowie, D. B., Flexner, L. B., *Amer. J. Physiol.*, **147**, 360 (1946).
160. Schoenheimer, R., *The Dynamic State of Body Constituents*, Harvard Univ. Press, Cambrige 1942.
160*a* Boursnell, J. C., Dewey, H. M., Francis, G. E. and Wormall, A., *Nature*, **160**, 339 (1947).
161. Flock, E. V. and Bollman, J. L., *J. Biol. Chem.*, **156**, 151 (1943).
162. Taurog, A., Lorenz, F. W., Entenman, C. and Chaikoff, I. L., *Endocrinology*, **35**, 483 (1944).
162*a* Nielson, P. E., *Amer. J. Physiol.*, **135**, 670 (1941/42).
163. Hevesy, G., Levi, H. and Rebbe, O., *Biochem., J.*, **32**, 2147 (1938).
163*a* Pojak, G., *Nature*, **160**, 841 (1947).

164. Haven, F. L. and Bale, W. F., *J. Biol. Chem.*, **129**, 23 (1939).
165. Hevesy, G., Levi, H. and Rebbe, O., *Biochem. J.*, **34**, 532, (1940).
166. Armstrong, W., *Am. Rev. Biochem.*, **11**, 441 (1942); *Fed. Proc.*, **6**, 253 (1947); cf also Manly, R. S., Hodge, H.C. and Manly M., *J. Biol. Chem.*, **134**, 293 (1940).
167. Hevesy, G., Linderström-Lang, K. and Olsen, C., *Nature*, **137**, 66 (1936).
168. Hevesy, G., Linderström-Lang, K. and Olsen, C., *ibid.*, **139**, 149 (1937).
169. Brewer, A. K. and Bramley, A., *Science*, **91**, 269 (1940).
170. Biddulph, O., *Science*, **89**, 393 (1939).
171. Biddulph, O., *Amer. J. Bot.*, **28**, 348 (1941).
171*a* Brewer, A. K. and Bramley, A., *Science*, **91**, 269 (1940).
172. Arnon, D. J., Stout, P. R. and Sipos, F., *Amer. J. Bot.*, **27**, 791 (1940).
173. Mullins, L. J., *Proc. Exper. Biol. and Med.*, **45**, 856 (1940).
174. Stout, P. and Hoegland, D.-R., *Am. J. Botany*, **26**, 320 (1939).—Cf. also Gustafson, G. and Darken, M., *ibid.*, **24**, 615 (1937).
175. Ballard, S. S. and Dean, L. A., *Phys. Rev.*, **59**, 467 (1941).
176. Henderson, W. J. and Jones, U. S., *Soil. Sci.*, **51**, 283 (1941).
177. Eklund-Ehrenberg, C., v. Euler, H. and Hevesy, G., *Sv. Vet. Akad. Ark. f. Kemi, Mineral. Geol.*, **23B**, n° 5 (1946).

Discussion du rapport de M. de Hevesy

M. Aten. — With regard to the question of insoluble phosphate in the body, which is of extreme importance in the treatment of tumors, some experiments have been performed in Holland. Suspensions in paraffin oil of the radioactive insoluble substance were injected in such a way that this substance surrounded the tumour as accurately as possible. Radiophosphorus was chosen as the source of the radiation, because of its relatively short half-life and also because of the absence of gamma-rays. It was anticipated that this procedure would prevent damage as well as any appreciable transfer of the injected substance to other areas of the body. Zirconium phosphate, which is well-known as a very insoluble substance, was chosen in our case. (Recently in America similar experiments have been performed with chromiumphosphate.) Good results were obtained. In some cases the tumours disappeared, but this does not mean much, since experimental tumours are known to be affected by chemical changes in their surroundings. More significant was the fact that in no case a displacement of the radiophosphorus was observed from the site of the injection to other locations. (Observations by *Siemeling*, Utrecht and by *Heijn*, Eindhoven).

Another point I want to raise is the following :

Professor Jansen in the Department of physiological chemistry in Amsterdam suggested that the healing action of very large quantities of vitamin D on lupus might be due to an influence of this vitamin on the rate of metabolic processes in the skin. A simple procedure to test this assumption was to examine the influence of vitamin on the rate of formation of phosphatides in the skin. Measurements of the specific activity of phosphatides in the skin of treated and of untreated rats did not show any differences, nor was the rate affected at which phosphate ions enter the skin.

M. de Hevesy. — The negative result mentioned by Dr. Aten may possibly be explained in the following way. The effect of vitamine D on bone tissue is a comparatively slow process while already in the

course of a few hours an appreciable part of the phosphatides of the skin gets labeled. The appreciable « background » due to the rapid rate of renewal of many phosphorus compounds may be a serious obstacle in investigations like that mentioned by Dr. Aten.

M. Brinkman. — The question of continual and simultaneous synthesis and breakdown of proteins in the body has been referred to by Prof. Rittenberg in his report, but no attempt has been made as to the reason for such a labile condition. Does Prof. de Hevesy agree with Prof. Rittenberg's statement concerning the instability of those molecules under the influence of such a set of hydrolysing enzymes? Does the concentration of inorganic phosphate influence the rate of those reactions?

M. de Hevesy. — I have not much to add to Prof. Rittenberg's statement. The enzymic breakdown of the phosphorus compounds in the living organism and their enzymic new-formation are fundamental biological processes. An organism constructed on such a dynamic basis may be better suited to perform its biochemical tasks.

Desoxyribonucleic acid has a unique position among the phosphorus compounds. Its renewal, at least to a large extent, goes hand in hand with mitotic and similar processes. It is much slower in an adult than in a rapidly growing organism, in contrast to other phosphorus compounds, as for example phosphatides which are renewed in most organs of young and full-grown animals at a not much differing rate.

As to the effect of the concentration of inorganic phosphate on the rate of renewal of organic phosphorus compounds I cannot make any definite statement. In view of the fact that inorganic phosphate and, what is often of greater importance, phosphate donators as adenosinetriphosphate are present in the cells in appreciable amounts, it is doubtful whether a change in the concentration of inorganic phophate has a profound effect on the rate of renewal of organic phosphorus compounds. As the inorganic phosphate concentration of the red corpuscles can easily be varied by varying the phosphate concentration of the plasma, the effect of the change of the inorganic phosphate concentration on the rate of renewal of glycerophosphate in the red corpuscles could be easily investigated. This compound is renewed at a convenient rate.

M. Calvin. — Has Prof. de Hevesy examined the effect, *in vitro*, of oxygen on the rate of incorporation of ^{32}P into the organic phosphorous compounds of the red cells?

M. de Hevesy. — In experiments with avian corpuscles which have an appreciable oxygen consumption, replacement of oxygen by nitrogen in the gazeous phase suppresses almost entirely the formation of labeled phytates and phosphatides.

M. Calvin. — As far as chlorella is concerned, the effect of oxygen tension upon the rate of incorporation of radiophosphate is very marked. A fifteen minute exposure under aerobic conditions will produce as much as 10 times the incorporation observed under anaerobic conditions.

M. G. Guében. — La méthode des indicateurs radioactifs introduite par Monsieur le Professeur de Hevesy a déjà conduit à des résultats remarquables dans divers domaines et notamment en biochimie. Elle ouvre des champs d'investigation extrêmement vastes. Elle donne toutefois lieu à l'objection suivante : est-on absolument sûr que le caractère radioactif de l'indicateur utilisé n'est pas de nature à modifier, à perturber dans certains cas le processus que l'on veut étudier? Je pense, quant à moi, qu'il n'y a pas intervention du rayonnement propre de l'élément employé, que les doses ne sont pas suffisantes pour produire cet effet. A ce point de vue on pourrait peut-être craindre que la considération de doses constitue une extrapolation hardie pour le cas de phénomènes se passant dans le milieu intra-cellulaire. Je voudrais demander à Monsieur le Professeur de Hevesy s'il connaît des expériences directes qui permettent de conclure avec certitude à l'absence d'une action du rayonnement des indicateurs employés.

M. de Hevesy. — There is hardly any danger that the minute doses we are usually using influence the physiological processes taking place in the organism. In most investigations just a few microcuries or less are administered to a rat and in experiments *in vitro* in which the permeability of red corpuscles is investigated, the addition of 1/100 of a microcurie to the blood sample may be sufficient. Now, one microcurie distributed in one g. tissue produces in the course of one day an ionization corresponding to the effect of a Röntgen ray of 43 *r* units. In the course of an experiment taking 2 hours in which 10 microcuries are administered to a rat weighing 100 g.

assuming a uniform distribution, each gramme of tissue will be exposed to an ionization corresponding to the effect of about 1/3 *r* units. This is a very modest dose.

I want to mention the fact that we raised tubercle bacteria in a medium (50 ml.) containing several *milli*curies of ^{32}P. The bacteria were found to grow exactly at the same rate as in an inactive culture solution. However it should be mentioned that tubercle bacteria are known to be exceedingly resistent to the effect of X-rays.

Now that large pile-produced activities are available there is some danger that tracers may be lavishly used. Such a course is to be avoided, the general rule being that we shall avail ourselves in animal, and still more in human experiments, of as restricted activities as possible.

M. Rittenberg. — Does Prof. de Hevesy know the spacial distribution of the labeled substances in the red cells?

M. de Hevesy. — In the mammalian red corpuscles while the labeled phosphatides are to a large extent present in the stroma, most other phosphorus compounds are to be found in the plasma. In nucleated red corpuscles desoxyribonucleic acid is present in the nucleus, which may contain minor amounts of phosphatides and acid-soluble P compounds but minimal amounts of ribonucleic acid only.

M. Joliot. — Faisant suite à ce qu'à dit Monsieur Guében, une cause d'erreur plus importante que l'action du rayonnement radioactif de l'indicateur est celle due à l'introduction parfois trop importante dans l'organisme des atomes stables isotopes du radioélément utilisé. On risque ainsi de modifier le processus physiologique normal. Il y a une plus grande sécurité si l'on introduit les plus grandes activités spécifiques possibles absolues minima compatibles à la sensibilité et à la précision des mesures radioactives. La radioactivité même faible de l'indicateur peut avoir de grandes conséquences dans les cas des processus biologiques très sensibles, par exemple en génétique. On peut imaginer à ce propos l'expérience suivante : préparer des levures chargées en phosphore contenant du radiophosphore et nourrir des larves de drosophyle par exemple avec cette levure. Les chromosomes se chargent en phosphore inactif et radioactif en des places déterminées et au cours du temps certains atomes de

radiophosphore se désintègrent, l'énergie de recul et le changement de nature chimique du Phosphore en Soufre coupe la molécule en des places déterminées. On observe ainsi des mutations de certains types. Si on répète l'expérience avec un autre radioélément contenu dans les chromosomes à d'autres places de celui-ci, on pourra produire des mutations de types différents. Il faut noter que c'est surtout l'énergie de recul et le changement de nature chimique du radioélément qui est responsable de la coupure et non l'ionisation due au rayon β émis. Des expériences de ce genre ont-elles été tentées?

M. de Hevesy. — Now that large activities are available, such kind of experiments can be carried out, though the difficulties involved should not be underestimated.

M. Courrier. — En qualité de biologiste, j'admire vivement le beau rapport de M. de Hevesy. Je serais heureux s'il voulait bien nous donner quelques explications complémentaires au sujet de l'étude des centres nerveux avec le phosphore marqué.

M. de Hevesy indique dans son travail que la pénétration du radiophosphore dans le cerveau est particulièrement lente. Puis-je lui demander ce qu'il pense des récentes recherches de *Borell*, *Westman* et *Oerström* (1947)? On sait que la lapine est une femelle à ponte provoquée par l'accouplement; le coït déclenche un réflexe neurohormonal; dans un premier temps, l'hypophyse est excitée par voie nerveuse; dans un deuxième temps, l'hypophyse excrète une gonadostimuline qui provoque l'ovulation. Cette ovulation s'effectue dix heures environ après le rapprochement sexuel. Or Westman et ses collaborateurs ont eu l'heureuse idée de rechercher la pénétration du radiophosphore dans l'hypothalamus (1) chez la lapine qui vient d'accepter le mâle et ils ont pu faire une observation remarquable : la quantité de radiophosphore fixée dans l'hypothalamus augmente sous l'influence de l'accouplement; cette augmentation est déjà nette *deux minutes* après le coït. Quelle est l'opinion de M. de Hevesy sur la rapidité de cette fixation du phosphore marqué dans les centres nerveux hypothalamiques?

M. de Hevesy. — The rate of penetration of ^{32}P into the brain tissues and still more in the brain cells is very slow. We found after

(1) L'hypothalamus est une région du cerveau qui est réunie à l'hypophyse par la tige pituitaire.

the lapse of 250 minutes the specific activity of the inorganic P of the brain tissue of the rabbit to make out only 1.5% of the specific activity of the plasma inorganic P. As a part of the inorganic ^{32}P of the brain tissue was present in the extracellular space the specific activity of the inorganic P of the brain cells is still lower than indicated by the above figure. The low permeability of the brain tissue cells is by no means disturbing in experiments of Westmann and his colleagues, who succeeded in showing that coitus accelerates the rate of penetration of labeled phosphate into the hypothalamique centers. In a few cases the rate of formation of labeled acid-soluble phosphorus compounds in the brain tissue was also found to be increased.

M. Courrier. — M. de Hevesy a insisté sur l'importance du radiophosphore dans l'étude biologique des globules rouges. Peut-il nous dire si cette étude a apporté des précisions sur la durée moyenne de la vie de ces éléments?

M. de Hevesy. — By using ^{32}P as an indicator we succeeded only in determining the life-cycle of nucleated corpuscles, namely those of birds, reptiles and so on. These determinations are based on the fact that the desoxyribonucleic acid present in the avian red corpuscles when labeled during their formation retains its label throughout their life. We can label with ^{32}P mamalian red corpuscles as well, but these corpuscles do not keep their label for several months which is required when the labeled corpuscles are to be used in life-cycle determination. ^{15}N administered as glycine remains in the heme of red corpuscles all through their life and Rittenberg and Shemin succeeded in determining the life-cycle of human red corpuscles by making use of ^{15}N labeled erythrocytes.

M. Courrier. — Il est étrange de noter que le globule rouge du mammifère, qui est dépourvu de noyau, paraît avoir une existence plus longue que celui des oiseaux qui est nuclé.

M. de Hevesy. — The mammalian and avian red corpuscles are so different that it is difficult to make any forehand statement on their relative life-time.

M. E.-J. Bigwood. — M. Courrier nous a rappelé la rapidité avec laquelle des influx nerveux centripèdes, d'origine sexuelle, pouvaient retentir sur le métabolisme de la cellule du système nerveux central

et provoquer une riposte de ce système nerveux central sur la fonction gonadotrope de l'hypophyse antérieure. Il lui semblait que ce processus était à opposer à la stabilité et au caractère peu réactionnel des constituants chimiques de la substance nerveuse, dont parlait M. de Hevesy; selon ce dernier, on peut concevoir que l'action des influx nerveux mentionnés par M. Courrier se traduit par un accroissement momentané de la perméabilité de la substance nerveuse, ouvrant transitoirement la barrière qui sépare sinon le tissu nerveux du plasma sanguin circulant.

On peut toutefois se demander s'il y a vraiment une opposition entre le phénomène signalé par M. Courrier et celui dont parlait M. de Hevesy. En réalité, ce dernier fait surtout allusion à la stabilité très grande des phospholipides du système nerveux central, c'est-à-dire aux constituants chimiques structurels de ce système, tandis que M. Courrier se réfère en somme au *métabolisme* de la cellule nerveuse. La notion de barrière sauvegardant une sorte d'autonomie du système nerveux par rapport au milieu humoral ne peut être prise dans un sens trop absolu. On sait, par exemple, que les modifications de la glycémie dans la circulation générale retentissent très rapidement sur le métabolisme du tissu cérébral.

In his chapter on the transfer of phosphate groups, M. de Hevesy recalls the interesting instance of direct transfer of a phosphate group from one molecule to another without passing through the inorganic stage and that namely in muscle extracts, non labeled phosphorus in 3 phosphoglyceric acid can be found to be converted into unlabeled 2 phosphoglyceric acid in spite of the presence of labeled inorganic phosphate. A similar transfer was shown by Parnas to occur in glucose monophosphoric esters, such as the conversion of a non labeled Cori's ester into non labeled Robinson's ester, notwithstanding the presence of labeled inorganic phosphate in the solution.

It would be most interesting to know whether similar phenomena can be found to take place in the case of transamination of non labeled N in spite of the presence of ammonium salts containing labeled nitrogen. Positive findings in this instance would render more convincing the cases where transamination is assumed to take eventually place.

M. Rittenberg. — There are several analogies to the transfer of phosphate from 3 phosphoglyceric acid to 2 phosphoglyceric acid.

For example diacetyldiketopiperazine will acetylate sodium leucinate without the intervention of free acetate ions. Another example studied by Bonhoeffer is the Canizzaro reaction in which a hydrogen atom from one aldehyde molecule is transferred to a second aldehyde. Many other examples could be given though I cannot at this moment recall such an experiment on transamination.

M. de Hevesy. — In our joint investigations with Professor Parnas we found several systems containing labeled inorganic phosphate in which phosphate transfer took place without participation of the inorganic phosphate. We found also that when adenylic acid is formed from adenosine in the presence of hexosediphosphate and labeled inorganic phosphate in yeast, one-half of the phosphorus atoms were found to be those originally located in the hexosediphosphate molecules, while the other half were originally present as inorganic phosphate.

M. Courrier. — M. de Hevesy a mentionné les recherches de *Flock* et *Bollman* concernant l'effet du diéthylstilbestrol sur le métabolisme des phosphatides dans le foie. Etant donnée l'action différente du foie sur les oestrogènes artificiels et sur les oestrogènes naturels, a-t-on étudié l'influence de ces derniers sur les phosphatides hépatiques?

M. de Hevesy. — The only oestrogenic substance the effect of which *Flock* and *Bollman* and also *Chaikoff* and his associates investigated was stilbestrol.

M. Govaerts. — Prof. Guében a posé la question de savoir si les radiations émises par les radioéléments artificiels utilisés comme indicateurs ne peuvent pas modifier les processus biologiques normaux. Prof. de Hevesy vient de donner quelques arguments qui montrent que les effets biologiques éventuellement produits par les radiations ne peuvent pas influencer les résultats observés. Je pense qu'il est intéressant de signaler un autre argument fourni également par le Prof. de Hevesy il y a quelques années. La perméabilité des globules rouges aux ions K et Na a été étudiée à l'aide des isotopes radioactifs. Les résultats obtenus montrent que pour l'un de ces ions la vitesse de pénétration est très grande et pour les autres elle est très petite. Aussi est-il difficile de concevoir que les radiations émises puissent, dans des conditions comparables, produire des effets totalement opposés.

L'utilisation des indicateurs isotopiques permet d'expliquer le mécanisme intime de différents processus biologiques. Il est cependant important d'être très prudent lors de ces interprétations et certains résultats que j'ai obtenus indiquent effectivement que ces processus ne sont pas toujours très simples. Jusqu'à présent il était admis que le phosphore acido-soluble du plasma est constitué en grande partie de phosphore minéral semblable à celui que nous trouvons dans nos flacons du laboratoire. L'injection d'une solution de phosphate marqué chez le chien a permis de montrer que le rein distingue le phosphate minéral injecté du phosphate « dit minéral » du plasma. Aussi, grâce à l'utilisation simultanée des méthodes physiques et des techniques délicates de la physiologie, j'ai pu mettre en évidence que la plus grande partie du phosphate « dit minéral » du plasma est dans un état physico-chimique différent de celui du phosphate injecté.

Radiocarbon and its Application in Chemistry and Biology

by
M. Melvin CALVIN

I.

INTRODUCTION

The significance of having available to us considerable quantities of more or less separated isotopes of carbon in both chemistry and biology need hardly be repeated. The usual definition of organic chemistry as the chemistry of carbon compounds, and its very name, « organic », can hardly fail to impress even the uninitiated with the importance of this element in both life and industry. It is my hope, in this brief paper, to outline not only the nature of these isotopes, how they are produced and measured, but also to indicate some of the applications which have already been made in the brief time of their availability. The fields of application are so diverse that it would be little short of impertinence for one man to pretend to encompass all of them adequately. They run the gamut from the most abstruse of technical and industrial production problems, through some of the very fundamentals of chemical transformation, to the very nature of life itself. If, therefore, some topics are only briefly mentioned or omitted entirely, it is not because of their lesser importance or usefulness, but rather due to unfamiliarity or total ignorance on my part, together with the limitations of time and space.

II.

CARBON ISOTOPES

a) The Stable Isotopes:— There are presently known five isotopes of carbon, ranging in mass from 10 through 14 of which two, C^{12} and C^{13}, are stable. Natural carbon consists of about 99% C^{12}

and 1% C^{13} (1). Already, in the years prior to the war, methods had been developed for the concentration of this heavier stable isotope in appreciable quantities. At present in the United States, there are two methods in use on a considerable scale. One is based on the thermal diffusion principle of Clusius using methane as the diffusing gas, and the other depends upon the equilibrium between KCN, H_2O and HCN (2) (3) multiplied in a contercurrent column. The thermal diffusion method has been developed by the Houdry Company at Marcus Hook, Pennsylvania, while the chemical equilibrium is the result of the work of Urey and has been developed by the Eastman Company in Rochester, New York. These methods have made available carbon with a C^{13} concentration up to 60%, at least on a semi-commercial basis, with the prospect of regular commercial availability in the very near future. The C^{13} concentration in a sample is most readily measured by means of some variety of mass spectrograph (Nier, etc.). For measurement, the samples should preferably be in the form of some simple gas, such as CO, CO_2, or methane. Some instruments claim to measure the isotopic composition of carbon to .01%, however, the more usual figure is .02%. Thus, if 100% C^{13} were available for an experiment, it would theorectically be capable of undergoing a 5000-fold dilution and still be detectable. For practical measurements, a 500 to 1000-fold dilution is the usual limit allowable in any experiment. A great deal of work has been done in the past ten years using C^{13} as a tracer, but I believe that because of the cumbersome and expensive methods of the measuring device and the dilution limits, it will be largely superseded by C^{14} as this becomes more available (4).

b) The Radioactive Isotopes:— The three remaining isotopes of carbon, C^{10}, C^{11} and C^{14}, are all radioactive. C^{10}, produced by the reaction of protons on B^{10} (5), is practically useless for tracer work because of its very short half-life of 8.8 seconds (6). C^{11} may be produced by a variety of reactions of which deuterons on B^{10} is the most common (7). It has a half-life of twenty-one minutes (8) and emits a particle of about 1 Mev (8) energy. Because it could be produced in very high specific activity in the Berkeley cyclotrons, and because of the ease with which it is measured, it has, until now, found considerable use in spite of its relatively short half-life. Here again, it is doubtful that C^{11} will find a very wide application because of the increasing availability of C^{14}. There are certain special applications for which C^{11} with its 1 Mev particle

and 0.51 Mev γ of the annihilation radiation, may have unique usefulness. These would, of course, be very short-term experiments in which a relatively penetrating radiation is to be followed behind some layer of absorber.

Finally, we come to C^{14} which will be the primary subject of this discussion. It has a half-life of 5000 years (10) (11) and emits a β-particle of about 150 KV maximum energy. It can be produced by any of three reactions:

$$N^{14} + n^1 \rightarrow C^{14} + p^1 \qquad (12)\ (13)$$

$$C^{13} + n^1 \rightarrow C^{14} + \gamma$$

$$C^{13} + d^2 \rightarrow C^{14} + p^1 \qquad (14)$$

It is now being produced in the uranium piles by the first of these three reactions (15). Nitrogen-containing compounds, at first calcium nitrate now beryllium nitride, are inserted into the pile and allowed to undergo thermal neutron irradiation for varying lengths of time, after which the C^{14} formed is isolated and prepared in the form of barium carbonate. Relatively large quantities of C^{14} are thus becoming available with very high specific activity. Barium carbonate having as high as 8% C^{14} has been prepared, while 2% preparations are now common. The specific activity of this 2% carbon is about 100 microcuries per milligram. From the point of view of tracer studies, the 5000 year half-life is very satisfactory, on the other hand, the low energy of the beta-particle makes its measurement a little more difficult than that of C^{11}. However, very adequate methods have been developed for the routine determination of C^{14} activity (16) (17). For example, we can now easily measure with a thin-walled Geiger tube (to be discussed later) 50 disintegrations per minute, per milligram of carbon. With the starting activity for approximately 2% C^{14} about 2.2×10^8 disintegrations per minute, per milligram, the allowable dilution for this carbon becomes approximately 5×10^6. With an ionization chamber-electrometer combination or by putting the carbon dioxide directly into a Geiger tube, it is possible to measure down to 5 disintegrations per minute, per milligram of carbon, thus bringing the allowable dilution for 2% C^{14} up to 5×10^7, and finally, with 20% C^{14}, wich will certainly be available soon, the allowed dilution reaches approximately 5×10^8. These figures should be compared with the 500 to 5000-fold dilution possible with the use of C^{13}. There is a further advantage of C^{14} over the stable isotope in experiments

in which the fate of the administered isotope is unknown and in which, therefore, the isotope must be hunted amongst the infinite variety of compounds found in living organisms. This process is very tedious and difficult when mass spectroscopic analysis is required, but becomes very quick and easy when all that is required is the insertion of some fraction or sample beneath a Geiger tube without the destruction of the sample itself.

III.

METHODS OF MEASUREMENT OF C^{14}

There are three systems now being developed for the routine measurement of C^{14}. They are:— (*a*) The thin-walled Geiger tube for which the sample is mounted as a solid outside the window (16), (*b*) a Geiger tube into which the carbon to be measured is introduced as carbon dioxide (18) (19), and (*c*) the ionization chamber-electrometer combination which also requires the carbon to be introduced into the ionization chamber as a gas, usually carbon dioxide (11) (20).

a) Thin-walled Geiger Counter:— Of these, two types have been developed. First, the ordinary low-pressure counter for which the window may be as thin as 1.5 to 2 milligrams per square centimeter backed up by a supporting grid (Fig 36.). These usually have an efficiency of about 12 to 17 disintegrations per count. They are quite stable, operating usually in the voltage range of 1100 to 1300 volts with a plateau of the order of 200 volts. More recently, a helium filled counter tube, operating near atmospheric pressure, has been developed (21) (22). The windows on these generally run from .7 to 1 milligram per square centimeter and do not require a supporting grid. They have an efficiency of 4 to 7 disintegrations per count. As yet they do not appear to be quite so stable as the low-pressure counters and require 1500 to 1800 volts for operations with a relatively narrow plateau of 50 to 100 volts. The essential problem with all thinwalled counters for use on weak beta-radiation is, of course, the uniformity with which the solid samples can be prepared so as to make possible reliable correction for the self-absorption of the radiation in the sample itself. This difficulty can be largely avoided in those cases in which very thin samples may

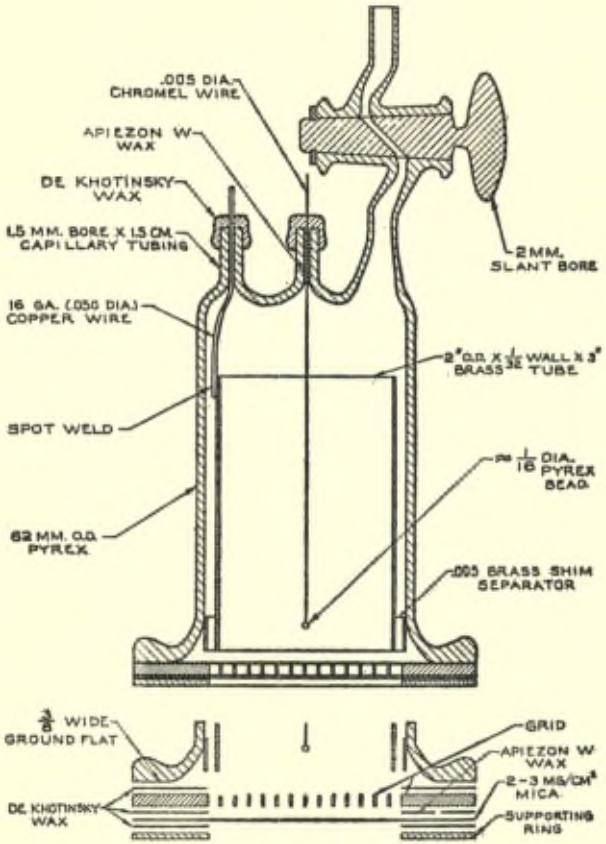

.005 DIA. CHROMEL WIRE
APIEZON W WAX
DE KHOTINSKY WAX
1.5 MM. BORE X 1.5 CM. CAPILLARY TUBING
16 GA. (.050 DIA.) COPPER WIRE
SPOT WELD
62 MM. O.D. PYREX
2 MM. SLANT BORE
2" O.D. X 1/32 WALL X 3" BRASS TUBE
1/16 DIA. PYREX BEAD
.005 BRASS SHIM SEPARATOR
3/8 WIDE GROUND FLAT
DE KHOTINSKY WAX
GRID
APIEZON W WAX
2-3 MG/CM² MICA
SUPPORTING RING

Fig. 36

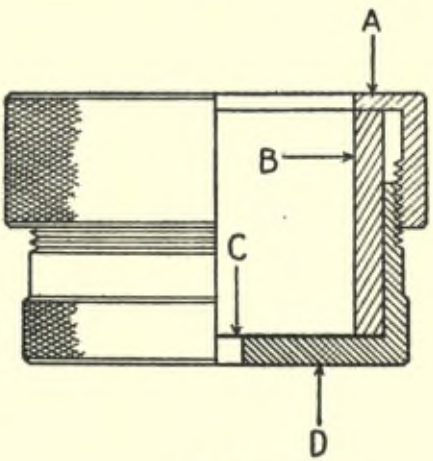

A
B
C
D

Fig. 37

be used that are less than .2 of a milligram per square centimeter of sample. However, when very weak samples are being counted, a larger amount of material must be mounted. We have been able to do this by developing a standard mounting technique for barium carbonate which has allowed us to make reproducible measurements to within 2% on samples running as high as 5 milligrams per square centimeter. This is achieved by grinding the barium carbonate in a mortar in a slurry of absolute alcohol and then evaporating this slurry on an aluminium plate held in a plate holder which confines the sample to a single well-defined area (Fig. 37). The self-absorption corrections for such a mounting have been determined so that in the rangs of 2 to 20 milligrams per square centimeter of sample, the results are satisfactory to within 2% (23).

b) Carbon Dioxide filled Counters:— The obvious way of avoiding these self-absorption problems most directly is, of course, to introduce the carbon as gas, usually carbon dioxide, directly into the Geiger tube. The sensitivity of this type of measuring device is quite high. However, because each sample requires a new filling of the Geiger tube, some difficulty has been encountered in its development for routine use. On the other hand, certain laboratories in the United States have done considerable work with this type of counting and claim not only very high sensitivity, but also routine reproducibility (18) (19).

c) Ionization Chamber-Electrometer Combination:— Sensitivity comparable to that achieved by putting the gas directly into the Geiger tube can be obtained also by putting the carbon dioxide to be counted into an ionization chamber and determining the ionization current by means of an electrometer (17) (20). This does not suffer from the need of reproducing a counter gas as does the previously mentioned method, but it does require considerably more sample to achieve the same sensitivity as the gas counting Geiger tube does. The limits for practical measurement of the three methods are:— For the thin-walled Geiger tube, 10^{-3} to 10^{-4} microcuries; for the gas-filled counting tube, 10^{-4} microcuries, and for the ionization chamber electrometer combination, 10^{-4} microcuries. The last two methods both suffer from the disadvantage of requiring the sample in the form of a gas, usually carbon dioxide, whereas, the thin-walled Geiger tube can measure the sample, at least approximately, in any solid form that can be spread on a plate.

In our laboratory in Berkeley, we have more or less settled on two types of instruments for routine use. First, the thin-walled Geiger tube for most preparations and second, the ionization chamber-electrometer combination for those cases in which the sample normally comes out in carbon dioxide or in which the specific activity of the sample is beyond the range of accurate determination with the thin-walled tube. Both instruments have been reduced to the stage of being operated entirely by laboratory technicians on a completely routine basis.

IV.

APPLICATIONS IN THE STUDY OF REACTION MECHANISMS

Although one of the most obvious applications of isotopic carbon would be in the study of the mechanism of a wide variety of organic transformations, relatively few of these have yet been investigated. Those that have, arose from the need for unequivocal methods of degradation of biochemical products or from the need to demonstrate the position of entering carbon atoms in a synthetic procedure during the course of the manufacture of some compounds required for biological study. Only in the past few months has the tool been used to study more complex organic rearrangements from the point of view of fundamental chemistry.

a) Oxidation of Propionic acid:— One of the first studies of a reaction mechanism arose from the need to degrade propionic acid resulting from bacterial fermentation (24). The method selected was oxidation by alkaline permanganate which gives quantitatively oxalate and carbon dioxide.

$$CH_3—CH_2—CO_2^- + 4MnO_4^- \rightarrow$$
$$C_2O_4^{--} + CO_3^{--} + 4MnO_2 + OH^- + 2H_2O$$

The obvious presumption to make was that the oxalate originated from the alpha and beta carbon atoms, while the carbonate came from the carboxyl group. A test of this oxidation with synthetic carboxyl-labeled propionic acid made by carbonating an ethyl Grignard with carbon dioxide, showed this not to be the case. The results, table I (25), clearly demonstrate that more of the oxalate arises from the carboxyl group and the alpha carbon atom than does from the methyl and alpha carbon atom.

TABLE I.

Permanganate oxidation of labeled Propionate.

Expt.	Concentration of NaOH moles/liter	Percent of C* in CO_3^{--}		$C_2O_4^{--}$		$\frac{\alpha C-\beta C \text{ rupture}}{\alpha C-COO^- \text{ rupture}}$
1	$10^{-4}-10^{-5}$ (HCO_3^- Buffer)	28.2		71.8		
2	$10^{-4}-10^{-5}$ (HCO_3^- Buffer)	27.6	28.9	72.4	71.1	2.5
3	0.1	30.7		69.3		
4	2	27.8		72.2		
5	2	30.4		69.6		
6	6	16.5		83.5		5.1
7	11	14.2	13.1	85.8	86.9	6.6
8	11	12.1		87.9		

Furthermore, the frequency of the alpha-beta rupture increases with increasing alkalinity. Similar experiments were performed on both alpha and beta hydroxypropionic acid which showed a ratio of alpha-beta rupture to alpha-carboxyl rupture of between 2 and 3, even at very high alkalinities (12N NaOH). These experiments show quite conclusively that the beta carbon atom is attacked before the three carbon chain is broken, in at least one path of the oxidation, and furthermore that some of the propionic acid oxidation does not proceed through either alpha or beta hydroxypropionic acid.

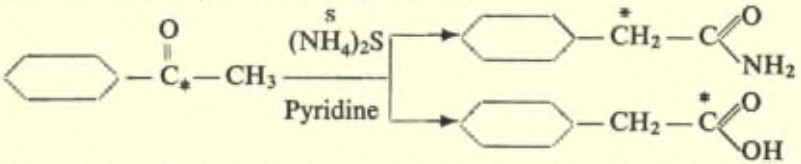
The acid dichromate oxidation of propionic acid yield carbon dioxide and acetic acid in which the carbon dioxide originates entirely from the carboxyl group of the propionic acid (24). The acid oxidation of lactic acid, is now being tested in view of the fact that this reaction is very commonly used for the degradation of biologically formed lactate.

b) The Wolff Rearrangement:— There are a considerable number of rearrangement reactions which seem to involve the breaking and making of new carbon bonds, one of these is the Wolff rearrangement.

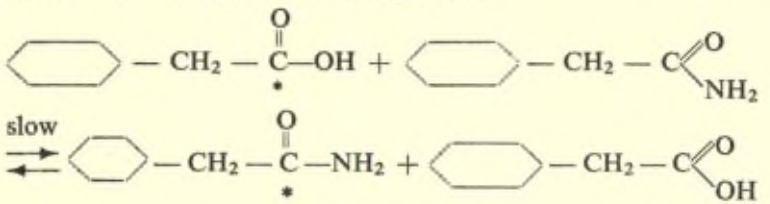
$$R-\overset{*}{C}(=O)-CHN_2 + R'OH \xrightarrow{Ag} R-CH_2-\overset{*}{C}(=O)-OR' + N_2$$

This was one of the first of the rearrangement reactions to be studied with isotopic carbon. It was first done by Arnold (26) with C^{13} and has since been repeated in our laboratory (27) with C^{14}. The results clearly indicated a shift of the R group from the carbonyl group to the diazomethane carbon atom.

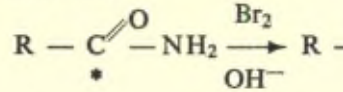
c) The Willgerodt Reaction (27):— In recent years this reaction has been extensively studied by kinetic and substitution methods. In view of its importance as a synthetic tool, we have examined this reaction in carbonyl-labeled acetophenone. A rather unusual result has appeared which is being further investigated. It may be summarized by the following reaction.



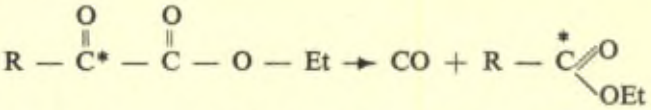
From this it is clear that the amide and the acid are formed by two different mechanisms. The formation of the amide does not involve the making or breaking of carbon bonds but rather is the result of a reduction and oxidation along the chain. The formation of the acid, on the other hand, must involve a carbon rearrangement. Furthermore, our ability to isolate both acid and amide from this reaction mixture having different radiocarbon distribution implies that the exchange reaction between acid and amide must be slow under the conditions of the rearrangement.



This was tested in a separate experiment and confirmed. Another reaction (27) of considerable importance in the degradation of acids is the Hoffman degradation with alkaline hypobromide. The result here could hardly be anything but the expected one and it was done incidental to the degradation work on the amide resulting from the Willgerodt reaction.

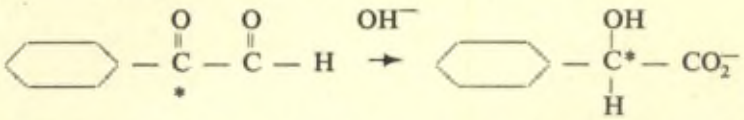


d) The Decarbonylation of Alpha Keto Esters (28):— A reaction that is of considerable interest, not only as a synthetic method but also because of its close relationship to the biological decarboxylation of keto acids, is the loss of carbon monoxide when an alpha keto ester is heated.



It would be of considerable importance to know something about the mechanism of this reaction especially with respect to which of the two possible carbons comes out as carbon monoxide. The reaction was tested by the thermal decomposition of carbonyl-labeled ethyl pyruvate. The carbon monoxide evolved contained no radioactivity. This indicates that it is the carbon of the carboxylate group which is eliminated even when that elimination involves the splitting up of the carboxyl group itself. A similar test is being performed on the free pyruvic acid. The results of this, however, can hardly be in question.

e) The last reaction upon which we have as yet any data involves the rearrangement of an alpha keto-aldehyde (29).



The alpha-labeled phenyl glyoxal was rearranged in alkali to produce mandelic acid, the label remaining on the alpha carbon atom. This is, therefore, also the result of a rearrangement of hydrogen atoms only, that is an oxidation and reduction along the chain similar to that observed in the Willgerodt rearrangement for the formation of amides.

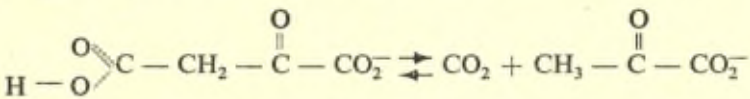
V.

APPLICATIONS IN BIOLOGY

a) Animal Metabolism:— Although most of the so-far published work involving isotopic carbon in biology has been in the field of bacterial and animal metabolism, we will be confined here to the briefest review of the most definitely established results serving

only as a background for the more extensive discussion of photosynthesis which is to follow. For more detailed examination of this subject, reference should be made to several excellent reviews which have already been published (20, 31, 32, 33, 34).

Probably the first definite impact which the use of isotopic carbon made on biochemistry was the establishment of the fact that carbon dioxide is not merely a terminal metabolic waste product, but actually plays a part itself in the various metabolic cycles that are being discovered. This was first shown by the simple experiment of exposing a variety of biological preparations—bacteria, yeasts, and minced animal tissues of various types—to isotopically marked carbon dioxide and then demonstrating that this carbon dioxide has actually been incorporated into the organic compounds present in the biological material used. More recently, the particular compounds into which the carbon dioxide has been incorporated have been determined as well as the particular position occupied by the carbon atom. These compounds have generally turned out to be the various acids involved in the di- and tri- carboxylic cycles, going all the way to glycogen, in the case of liver. The point of entry of carbon dioxide was presumed to be the beta-carboxylation of pyruvic acid to give oxalacetic acid which is known as the Wood-Werkman reaction since it was first proposed by them in connection with the formation of succinic acid by propionic-acid bacteria dissimulating glycerin.



This has since been shown to be a clearly reversible reaction using cell free extracts of various types as a source of the beta carboxylase enzyme in the presence of ATP (adenosine triphosphate). The experiment was done by simply allowing the enzyme to act on oxalacetic acid in the presence of isotopic carbon dioxide and then demonstrating the presence of the isotope in the remaining oxalacetic acid. The following scheme, can then be set up to account, not only for the presence of the isotopic carbon in the intermediate acids, but also for the particular positions which they occupy in these acids and in the glycogen form. Experiments of this type are being extended using laboratory synthesized intermediates containing the isotope in various positions and isolating and degrading the proposed intermediates. For example, carboxyl-labeled acetate, carboxyl-labeled

lactate, and alpha, beta-labeled lactate have all been used and their incorporation into the intermediates of the di- and tri-carboxylic cycles demonstrated. As this work progresses and the various enzymes involved are isolated and their reactions demonstrated, we can look forward to a rapidly unfolding picture of inter-relationships of fat, protein and carbohydrate metabolism. Already there is no question but what intermediates are common to the synthesis and degradation of all three types of material.

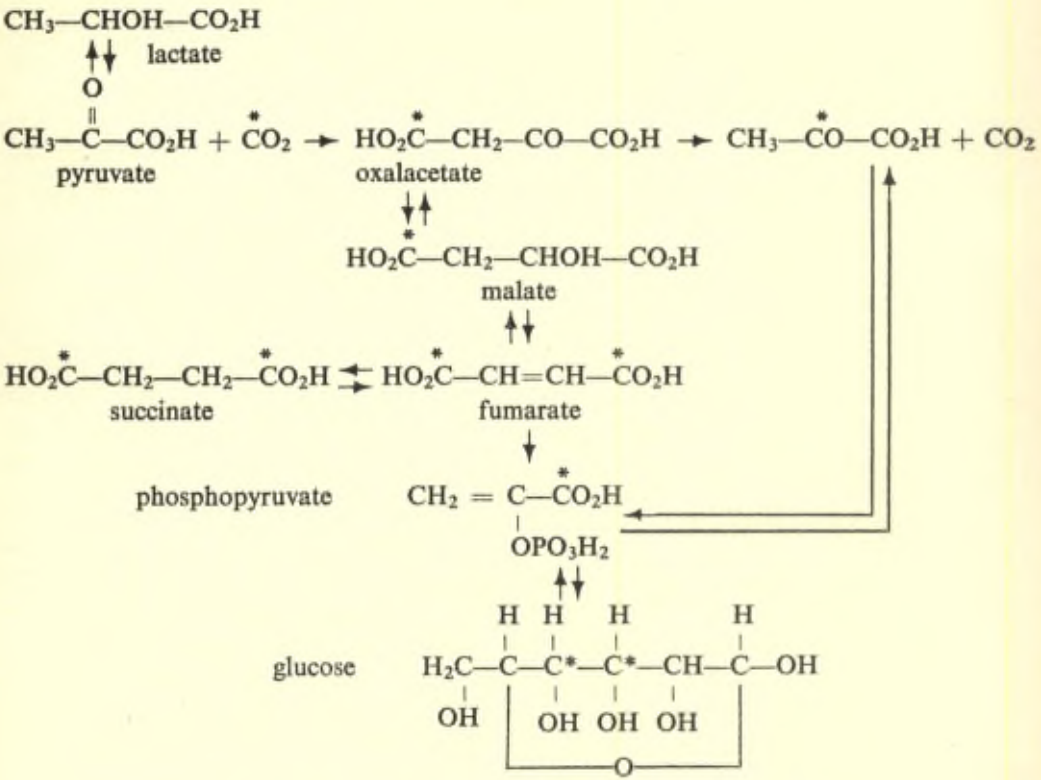
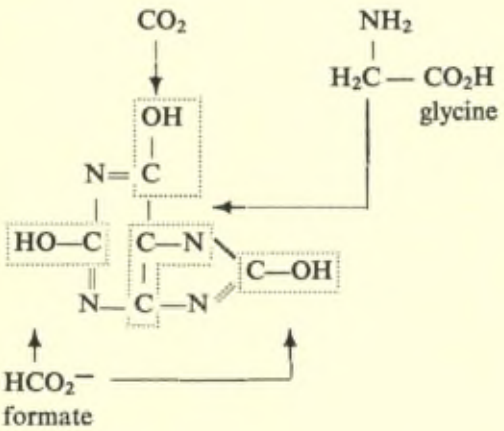


Chart showing the paths by which isotopic CO_2 and lactate may be incorporated into glycogen and its intermediates.

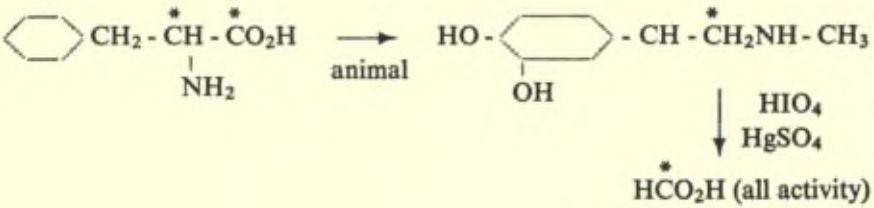
Isotopic carbon has already been used to determine the source or fate of a number of other special compounds of biological interest such as urea, uric acid, tyrosine, phenylalanine, and the porphyrins, and work is now progressing on studies of the metabolism of the

sterols, vitamins, hormones, etc. Still another type of study which is in progress is the examination of the fate of many compounds not normally present in the organism, such as synthetic drugs, carcinogens, and the like. It will be of interest to mention the results of some of these researches.

The biological precursors of uric acid carbon (35, 36, 37) were determined by administering labeled compounds to pigeons. The compounds used were carbon dioxide, carboxyl-labeled acetate, carboxyl-labeled lactate, alpha and beta -labeled lactate, carboxyl-labeled glycine, formic acid and nitrogen-labeled glycine. The excreted uric acid was isolated and degraded. The results are summarized in the following chart.



By feeding labeled phenylalanine (38), it has been shown that adrenaline is formed from it directly by decarboxylation and oxidation without rupture of the ring side chain structure as shown in the following reaction.



In another set of experiments, β-labeled tyrosine (39) was injected into the tail vein of melanomous mice which were then subjected to an anatomical fractionation after varying periods of time. The highest specific activities were found in the intestinal mucosa, the adrenals, the thyroid, and the melanoma. The latter three findings were to be expected as tyrosine is a precursor of adrenaline, thyroxine and melanin. The finding in the intestinal mucosa seems to indicate an active protein synthesizing system in this organ, a result comparable to that obtained using S^{32} labeled methionine (40). It should be pointed out that tyrosine administered in this fashion is very rapidly metabolized, 20% of the radioactivity appearing in the expired carbon dioxide in the first 12 hours.

Another problem of considerable interest which shows promise of being solved by these methods, is the fate of carcinogenic hydrocarbons. Heretofore, only a small percentage of the administered carcinogen (dibenzanthracene) has been accounted for by the ordinary analytical methods, such as straight chemical analysis or spectroscopic analysis. These, of course, were limited to a recognition of the condensed ring system and could not detect more extensive degradations. 9,10-labeled dibenzanthracene has been synthesized and its fate, following administration to mice, is now being examined (41). It has already been discovered that upon administration by injection of an aqueous colloid, a considerable fraction (50%) is rapidly excreted through the bile. The feces of animals injected intraperitoneally with the carcinogen in tricaprylin have been examined and of the radioactivity found in the feces, some 80% turns out to be water soluble. This is indicative of an extensive degradation into water soluble aromatic acids, as the methods of fractionation rule out conjugation with water-solubilizing substances.

Rather than try to discuss any more of these examples, I would like to use the remaining time for a somewhat detailed presentation of the use of isotopic carbon in the study of the mechanism of photosynthesis as an example of the problems, pitfalls and techniques involved in a biological investigation with isotopic carbon.

VI.

PHOTOSYNTHESIS

The problem of photosynthesis is probably one of the most easily defined of present-day biochemical problems and most difficult of solution. It is already apparent that a most involved kind of physics, chemistry, and biology must be concerned with its solution. Here again, this is not the place to attempt a complete review of all that is known or has been surmised about the process (42, 43, 44).

The overal problems involved can be most readily appreciated in terms of the chart shown below. The green plant is able to take carbon, hydrogen and oxygen in the lowest energy forms, as carbon dioxide and water, together with electromagnetic energy, in the form of visible light, and convert this system into compounds of higher energy content, that is gaseous oxygen and reduced carbon, generally in the form of carbohydrate.

$$(CH_2O)_6 \qquad\qquad O_2$$
$$\underset{h\nu}{\rightleftharpoons}$$
$$CO_2 \qquad\qquad H_2O$$

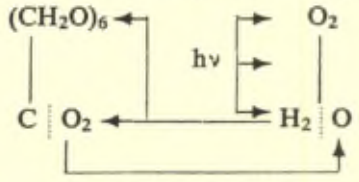
The questions may then be asked, by what path is the quantum of light primarily converted into chemical energy, and what is the path taken by the three elements, carbon, hydrogen, and oxygen (and presumably the other elements constituting the living organism) to pass from their initial low energy condition into their final high energy states. It is thus obvious that at least for the latter part of the problem, the availability of isotopic tracers of carbon, hydrogen, and oxygen provide the method par excellence of mapping what now appears to be a very intricate network of paths. The use of isotopic carbon (and in some experiments, O^{18}) has already provided considerable information, not only about the nature of the intermediate steps involved in the transformations of carbon dioxide and water, but also some clues as to the nature of the primary action of the light conversion. Earliest of this work is that of Ruben and co-workers using C^{11} (45, 46, 47, 48). They were able to confirm the suggestions arising out of the flashing light experiments that there existed a preliminary fixation of carbon dioxide not directly connected with the act of light itself and that this action was at

least partially reversible. Furthermore, by using isotopic oxygen, it was demontrated (49) that the gaseous oxygen produced in photo synthesis had its origin in the water molecule rather than in the oxygen of the carbon dioxide molecule. They were unable, however, to characterize the compounds into which the carbon dioxide was incorporated in the dark, primarily due to the short times available because of the short half-life of C^{11}.

All of the work so far described was done prior to the war. It ceased during the war, and Dr. Ruben lost his life in war service. One of the major activities of our laboratory is the continuation and extension of the work on photosynthesis using C^{14} with which tool we will not be subjected to the inconvenience of a short half-life. One of the first results to come from this laboratory, was the demonstration of the ability, at least of chlorella, to store reducing power during illumination in the absence of carbon dioxide.

The experiment was performed as follows. A sample of actively photosynthesizing fresh chlorella was divided into two parts. One part was kept in the dark in the presence of normal carbon dioxide, while the other part was illuminated in the absence of carbon dioxide, both for a period of about one hour. The two samples of chlorella were then exposed immediately (within a minute after the cessation of the illumination) to the same radioactive carbon dioxide in the dark. The sample which had been pre-illuminated was able to fix as much as ten times the amount of carbon dioxide as the one which had been kept in the dark. Furthermore, upon analysis of the fixed carbon in the sugar fractions of both samples, it was found that the dark sample had very little radioactivity (less than .1 %) in the sugar fraction (glucose). In the pre-illuminated sample, as much as 2 % of the total activity was found in the sugar fraction. It is thus apparent that not only is reducing power stored during illumination in the absence of substrate, but that this reducing power is capable of carrying subsequently administered carbon dioxide clear up to the state of carbohydrate.

We can now fill in a number of items on the originally blank sheet with which we defined the problem of photosynthesis.



It thus appears that the line representing the path to molecular oxygen has its origin in water and that the oxygen originally present in carbon dioxide must first pass through water on its way to gaseous oxygen. Furthermore, we can now say with certainty that the primary action of light absorption and its initial conversion into some form of chemical energy does not take place along the carbon dioxide-carbohydrate line. This confirms in a particular way, the very broad generalizations made by Van Niel (43) in which he indicated that the function of light was to make available active hydrogen from water which could then be used to reduce carbon dioxide in the same way as other forms of energy may be used by nonphotosynthetic organisms. Whether the photochemical reaction directly involves water or some complex of it at very nearly that energy level, or whether the photochemical reaction operates at some intermediate energy level, is still a moot question. In terms of the chart, the point of entry of the photon along the water-oxygen line is unknown. A more detailed analysis of the fate of the dark fixation of carbon dioxide is now in progress. The following two charts show the method of separation and the information as we have it to date on the distribution by compounds of this dark fixed carbon dioxide in both preilluminated and dark pretreated chlorella (see tables 2 and 3).

It is evident from these charts that there are still many gaps to be filled and some of them are very likely most important to an understanding of the processes involved. Beyond this, three of the isolated substances have been degraded and their radiocarbon distribution determined. The first of these is the succinic acid. This was degraded by an unequivocal series of reactions so as te enable us to specify the percentage of the radioactivity to be found in the carboxyl groups and in the methylene groups.

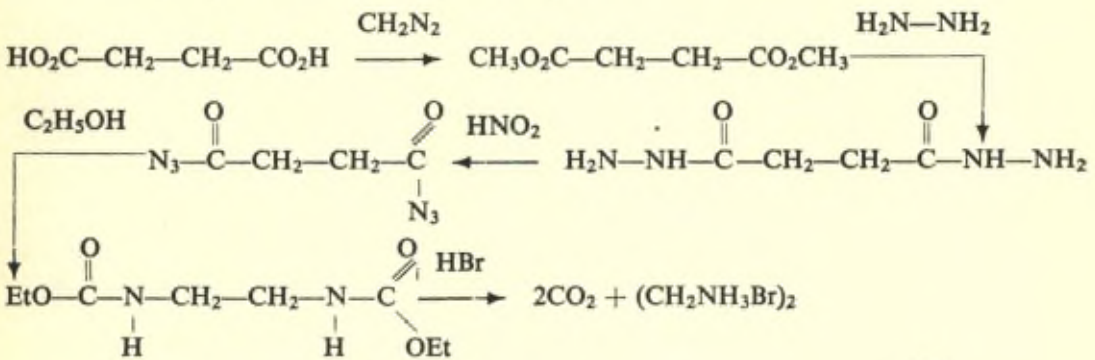


TABLE 2 (51).

METHOD OF FRACTIONATION.

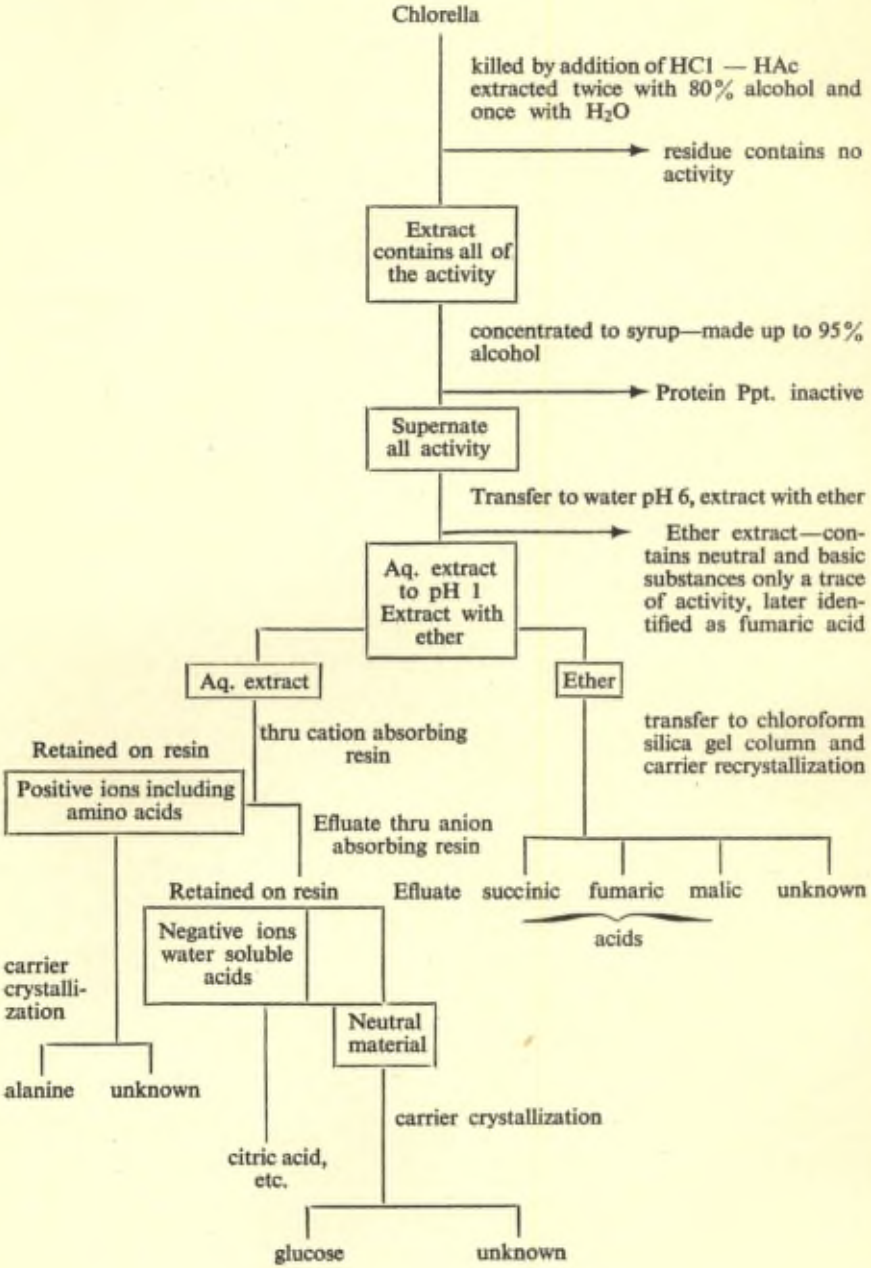


TABLE 3 (50).

Dark Fixation of CO_2 by Chlorella.

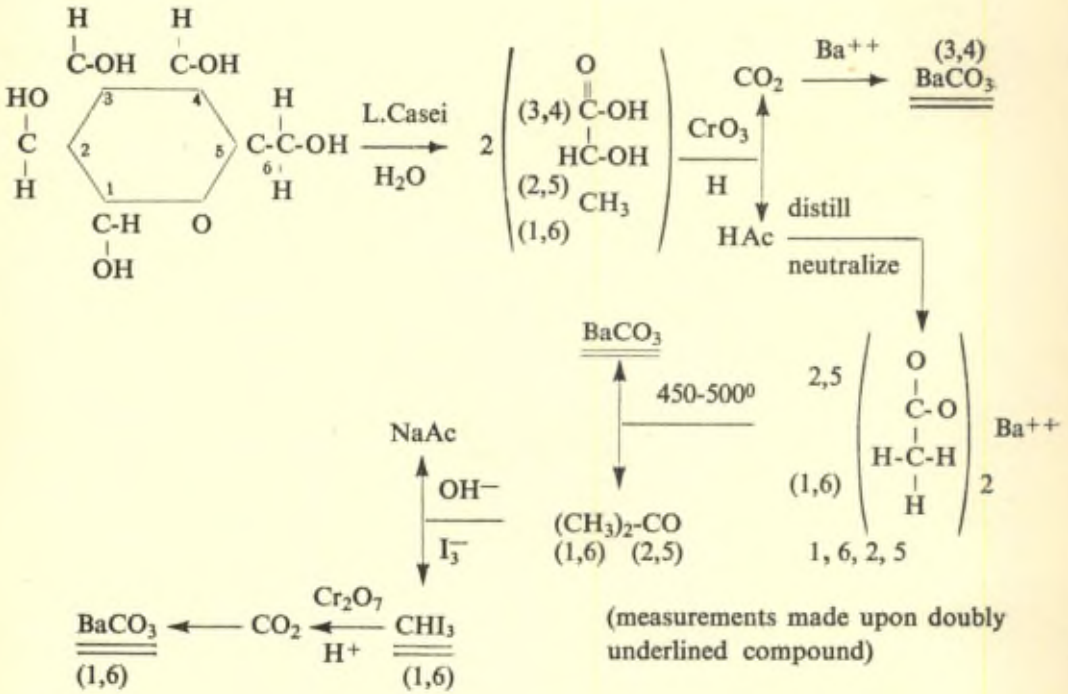
Preparation No.	I	II	III
Pretreatment	CO_2 in dark	Light in absence of CO_2	
Total (relative units)	1	5.5	10
Succinic acid (extracted by ether from pH 1)	70%	6%	.3%
Fumaric acid (extracted by ether from pH 1)	3%	1%	.3%
Malic acid (extracted by ether from pH 1)	—	6%	1.75%
Cationic substances (Alanine)	15%	30% (18%)	20% (7%)
Anionic substances	9%	10%	9%
Neutral (sugars and glucose)	<.1%	1.5%	8%
Unidentified (extractable by ether from pH 6)	2%	6%	(4%) .2%
Unidentified (extractable by ether from pH 1)	—	25%	2.7%
Lost (Unidentified—probably α- and β-keto acids)	—	—	50%

The succinic acid from the dark pre-treated algae (I) contained no detectable amount of activity in the methylene groups, whereas that from the light pretreated (III) had 2% of its activity in the methylene groups. The next compound to be degraded was the alanine. It was treated as shown in the following reaction scheme (52).

$$CH_3 - \underset{\displaystyle NH_2}{\overset{\displaystyle |}{CH}} - CO_2H \xrightarrow[2)\ \text{Dichromate}]{1)\ \text{Ninhydrin}} CH_3CO_2H + CO_2$$

The results although not yet as unequivocal as those with the succinic acid seem to indicate 90-95% of the activity in the carboxyl group with 5-10% in the remaining fragment (*). The glucose derived from this dark fixation has not itself been degraded, but some glucose prepared in a short photosynthetic experiment has been degraded according to the following scheme (53, 54).

(*) This is most likely in the α-carbon atom although experiments to determine this have not yet been completed.

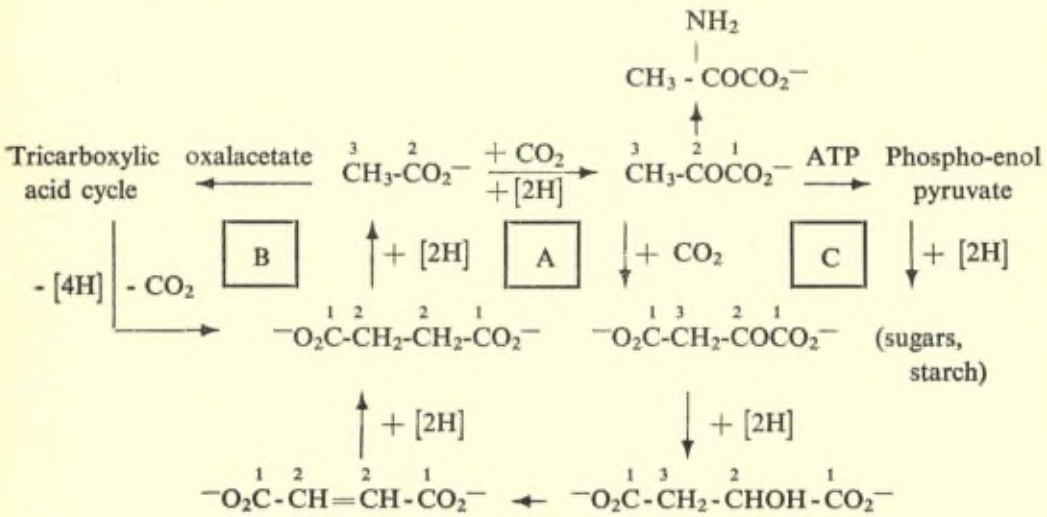


(measurements made upon doubly underlined compound)

The relative amounts of radioactivity found in the three pairs of carbon atoms were as 0.6 : 1.0 : 2.1 for (1,6) : (2,5) : (3,4). It may be of interest to report that this photosynthetic glucose represented some 35 % of all the radiocarbon incorporated into the plant (barley leaves) during the course of a one hour photosynthesis (55).

Before the significance for photosynthesis of the particular compounds into which the radiocarbon has been fixed can be described, it is necessary to arrive at some answer to the question of the possible relationship of these compounds to the respiration of the algae. Undoubtedly there exists in the algae, the same group of respiratory enzymes that has been found in animal tissues and it is conceivable that the fixation of carbon in the acids of the dicarboxylic cycle may have been achieved by virtue of the reversibility of the reactions of this cycle in much the same way that fixation into these compounds has been demonstrated in liver and muscle extracts and in rat livers. The first and most powerful argument that we are as yet able to present is the profound effect of preillumination upon the nature of the fixed carbon. The absence of any fixation in glucose in the

pre-dark treated algae and its presence to a very considerable extent in the pre-illuminated algae, both under anerobic conditions, seems to indicate that we are indeed dealing with the photosynthetic intermediates and not merely with the respiratory intermediates. If the latter were the case, one would expect at least some fixation of carbon dioxide in the glucose from the pre-dark treated algae—a situation which actually obtains for in vivo experiments with animals with certain isolated organs and in liver slices. It may well be that all or part of the succinic acid found in the pre-dark treated algae is the result of the reversal of the respiratory reactions; however, since most of the fixed carbon in the pre-illuminated experiments is to be found in other forms, it seems reasonable to suppose that these are indeed the intermediates in the photosynthetic reduction of carbon dioxide. It is possible to account for the results as they now stand by making use of a group of reactions each of which has been demonstrated previously either with animal tissue or bacteria (34). These constitute only an allowable scheme, most of which has been previously speculated upon. Undoubtedly, there are other allowable schemes which would account for the meager results so far available (51), but we can use this one as a working hypothesis for the conception of new experiments until such time as the data requires its modification or rejection.



REFERENCES

1. Nier and Gulbranson, *J. Am. Chem. Soc.*, **61**, 697 (1939).
2. Roberts, Thode and Urey, *J. Chem. Phys.*, **7**, 137 (1939).
3. Hutchinson, Stewart and Urey, *J. Chem. Phys.*, **8**, 532 (1940).
4. For several review articles on the subjects above see : *Prep. and Measurement Of Isotopic Tracers*, A. Symposium, Edwards Bros., *Ann. Arbor*, Michigan (1946).
5. Delsasso, White, Barkas and Creutz, *Phys. Rev.*, **58**, 586 (1940).
6. Barkas, Creutz, Delsasso, Fox and White, *Phys. Rev.*, **57**, 562 (1940).
7. Yost, Ridenour and Shinchara, *J., Chem. Phys.*, **3**, 133 (1935).
8. Solomon, *Phys. Rev.*, **60**, 279 (1941).
9. Delsasso, White, Barkas and Creutz, loc. cit.
10. Libby, private communication.
11. Reid, Dunning, Weinhouse and Grosse, *Phys. Rev.*, **70**, 431 (1946).
12. Ruben and Kamen, *Phys. Rev.*, **59**, 349 (1941).
13. Norris and Snell, *Science*, **105**, 265 (1947).
14. Ruben and Kamen, *Phys. Rev.*, **57**, 549 (1940).
15. Yankwich, *J. Chem. Phys.*, **15**, 374 (1947).
16. Dauben, Reid and Yankwich, in prep.
17. Henriques and Margnetti, *Ind. Eng. Chem. Anal. Ed.*, **18**, 417 (1946).
18. Libby, *Anal. Chem.*, **19**, 2 (1947).
19. Miller, *Science*, **105**, 123 (1947).
20. Janney and Moyer, in prep.
21. Yankwich, Rollefson and Norris, *J. Chem. Phys.*, **14**, 131 (1946).
22. K. Scott, priv. communication.
23. Yankwich, Norris and Huston, *Anal. Chem.*, **19**, 439 (1947).
24. Nahinsky and Ruben, *J. Am. Chem. Soc.*, **63**, 2275 (1941).
25. Nahinsky, Rice, Ruben and Kamen, *J. Am. Chem. Soc.*, **64**, 2299 (1942).
26. Huggett, Arnold and Taylor, *J. Am. Chem. Soc.*, **64**, 3043 (1942).
27. Dauben, Reid, Yankwich and Calvin, *J. Am. Chem. Soc.*, **68**, 2117 (1946).
28. Calvin and Lemmon, *J. Am. Chem. Soc.*, **69**, 1232 (1947).
29. Neville and Brown, presentend at New York Meeting of *Am. Chem. Soc.*, Sept. 1947.
30. Werkman and Wood, *Adv. in Enzym.*, **2**, 135 (1942).
31. Evans, *Symp. on Resp. Enzym.*, **197**, Madison, Wisc.
32. Stotz, *Adv. in Enzym.*, **5**, 153 (1945).
33. Buchanan and Hastings, *Physiol. Rev.*, **26**, 120 (1946).
34. Wood, *Physiol. Rev.*, **26**, 198 (1946).
35. Sonne, Buchanan and Delluva, *J. Biol. Chem.*, **166**, 395 (1946).
36. Buchanan and Sonne, *J. Biol. Chem.*, **166**, 781 (1946).
37. Shemin and Rittenberg, *J. Biol. Chem.*, **167**, 875 (1947).
38. Gurin and Delluva, *Proc. Fed. Am. Soc. Exper. Biol.*, **6**, 257 (1947).

39. Reid, *Science*, **105**, 208 (1947).
40. Tarver and Reinhardt, *J. Biol. Chem.*, **167**, 395-400 (1947).
41. Heidelberger and Jones, presented at Chicago Meeting of American Assoc. for Cancer Research, (19th, May 1947).
42. Van Niel, *Adv. in Enzym.*, **1**, 263 (1941).
43. Franck and Gaffron, *Adv. in Enzym.*, **1**, 199 (1941).
44. Rabinowitch, *Phytosynthesis*, Vol. I., Academic Press (1945).
45. Ruben, Hassid and Kamen, *J. Am. Chem. Soc.*, **61**, 661 (1939).
46. Ruben, Kamen and Hassid, *J. Am. Chem. Soc.*, **62**, 3443 (1940).
47. Ruben, Kamen, Perry, *J. Am. Chem. Soc.*, **62**, 3450 (1940).
48. Ruben and Kamen, *J. Am. Chem. Soc.*, **62**, 3451 (1940).
49. Ruben, Randall, Kamen and Hyde, *J. Am. Chem. Soc.*, **63**, 877 (1941).
50. Benson and Calvin, *Science*, **105**, 648 (20th, June 1947).
51. Benson, Bassham, and Calvin (in press).
52. Van Slyke, Dillon, Mac Fadyen and Hamilton, *J. Biol. Chem.*, **141**, 627 (1941).
53. Wood, Lifson and Lorber, *J. Biol. Chem.*, **159**, 475 (1946).
54. Aronoff, Barker and Calvin, *J. Biol. Chem.*, **169**, 459 (July 1947).
55. Aronoff, Benson, Hassid and Calvin, *Science*, **105**, 664 (1947).

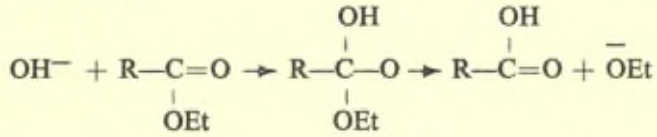
Discussion du rapport de M. Calvin

M. Ingold. — The immediate conclusions relevant to chemical mechanism which are derivable from isotopic tracer experiments such as Dr. Calvin has described are so clear that discussion can scarcely add anything to them; and therefore my remarks tend in a different direction, and are made because I am interested in the relationships that can be traced between different reactions in organic chemistry. I select three of Dr. Calvin's reactions *in vitro* for comments of this nature.

He has related that the predominant division of propionic acid between C_α and C_β in its oxidation by alkaline permanganate to carbonic and oxalic acits was not at first foreseen. It does, however, seem reasonable in the light of our empirical knowledge of the products of permanganate oxidations in general. The first tangible products in the oxidation of saturated compounds by alkaline permanganate are formed by the conversion of C-H into C-OH. An example is furnished by Lawrence's well known synthesis of terebic and terpenylic acids by oxidation of *iso*propyl-succinic and -glutaric acids. Analogy, with some support from theory, suggests that the α-and β-positions of propionic acid might be comparatively vulnerable to this type of attack, and that therefore glyceric acid might be an important intermediate. We know that the oxidation of olefins by alkaline permanganate involves first, the production of glycols, and then, fission between the hydroxyl bearing carbon atoms. Glyceric acid is a glycol and its fission could thus account for the result which the tracer experiment has actually established.

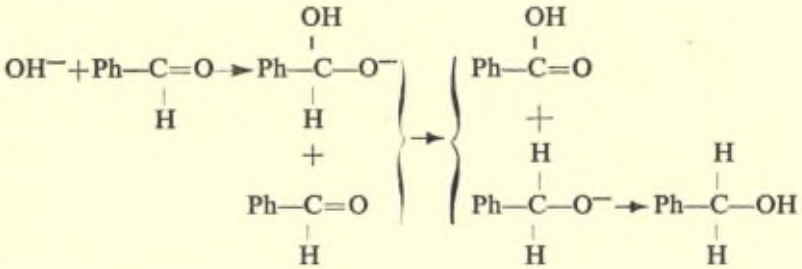
Dr. Calvin has described the experiments which were made to establish that no exchange of an amido group between an amido $R—CONH_2$ and an acid RCOOH took place under conditions which were neither strongly alkaline nor strongly acid. The negative result is reasonable, because the separation of the groups ($.NH_2$ and .OH) which might have exchanged, normally requires either the addition of a strongly nucleophilic reagent, e. g. OH^-, to the carboxyl carbon atom, or a proton uptake, followed by the addition, at the carboxyl carbon atom, of a much more weakly nucleophilic,

reagent e.g. OH_2, which the proton uptake has enabled the molecule to accept. As examples, one might cite the alkaline and acidic modes of hydrolysis of carboxylic esters, and of amides and oxygen exchange between carboxylic acids and alkaline or acidic heavy oxygen water, e.g.



Here are examples of a common underlying process of great generality, and it is difficult to see how this basic process could furnish means for the hypothetical amide-acid exchange, where none of the requirements which are found necessary in all its other applications, are fulfilled.

Connected with this matter is the alkali-induced conversion of phenylglyoxal to mandelic acid, which is obviously only an internal form of the Cannizzaro reaction, typically illustrated by the alkali-induced transformation of benzaldehyde into benzyl alcohol and benzoic acid. I think that the Cannizzaro reaction is a heterolytic process, involving preliminary uptake of the hydroxide ion (as in the alkaline hydrolysis of esters) and the subsequent loss of a hydride ion (just as the ester loses an alkoxide ion). The hydride ion H^- is, however, not stable enough to be simply ejected into the solution: it requires some acceptor and there can be no better acceptor than a carbonyl group, with its known affinity for cyanide ions, bisulfite ions, and, indeed many kinds of nucleophilic anions. Thus the Cannizzaro reaction may be pictured as a hydride ion transfer :



The picture fits Bonhoeffer's experiment in deuterium water, which showed that the transferred hydrogen never gets into the water. It fits the known reducing properties of alkaline aldehydes. However

the hydride ion transfer is more than a reduction in the general sense of electron addition : it is a nucleophilic anion addition in line with all that is most typical is carbonyl reactivity. The reducing agent is « tailor made » for the carbonyl group.

M. Guében. — I would like to ask Professor Calvin how he determines the self-absorption correction curve?

M. Calvin. — The self-absorption correction is determined by preparing a number of plates from the same sample of $BaCO^3$, each having a different weight and covering as wide a range as is desired. These plates are then counted and the apparent specific activity can be plotted against the density of the mounting. Extrapolation to zero weight would then give a satisfactory 100% point and the correction scale made from that. The self-absorption correction curve as obtained for $BaCO^3$ should not be used for other substances since the form of the solid and its distribution on the plate is not the same.

M. Rittenberg. — I believe Doctors Ruben and Kamen suggested that the first step in the fixation of CO^2 is condensation to yield a compound having a molecular weight of about 1,000 while your scheme seems to introduce CO^2 only by carboxylation of acetic and pyruvic acids. Would you care to comment on this?

M. Calvin. — Ruben and Kamen were measuring only tracer amounts of material and it is likely that their material was associated with dissolved protein or other high molecular weight particles thus producing the effect of a large molecular weight.

Actually all of the chemical characters reported by them can be accounted for by the mixture of compounds which we have found.

M. Rittenberg. — I should like to comment on Dr. Calvin's remark that C^{14} will largely supersede C^{13}. This is of course largely true. However, it should not be interpreted as meaning that C^{13} will have no further use. Actually much use will be made of both C^{13} and C^{14} where two carbon isotopes will be used to label two different carbon atoms in the same molecule.

M. Calvin. — I agree with Dr. Rittenberg completely, and am grateful for his calling the attention of the conference to what might be misconstrued. In fact there are some experiments in which

only C^{13} could be used. For example in the isomerization of butane to isobutane by such catalysts as $AlBr_3$ the question arises as to whether the reaction is intra or inter molecular. This could be answered by carrying out the reaction on a mixture of butanes, some containing only C^{12} and some containing only C^{13}, followed by a mass spectrometer analysis of the isobutane formed. If it consists only of all C^{12} isobutane and all C^{13} isobutane the reaction does not involve the transfer of methyl groups from one molecule to another. If however there is present isobutane containing both C^{12} and C^{13} then there has accured such a transfer. It should be noted that here the success of the experiment depends upon the peculiar ability of the mass spectrometer to determine the weight of whole fragments and not merely upon an analysis of isotopic content.

M. Backer. — The method described by Dr. Calvin for the study of rearrangements of carbon compounds has greatly impressed me. With regard to the formation of two different propenes, by dehydration of labeled propyl alcohol, I should be inclined to ascribe it to the shifting of the double bond under the catalytic influence of the sulfuric acid or the aluminium oxyde. I agree with Professor Ingold that the observed oxydation of propionic acid (chiefly α-β- fission) is interesting, but not surprising.

M. Karrer. — I should like to suggest that acrylic acid may be one of the intermediates in the oxydation of propionic acid.

M. Calvin. — In view of the continued interest in the propionic acid oxydation and the remarks of Professor Ingold, I feel it necessary to point out that it has already been shown that α-hydroxy and β-hydroxy propionic acids are not the only intermediates, if indeed they are intermediates at all, in the oxydation of propionic acid by alkaline permanganate. These experiments have been brought up not only for their interest to theoretical organic chemists but especially to illustrate the requirement of demonstrating directly and unequivocally the validity of any degradation procedure for determining the isotope distribution in a biologically formed compound, and this notwithstanding the most plausible guesses which the theoreticians may make. Another example of this is the supposition that all the acid dehydrations of n-propylalcohol to propylene would result in an aquilibration of the double bond between

both ends of molecule. This has turned out to be the case with H_3PO_4 but not completely so with Al_2O_3.

M. Briner. — Dans le remarquable rapport présenté par le Dr Calvin on a surtout discuté les résultats concernant le mécanisme des réactions organiques, mais ceux qui portent sur la photosynthèse méritent aussi de retenir l'attention.

On sait en effet combien les théories photochimiques énoncées jusqu'à présent pour expliquer le mécanisme photochimique de l'assimilation chlorophyllienne sont peu satisfaisantes. Or les recherches faites à l'aide de l'isotope radioactif du carbone ont conduit à un processus photochimique primaire bien déterminé. A-t-on soumis ce processus au calcul en se basant sur les relations d'équivalence photochimique (unissant l'énergie de la réaction au quantum de la lumière active)? Et dans cet ordre d'idées, a-t-on utilisé une lumière simple au lieu de la lumière blanche pour procéder à des vérifications?

M. Calvin. — No information is as yet available on the quantum yield of the stored reducing power, nor on its dependence upon wave lenght.

M. Courrier. — Dans son intéressant rapport, M. Calvin fait allusion à des études entreprises sur les stérols et les hormones à l'aide du carbone radioactif.

Peut-il nous donner des précisions sur les résultats obtenus dans ce domaine?

M. Calvin. — There is much work in progress on the metabolism of vitamines, hormones, drugs, etc., but most of it has just reached the stage of achieving the synthesis of the labeled material. We can look forward to the biological results of these experiments in the coming few years. Some of the substances which I know to be under investigation in the United States are, certain sterols labeled in ring A, diethyltilbestrol, barbiturates, carcinogens, etc.

The use of N^{15} and D for the Study of chemical Processes in the living Cell

by

M. D. RITTENBERG

From the point of view of the chemist the living cell is a chemical machine which by oxidation of its dietary materials obtains the energy it requires for its special functions. The mechanism differs from all others known in as much as it can also use the same dietary materials for the building and repair of the metabolic machine. The study of these systems is rendered difficult by two factors. First the composition of the diet is quite similar to that of the cell itself, so that the moment a dietary component enters the cell it mixes with the same compound already present and the investigator loses trace of the substance. Second it is in general not possible to simplify the experimental conditions by holding all variables constant and studying the effect of the variation of a single factor; the cell must be studied as it is found in the natural state.

Various attempts had been made in the past to label organic compounds so that they could be traced through the living cell. Relatively little success was obtained by these methods for the labels employed so changed the properties of the compound being investigated that one could never be certain that the normal metabolism was being investigated.

With the discovery of isotopes a method of labeling compounds became available which did not appreciably alter the chemical or physiological properties of the labeled compound. Indeed compounds containing the heavier isotopes are not foreign to the living cell. Thirty-seven of each ten thousand molecules of naturally occuring glycine contain an atom of N^{15}. While the glycine molecules which are used for the study of the reactions of this compound in the living cell contain a much higher proportion of N^{15} this change is quantitative but not qualitative in nature.

The first application of the isotope technique for the study of processes in the living cell was made by de Hevesy in 1923. He investigated the transport of lead in a plant. The roots of the plant were immersed in a solution which contained a radioactive isotope of

lead. By determining the appearance of radioactivity in the various parts of the plant he was able to detect the appearance of the lead.

In 1931 Urey discovered a heavy isotope of hydrogen and in the next few years developed practical methods for the concentration of the heavier isotopes of hydrogen, carbon, nitrogen, oxygen and sulfur. It is from just these elements that the greatest number of compounds existing in the living cell are constructed. The researches of this physical chemist thus provided the tools for the investigation of the chemical processes of the living cell.

In 1934 the late Dr. R. Schoenheimer and I initiated our investigations on the fate of the fatty acids in the mouse. Fatty acids were prepared in which one or more of the hydrogen atoms were replaced by deuterium. The transport and deposition of these labeled fatty acids was followed by deuterium analysis. As concentrates of the heavier isotopes of nitrogen and carbon became available the study of the metabolism of the amino acids was undertaken.

Such a great volume of work has been published on the application of the isotope technique to biochemistry in the past years that I must of necessity restrict myself to but a few examples which illustrate the scope and power of the method. For the purpose of classification the applications of the isotope technique may be divided into four categories :

a) The study of transport problems;
b) The study of the conversions of one compound to another;
c) The study of the mechanism of the conversion reactions;
d) The study of the rates of reactions.

I shall choose my illustrations from problems studied in my laboratory not because of their greater inherent importance but because the data is more readily available to me than those of other investigators in this field.

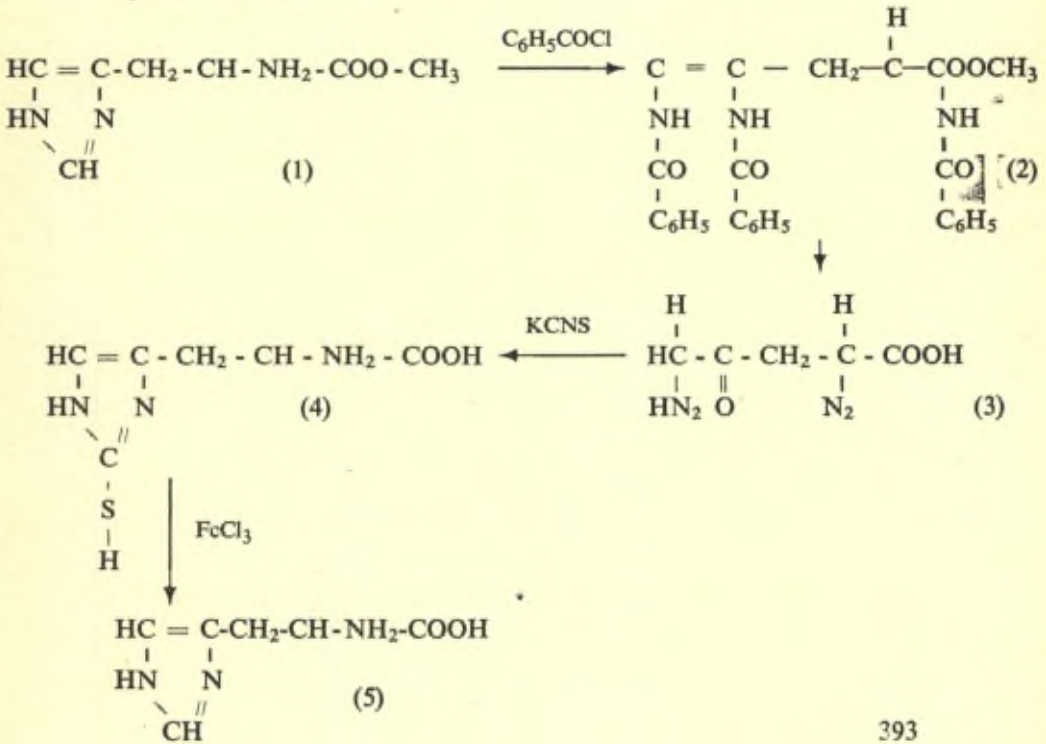
It is of the greatest importance that these investigations can be carried out in the normal, intact organism, under conditions which do not depart appreciably from the normal state.

A preliminary step to the investigation of the biological problem is the preparation of the labeled compound. This subject has already been discussed for the case of deutero compounds by Dr. Langseth and for C^{14} and C^{13} labeled compounds by Dr. Calvin. The synthetic problems which arise in the preparation of N^{15} labeled compounds are in general quite simple. In general a slight change of classical synthetic procedures yield the required compound. The

synthesis must be modified so as to give a maximum yield based not on the carbon chain employed but on the heavy nitrogen. As an illustration consider the changes required for the synthesis of alanine. Alanine is usually prepared by the treatment of α-chloropropionic acid with a large excess of concentrated ammonia (50 to 100 moles).

Due to the relative scarceness of isotopic ammonia such a procedure is not very practical. However ammonia can be converted to potassium phthalimide in good yield (~ 85%) which on condensation with the ester of chloropropionic acid and subsequent hydrolysis (yield ~ 95%) yields alanine in an overall yield of more than 80%. There is a second quite general reaction which can be employed for these synthesis. The α-keto acid if available, gives by reductive amination ($NH^3 + H_2 + Pd$ catalyst) the desired amino acid. This pyruvic acid can be converted in fair yield (~ 60%) to alanine In both these examples the N^{15} which is not incorporated into the amino acid can be recovered undiluted from the mother liquors.

There is one synthetic method which has no analogy in the field of classical organic chemistry. The starting material in this case is the compound which is finally to be labeled. An example is given below :—



l (+) Histidine methylester (I) is intensively benzoylated to yield the tribenzamido $\Delta\gamma$-pentenoic acid (II). A two step hydrolysis yields the α, δ diamino, γ-keto valeric acid (III). This condenses with KCNS, prepared with labeled NH_3, to yield thiolhistidine (IV) which is readily oxidized to histidine (V). The overall yield of the carbon skeleton from histidine back to histidine is quite poor (3-5 %) but the yield based on NH_3 is excellent (> 60%). If a complex synthetic procedure is required for the synthesis of a labeled compound it is important that as in this case the valuable isotopic material be introduced near the end of the reaction route. Not only do procedures such as the one described give in general high yields but they also in many cases yield the optically active isomer.

TRANSPORT PROBLEMS.

The study of transport problems is in general technically very simple. The labeled compound is administered to the animal by some convenient route and at some later time the same compound is isolated from one or more of the tissues. Analysis of the isotope content of the compound isolated directly gives the amount of the administered compound which has been transported to the tissue. Such investigations have been of the greatest value for the measurement of the rate of transfer of water, labeled with D_2O, and of inorganic ions, labeled with radioactive elements. Some years ago Dr. K. Bloch and I injected into a dog cholesterol labeled with deuterium. After 3 days cholesterol was isolated from various tissues. The isotope concentrations are shown in table I.

TABLE I

Atom % Excess D in tissue Cholesterol after intravenous Injection of Cholesterol containing 4.16% D

Organ	% D
Red blood cells	0.31
Liver	0.71
Kidney	0.31
Heart	0.39
Spleen	0.46
Pancreas	0.25
Brain	0.00
Spinal cord	0.00
Lung	2.00

This data combined with knowledge of the total cholesterol content of the various organs directly gives a quantitative measure of the amount of injected cholesterol which has been deposited in the various organs of the dog. It is striking that none of the cholesterol has been deposited in the central system despite the fact that this organ, as is well known, is very rich in cholesterol. The extension of this technique to other problems is so obvious that no further discussion is required.

CONVERSION REACTIONS

One of the striking characteristics of the living cell is the independence of its composition on rather large variations of its diet. Further, as not all of the compounds which the cell requires are always present in its environment, it is obvious that the cell must have chemical mechanisms available to synthesize the required compounds from those present in its diet. It is in the discovery of the conversion of one compound into another that the isotope technique has had its greatest successes. In principle the procedure is simple. A compound is prepared with an abnormal isotope abundance in one or more of its atoms and fed to an animal. Any compound subsequently isolated from the tissues which contains an abnormal isotope abundance has been derived from the compound administered. The more closely the isotope concentration of the isolated compound approaches that of the one fed, the more direct is the conversion route.

One such non essential compound for the rat is the amino acid glycine (CH_2NH_2COOH). It was known that the rat could synthezise this compound. Indeed an immature rat can grow on a glycine free diet. To discover the precurser of glycine it would be necessary to feed a labeled suspected precurser and isolate some glycine from the rat tissues. Since biochemistry is but a sub-division of an empirical discipline, organic chemistry, there are no theoretical principles to guide us in our choice of the few precursers which can practically be chosen for testing. However, some reasonable guesses can be made. It is known that the treatment of serine (α-amino β-hydroxy propionic acid) by hot aqueous alkali leads to the decomposition of serine with the formation of glycine.

My collaborator, Dr. O. Shemin, took up the study of the origin of glycine in the rat. He administered to rats a series of compounds labeled with N^{15}. He could of course have subsequently isolated glycine from the tissues. He, however, took advantage of a well known reaction of the rat. The administration of benzoic acid to this animal leads to its excretion in the urine in the form of benzoyl-glycine, hippuric acid. In table II is given the data obtained from such experiments.

TABLE II

Utilisation of various compounds for glycine formation in the rat.

(All values given are calculated on the basis that the test compound administered contained 100 atom % excess N^{15}.)

Compound administered	N^{15} concentration in isolated hippuric acid Atom % excess
Glycine	35.7
NH_3	0.3
l-serine	18.2
d-serine	0.6
l-glutamic acid	2.2
d-glutamic acid	0.1
dl-aspartic acid	1.6
l-alanine	1.1
dl-proline	2.0
l-leucine	0.8
Ethanolamine	0.3

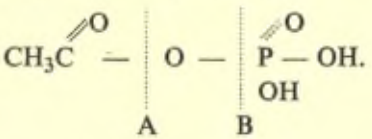
The results show that dietary glycine supplies about one third of the glycine used for conjugation with benzoic acid. Ammonia is a very poor source of the nitrogen of the excreted hippuric acid as are also all other amino acits tested and the unnatural isomer of serine. The natural isomer, l-serine however, is almost as efficient a source of glycine for hippuric acid formation as is glycine itself. Clearly we are here observing a rather rapid conversion of serine to glycine. Further since we have observed it in the normal intact animal it must be a normal process. As has been observed in the study of other conversions the reaction serine to glycine occurs

even though the diet contains an adequate supply of glycine. In general if the living cell has a mechanism for the formation of a certain compound the synthesis occurs regardless of the amount of the compound present in the diet.

The example just cited is rather simple because of the simplicity of the compounds involved. More complex cases have been studied. Thus the administration to rats of acetic acid labeled either with deuterium in the methyl group or C^{13} in the carboxyl group results in the formation of labeled cholesterol, $C_{27}H_{46}O$(8). The intermediate steps in this complex condensation are completely unknown. This utilization of acetic acid for the formation of cholesterol takes place not only in the whole animal but also in surviving liver slices. Incubation of these with deuterio acetic acid results in the formation of deuteriocholesterol. It is quite striking that contrary to the oft raised doubts to the value of deuterium as a label for organic compounds, the C-D bond in acetic acid is sufficiently stable to remain unbroken through the many reactions which must intervene between acetic acid and the end product, cholesterol. Indeed experiments in vivo show that even deuterium in positions adjacent to a carbonyl group, as in pyruvic acid and cholestenone, are not rapidly removed by enolization.

REACTION MECHANISM

The future importance of the isotope technique for the study of the mechanism of organic reactions is quite obvious. The elegance of this approach is illustrated by the results obtained simultaneously by Dr. Calvin and his collaborators and Dr. Shantz and myself on the mechanism of the Willgerodt rearrangement. In my laboratory we have been primarily interested in the mechanism of biochemical reactions. Dr. Bentley has during the last year studied the mechanism of hydrolysis of acetyl phosphate

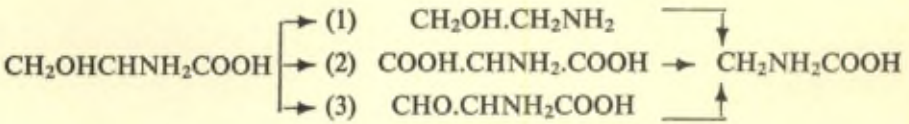


This compound in aqueous solutions rapidly hydrolyses to acetic and phosphoric acids. If, as has been suggested, acetyl phosphate is an acetylating agent the hydrolytic cleavage should occur between

the carbon and oxygen atoms (at A). If, on the other hand, the split takes place between the phosphorus and oxygen atom (at B) the compound will act as a phosphorylating agent. If the hydrolysis takes place in water labeled with O^{18} the point of cleavage will be indicated by the appearance of 0^{18} in one or the other of the resulting acids. Actually the split can occur at either A or B, depending on the *p*H of the medium. In alkaline solutions it occurs at A while in neutral and acid solutions it occurs at B. Depending therefore on the *p*H acetyl phosphate can act either as an acetylating or a phosphorylating agent.

As previously stated the isotope technique has made some of its most important contributions to biochemistry in its discovery of the precursers of many non-essential dietary components of the cell.

I have already indicated that, glycine, one of these non-essential dietary components, is made from serine. There are several plausible mechanisms to explain this reaction. Three are given below.



In mechanism (1) the serine is decarboxylated to ethanolamine which is then oxydized to glycine. In this process the β-carbon atom of serine becomes the carboxyl group of glycine. In mechanism (2) the serine is oxidized to amino-malonic acid which is then decarboxylated to glycine. In this process half of the carboxyl group of glycine are derived from the β atom of serine, the other half from the carboxyl group of serine, for the intermediate amino malonic acid is a symmetrical molecule. In mechanism (3) the β-carbon atom is oxidized to an aldehyde which by a reverse aldosation yields glycine. In this process the β atom of serine is lost. In order to determine which mechanism was operating, Dr. Shemin synthesized a serine molecule in which the animo group was labeled by N^{15} and the carboxyl carbon with C^{13}. Glycine isolated from rats fed this serine contained both N^{15} and C^{13}. This eliminates mechanism (1). The quantitative results show that relatively as much C^{13} and N^{15} is present in the glycine. This eliminates scheme (2) since in this case only half as much C^{13} as N^{15} should have been

present in the glycine. Scheme (3) or some variant of it must be the correct one.

This experiment was chosen because it illustrated the great advantage of doubly labeled molecules. Indeed it seems likely that in the future most researches will start with doubly or even more highly labeled molecules. Dr. Sprinson in my laboratory has shown by employing a leucine molecule which was labeled by N^{15} in the amino group and by deuterium in the α, β and γ positions that the reversible deamination-reamination is accompanied by the loss of the N^{15} and the deuterium in the α position but not deuterium in the γ and β positions. It is to be noted that investigation of such reaction mechanism is possible only by the use of an isotope of hydrogen.

REACTION RATES.

In order to evaluate the importance of a reaction in the cellular economy it is necessary to know something of its rate. Unfortunately but little kinetic data exist for the reactions occuring in the living cell. The isotope technique has begun in the past few years to supply such kinetic data.

The simplest way to study the rate of formation of one of the constituents of the living cell is to abruptly increase the deuterium concentration of the cellular water by injection of D_2O and then to determine the rate of increase of the deuterium concentration in the carbon bound hydrogen atoms of the organic compound. Nothing need be known of the mechanism of the synthetic reaction and since water (and D_2O) rapidly distributes itself uniformly through the entire system, the problem which sometimes arises with the use of other labeled compounds namely diffusion across cell membranes does not exist. Further because of the uniformity of distribution, the isotope concentration available at the cell for synthetic reactions can be determined by isotope analysis of water obtained from any convenient part of the animal (blood, urine, respiration, etc.). By this technique the rate of formation of the fatty acids and cholesterol have been measured (9).

For the determination of rates of reaction of the amino acids and the proteins the heavy isotope of nitrogen has some advantages. If a small amount of a labeled amino acid is given to an animal, about one fourth of the amino nitrogen is excreted in the urine

within 24 hours. The remainder is very slowly excreted over a period of months. Since the animal does not have a large reservoir of amino acids, the remainder of the amino acid fed, must have been incorporated into the tissues proteins. As in an adult animal in nitrogen equilibrium, the total amount of protein is nearly constant, the incorporation of the dietary amino acid must have gone parallel to a liberation of an equal amount of the same amino acid; peptide bonds must have been ruptured and reformed. From a chemical stand point this is equivalent to saying that protein has been degraded and resynthesized. Such experiments do not give direct data on the rates of these reactions; they merely suggest that the reactions are rapid.

In our laboratory we have attempted to evaluate the rates of these reactions in both the rat and the human (10). The technique is as follows. The experimental animal is fed for a few days with glycine labeled with N^{15}. After cessation of feeding of the labeled compound, samples of one of the tissue proteins are obtained and analysed for N^{15}. In the case of the rat the liver proteins were analysed. The isotope concentration in the liver proteins rises during the period in which labeled amino acid is being administered and falls after that. From the rate of fall of the N^{15} concentration it can be estimated that about 10% of the rat liver proteins are daily being degraded and resynthesized. Approximately the same rate is found for the formation and degradation of the human plasma protein.

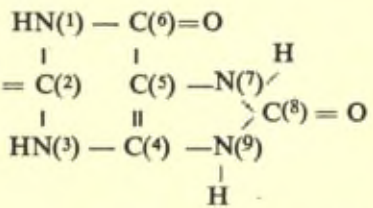
What is the purpose of this apparently useless synthesis and degradation of the structural components of the living cell? All of the complex compounds which form the living cell, the fats, the proteins and the carbohydrates are thermodynamically unstable with regard to hydrolysis to their constituent building blocks, the fatty acids, the amino acids and the monosaccharides. Further these latter compounds in the presence of oxygen are unstable with regard to their oxidation products. Most living cells are richly endowed with enzymes to catalyse these degradative reactions. Under the influence of these catalysts the structural components of the cell disintegrate. To compensate for this erosion another set of enzymes rebuild the structural components as fast as they are broken down. The secular stability of the living cell results not from an absence of reactions but from the detailed balancing of the synthetic and degradative reactions. The discovery and description of the continuous breakdown and reformation of the structural

elements of the living cell, which has been observed for the carbohydrates and lipids, as well as the proteins is the most important contribution the isotope technique has made to biochemistry.

In developing this new viewpoint the isotope technique has solved some problems but it has simultaneously developed a whole new set of unsolved ones. Fortunately it appears that the method is capable of attacking the problems it has raised.

In the last two years our laboratory, taking advantage of the fact that the isotope technique is capable of studying the normal organism, has begun the study of human biochemistry. Administration of labeled glycine to humans permitted us to determine the rate of plasma protein formation. As in the case of the liver proteins of the rat about 10% of human plasma proteius are daily being degraded and resynthesized.

From these experimental subjects we have also obtained samples of urinary-uric acid (11). Uric acid is in man the end product of purine metabolism.



The uric acid excreted, during the period of feeding of labeled glycine and for a short period there after contained considerable concentrations of N^{15}. Since uric acid contains four nitrogen atoms it was desirable to know the distribution of N^{15} in the individual positions. Hydrolysis of uric acid with concentrated HCl results in the formation of NH_3 and glycine. Previous investigations have clearly demonstrated that the ammonia is derived from the nitrogen atoms at positions 1, 3 and 9 while the glycine nitrogen is derived from the atom at position 7.

Analysis of the fragments resulting from the hydrolysis of uric acid show that the N^{15} concentration in the 7 position is far higher than that in positions 1, 3 and 9. It is quite clear from this data that at least the amino group of glycine is specifically utilized for uric acid synthesis. Shortly prior to this work Sonne, Buchanan and Delluva (12) at the University of Pennsylvania had found that in the pigeon the carbon atom in position 4 of uric acid is derived

from the carboxyl group of dietary glycine. It thus appears that the glycine molecule is employed for the atoms in position 4, 5 and 7 of uric acid. They have also found the sources of the carbon atoms at position 2, 6 and 8. Since the nitrogen atoms at positions 1, 3 and 9 probably arise from ammonia we now know the origin of each atom of uric acid. Similar investigations of other complex constituents of the living cell should yield similar results.

BIBLIOGRAPHY

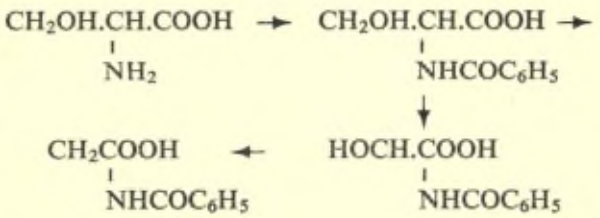
1. de Hevesy, G., *Biochem. J.*, **17**, 439 (1923); *Biochem. Ztschr.*, **173**, 175 (1926).
2. Urey, H. C., Buckwedde, F. G. and Murphy, G. M., *Phys. Rev.*, **39**, 164 (1942); **40**, 1 (1932).
3. Schoenheimer, R. and Rittenberg, D., *J. Biol. Chem.*, **111**, 163 (1935); **111**, 169 (1935); **111**, 175 (1935).
4. Schoenheimer, R. and Ratner, S., *J. Biol. Chem.*, **127**, 301 (1939).
5. Tesar, C. and Rittenberg, D., *J. Biol. Chem.*, **170**, 35 (1947). — Bloch, K. and Schoenheimer, R., *J. Biol. Chem.*, **138**, 167 (1941).
6. Bloch, K., Berg, B. N. and Rittenberg, D., *J. Biol. Chem.*, **149**, 511 (1943).
7. Shemin, D., *J. Biol. Chem.*, **162**, 297 (1946).
8. Bloch, K. and Rittenberg, D., *J. Biol. Chem.*, **145**, 625 (1942); **162**, 441 (1946).
9. Schoenheimer, R. and Rittenberg, D., *J. Biol. Chem.*, **121**, 235 (1937).
10. Shemin, D. and Rittenberg, D., *J. Biol. Chem.*, **153**, 401 (1944).
11. Shemin, D. and Rittenberg, D., *J. Biol. Chem.*, **167**, 875 (1947).
12. Sonne, J. C., Buchanan, J. M. and Delluva, A. M., *J. Biol. Chem.*, **166**, 395 (1946).

Discussion du rapport de M. Rittenberg

M. Calvin. — Have you any idea what becomes of the hydroxymethylene group of the serine when the serine is converted into glycine?

M. Rittenberg. — Our observations do not provide an answer to that question.

M. Karrer. — M. Rittenberg a signalé que la serine est transformée dans l'organisme animal en glycine et il a retrouvé celle-ci sous la forme d'acide hippurique. Il est possible que la réaction se développe de la façon suivante : la serine est transformée d'abord en benzoylalanine, dont l'oxydation, d'abord suivie d'une réduction donnerait naissance à l'acide hippurique :



M. Rittenberg. — The benzoic acid does not seem to be essential in the conversion of serine to glycine since the same results can be obtained in experiments in which no benzoic acid is fed.

M. Bigwood. — Professor Calvin and Professor Rittenberg both mentioned that they could not survey the entire literature covering the field of research in metabolism with the use of tracers, but that they would point out the essential facts. There are however two very important problems in animal metabolism which are worthwhile raising here.

The first one concerns the metabolism of phenylalanine. Most of the experiments have to do with the fate of this amino acid. Pro-

fessor Calvin referred to its conversion into adrenalin, using β-carbon labeled phenylalanine. There are also Moss and Schoenheimer's observations with deuterophenylalanine showing its conversion into deuterotyrosine. But the reverse reaction is a most interesting one to investigate, in the light of the very remarkable findings of Rose in Chicago based on nitrogen balance experiments with mammalians fed practically exclusively on known mixtures of pure aminoacids as far as their protein supply is concerned; their observations point to the fact that phenylalanine is an essential amino acid in so far that it must be found in the diet in order to maintain nitrogen balance whatever the total nitrogen intake may be, whereas tyrosine is not an essential one. Indeed it is interesting therefore to see that deuterium in labeled phenylalanine can be traced in tyrosine, but it would be a most valuable confirmation of Rose's findings to be able to show that labeled carbon or nitrogen in tyrosine is *not* to be found in phenylalanine. Could Professors Calvin and Rittenberg tell us whether any attempt has so far been made to clarify this very essential point?

The other question I want to raise concerns the breakdown of long chain fatty acids, according to the well known Knoop's theory of β oxydation. Knoop's fundamental observation at the beginning of this century was made on animals fed with fatty acids chemically « labeled » in such a way that the animals were fed with phenyl derivatives of the fatty acids (a C_6H_5 group substituted to a hydrogen atom in the terminal CH_3 group of the fatty acid, hence in the « ω » position in the carbon chain). He found that when the fatty acids contained an even number of C atoms, the phenyl group was found in the urine in the form of phenylacetic acid (or rather this acid conjugated to glycine), whereas if the fatty acid contained an odd number of C atoms, benzoic acid was excreted instead of phenylacetic (again in the conjugated form with glycine). This was explained in the light of successive β oxydations splitting off, each time two carbon atoms at the time, presumably in the form of acetic acid, which was supposed to be easily and rapidly burned to CO_2 and H^2O.

Natural edible fats are formed of fatty acids containing an even number of C atoms. According to this β-oxydation process they will pass through the 4 carbonatom stage, namely butyric acid and a further step of β oxydation will lead to acetylacetic acid, a trace of which is found in normal urine together with its spontaneously

split products acetone and CO_2. When large amounts of fats are incompletely metabolised, acetylacetic acid, β-hydroxybutyric acid and acetone are accumulated and passed through the kidney, a condition which is referred to under the name of ketosis.

In the case of a fatty acid with an odd number of C atoms, one of the last stages of the same process of β-oxydation corresponds to propionic acid (instead of butyric) and this moves in the body towards the building up of sugar and glycogen; it is glycogenic instead of ketogenic. The validity of Knoop's theory was questioned however, since one did not know whether the living cell handled fatty acids in the same way it used up its phenyl derivatives.

A few years later, Embden found in experiments on perfused liver, that fatty acids (non phenylated) were ketogenic when they contained an even number of C atoms; whereas they were not when they had an odd number of them, in which case they were glycogenic. This finding was considered to be a definite confirmation of Knoop's theory. Very much later, however, things began to look less clear. According to the theory, any ketogenic fatty acid was considered to be able to yield one molecule of acetylacetic acid (or one of its derivatives) per molecule of fatty acid metabolised, and no tmore. But, Quastel and his colleagues working on liver slices in Warburg's apparatus, and other workers too, were able to confirm most of the previous findings but they also found that the yield in ketonbodies per fatty acid metabolised was greater than *one* to *one*. Moreover, it was found that rather short chain fatty acids with an odd number of C atoms, such as valeric or heptanoic acids were both ketogenic and glycogenic at the same time. Then came the observations of Weinhouse, Medes and Floyd in 1943, who labeled the carboxyle group of octanoic acid with ^{13}C and found the label both on the carbonyl and the carboxyl of the acetylacetic acid formed. This indeed was unexpected in the light of the original conception of Knoop and Embden; it helped, however, to understand the more recent findings referred to above; it confirms indeed the β oxydation theory although its interpretation must now be given rather differently, in so far that the 2 carbon splits (presumably acetic acid or some similar compound) associate in pairs to form acetylacetic acid :

$$2\ CH_3CO_2H \rightarrow CH_3CO.CH_2CO_2H + H_2O$$

This view is in conformity with the so-called « β-oxydation condensation » theory. We cannot forget, however, that in the present

state of our knowledge there subsists, so far as facts are concerned, a serious contradiction between Embden's original findings (see above) and the more recent ones including those with the use of isotopic tracers. According to the latter, long chain fatty acids, whether they contain an even or an odd number of carbon atoms, are to be considered as ketogenic, whereas Embden claimed that those with an odd number of C atoms were not ketogenic.

As long as this contradiction subsists, any interpretation of the fundamental breakdown process of fatty acids in the body will stay questionable. It is probable that the length of the carbon chains used in those various experiments has a lot to do with the divergencies concerned and that the contradiction is but an apparent one rather than a true one. This, however, still needs to be proved. As far as labelling is concerned, it would be interesting to know whether there is any technical difficulty in the way of preparing labeled ^{13}C in the carboxyle group of much longer chains than octanoic acid, namely those containing as well odd or even number of C atoms, and with regard to the latter, those containing 16 or 18 carbon atoms such as those found in the fatty acids of the most common natural edible fats and to repeat Weinhouse's et al. experiments with such labeled chains. It would also be essential to repeat Embden's experiments with longer chains than those he used.

Finally, with regard to the nature of the two carbon-atom group split off at each β-oxydation step, do the investigations with tracers give any definite evidence as to whether it is acetic acid or not? When acetic acid with labeled carbon is fed to an animal, isn't the label found in acetylacetic acid?

M. Calvin. — There has been an experiment performed in the Biochemical Department at Berkeley in which β-labeled tyrosine was fed and some indication of active phenylalanine found. But this was done by adding carrier phenylalanine and crystallizing it out. Since it is very difficult to separate small amounts of tyrosine from phenylalanine by a simple recrystallization, it is very likely that this indication of a reduction of tyrosine to phenylalanine is due to contamination of the isolated phenylalanine by the original tyrosine. Furthermore, even if some active phenylalanine were found it would not necessarily indicate a mass conversion of tyrosine into phenylalanine but only that the enzymatic oxydation of the benzene ring is a reversible one.

M. Rittenberg. — Dr. Calvin has touched on a very important aspect of isotope research. One must clearly distinguish between a mass conversion of one substance to another and the results of a cyclic process or reversible system. While the feeding of acetate labeled with isotopic carbon leads to the formation of both fatty acids and glucose only the first, the fatty acids, actually utilize the carbon atoms of acetate for a net increase of fatty acid.

Acetate carbon is not a source of atoms for new glucose formation since in the process of introducing the acetate carbon atoms into a glucose molecule another molecule is involved which itself is formed from one molecule of glucose. The net result thus does not increase the total amount of glucose.

With regard to Prof. Bigwood's last question as to the nature of the C_2 fragments split off in the course of β oxydation of fatty acids, I do not believe we should too readily discard the simple notion that acetic acid itself is involved in the metabolic reactions.

Bernhard has demonstrated that administered acetic acid is used to acetylate γ phenyl α amino acetic acid and Hemingway et al. have demonstrated that acetic acid is converted to acetoacetic acid. On the other hand, we have demonstrated that pyruvic acid which can acetylate γ phenil α amino butyric acid but not *p*-aminobenzoic acid, does not do so via acetic acid.

It is quite possible that there are several forms of the C_2 unit. Among these must certainly be considered the N acetyl group and acetoacetic acid.

It is difficult to explain the apparent contradiction mentioned by Professor Bigwood. I think there can be little doubt that both the odd and the even fatty acids are degraded with the production of acetate molecules and that acetate can be condensed to yield acetoacetate. Indeed Mac Kay has recently shown that valeric acid is both ketogenic and glycogenic. Clearly in this case the acetoacetic acid arises from a condensation of acetic acid to aceto-acetic acid.

M. Bigwood. — Dans son exposé verbal, le Professeur Rittenberg a signalé qu'au cours de la désamination d'un acide α aminé par oxydation, l'hydrogène en α se sépare d'abord et le groupement α aminé ensuite. Comment faut-il concevoir cette suggestion d'étapes intermédiaires? Dans la réaction $R—CH.NH_2—CO_2H + 1/2\ O_2 \rightarrow R—CO—CO_2H + NH_3$, la séparation du groupement

amine doit s'accompagner de celle de l'hydrogène en α pour donner l'ammoniac. D'autre part, quand la désamination se fait, par ailleurs, par hydrolyse ou par réduction, donnant respectivement un acid-alcool ou un acide gras (au lieu d'un acide α-cétonique en cas d'oxydation), c'est le groupement amine qui se sépare tandis que l'hydrogène en α reste au contraire en position.

M. Rittenberg. — The more rapid rate at which the α hydrogen atom is removed compared to the α amino group is susceptible to several explanations at present. It is possible that the reversible dehydrogenation of the amino compound to the imino one is faster than the hydrolysis of the imino to the keto acid. This would account for the more rapid loss of the α hydrogen atom compared to the α nitrogen. On the other hand it is possible that another reaction is involved, possibly the dehydrogenation of a peptide bond to yield an acyl imino compound.

M. R. Courrier. — 1. Certains travaux récents effectués dans le laboratoire du Professeur Rittenberg ont mis en évidence des relations entre le cholestérol marqué par l'hydrogène lourd et le prégnandiol urinaire. M. Rittenberg voudrait-il nous dire s'il existe de nouveaux résultats dans ce domaine?

2. M. Rittenberg signale dans son rapport qu'après injection de deutérocholestérol au chien, on ne trouve pas trace de cette substance marquée dans le cerveau, ni dans la moelle épinière. Un tel résultat n'est-il pas surprenant, étant donnés les rapports étroits entre le cholestérol et le tissu nerveux?

M. Rittenberg. — The experiment describing the relationship of pregnandiol to cholesterol was carried out by Dr. K. Bloch. We have not investigated the relation of the other steroid hormones to cholesterol.

The absence of cholesterol in the central nervous system is probably a reflection of the much discussed blood-brain barrier.

M. Govaerts. — Prof. Rittenberg vient de signaler la possibilité de déterminer la vitesse de transport de certains composés dans l'organisme. L'eau est un des premiers corps étudiés et les travaux du Professeur de Hevesy ont montré tout d'abord que l'eau admi-

nistrée chez l'animal se répartit très rapidement dans toute l'eau de l'organisme. Les auteurs américains ont montré que l'eau pénètre très rapidement dans l'humeur aqueuse. De notre côté, nous avons observé que l'eau pénètre aussi très rapidement dans les globules rouges et pour l'eau tout se passe comme s'il n'existe pas de membrane. Dans une enceinte fermée, nous avons observé également à l'aide de l'hydrogène lourd des transports d'eau très rapides. Tous ces résultats permettent de conclure que d'une manière générale l'eau est douée d'une très grande mobilité dans la nature.

TABLE DES MATIÈRES

LES PREMIERS CONSEILS DE CHIMIE SOLVAY

Premier Conseil, 1922 : *Cinq Questions d'Actualité.*

Rapports de MM. F. Soddy, F.-W. Aston, J. Perrin, W.-H. Bragg, W.-J. Pope, T.-M. Lowry, Ch. Mauguin et A. Job.

Deuxième Conseil, 1925 : *Structure et Activité Chimiques.*

Rapports de MM. W.-B. Hardy, W.-L. Bragg, J. Duclaux, T-M. Lowry, F. Swarts, M. Tiffeneau et Orékhoff, J. Perrin, A. Job, E. K. Rideal, E.-F. Armstrong et P. Hilditch, Ch. Moureu et Ch. Dufraisse, H.-E. Armstrong, J. Duclaux et H. van Euler.

Troisième Conseil, 1928 : *Questions d'Actualité.*

Rapports de MM. E.-K. Rideal, W. Mund, A. Berthoud, S. Price, F.-G. Donnan, P. Girard, G. Urbain, N.-V. Sidgwick, P. Walden et P. Karrer.

Quatrième Conseil, 1931 : *Constitution et Configuration des Molécules organiques.*

Rapports de MM. W.-H. Mills, J. Boeseken, H. Staudinger, J. Timmermans, Mme Ramart-Lucas, MM. S. Sugden, R. Kuhn, B. Holmberg, R. Robinson et W. Schlenk.

Cinquième Conseil, 1934 : *L'Oxygène, ses Réactions Chimiques et Biologiques.*

Rapports de MM. M. Bodenstein, J.-A.Christiansen, H. Wieland, W.-P. Jorissen, W.-H. Bone, Ch. Dufraisse, R. Wurmser, O. Warburg et O. Meyerhof.

Sixième Conseil, 1937 : *Les Vitamines et les Hormones.*

Rapports de MM. G. Bertrand, P. Karrer, A. Szent-Györgyi, A. Windaus, H. von Euler, W.-N. Harworth, L. Ruzicka, E. Laqueur, F. Kögl et E.-C.Dodds.

Les Rapports et Discussions des six premiers Conseils ont été édités par MM. Gauthier-Villars et C^ie^, à Paris.