Journal of Labelled Compounds — October-December — Vol. II, No. 4

Abstracts of the papers presented at

The Second International Conference on methods of preparing and storing labelled compounds

BRUSSELS – 28th November – 3rd December 1966

1 — COMMUNICATIONS

F. PIETIG and H. W. SCHARPENSEEL (Institut für Bodenkunde, Bonn, Deutschland) :

^{14}C-Labelled benzenes of high specific activity.

The process is one of benzene synthesis through catalytic polymerization of acetyls. Various attempts without catalyst are compared as regards yield and radiochemical purity with syntheses using diborane and especially a new activated silica-alumina-based "cracking catalyst". This gives a yield of 90 to almost 100%.

P. VERCIER (Compagnie Française de Raffinage, Harfleur, France):

Improvements in the synthesis of cyclopentadiene-x-^{14}C – A novel synthesis of alkali cyanides

1. A new synthesis of alkali cyanides, starting from barium carbonate, is described. First, Barium cyanamide produced by passing NH_3 gas on $^{14}CO_3Ba$. In a second phase, Barium is replaced by divided sodium in order to make sodium cyanamide (more readily heat degradable) by a 20 minutes heating period at 350°C. Temperature is then raised to 850° and pyrolisis proceeds for another 10 minutes with a plug of Iron. One obtains a 68% yield of water soluble cyanide-^{14}C; 19% of metallic-^{14}C-cyanides and 11% of undecomposed radioactive cyanamide. Chemical yield is somewhat higher, due to CO_2 absorption on metallic sodium.

2. Methods available in the literature of the transformation of trans-1,2-dibromocyclopentane in cyclopentadiene give rather poor yields and the isolated product is often soiled by a number of impurities.

A pure sample of cyclopentadiene-x-^{14}C has been prepared on the 5 mM scale by adding dropwise under nitrogen a carbon tetrachloride solution of 1,2-trans-dibromocyclopentane over a definite mesh-size calcium oxide bed at a temperature not over 230°C. At the furnace outlet, the cyclopentadiene contained 15% of CCl_4, 2 to 7% of cyclopentene and dicyclopentadiene in amounts under 0,5%. After preparative gas phase chromatography, the purity of the isolated cyclopentadiene was over 99%. It was obtained in 70-75% yield from dibromocyclopentane (including losses at the chromatography stage).

M. A. MUHS (Shell Development Company, Emeryville, U. S. A.):

Carbon-14 labelling using carbene insertion — Potentialities of selective insertion into alkyl halides

The methylene insertion reaction has offered intriguing possibilities as a one-step procedure for the introduction of carbon-14 into organic molecules. We have recently reported

on the use of this reaction for the synthesis of ^{14}C-labelled hydrocarbons. With saturated hydrocarbons, the methylene inserts randomly at all C-H bonds and in some cases this may be a drawback since the desired material may be present in a relatively low amount. This difficulty could be overcome to some extent if one bond in a molecule underwent methylene insertion to a greater extent than the other bonds. Indications of such behaviour have been reported for alkyl halides which suggests that a labelled alkyl halide could be prepared in a relatively large amount by choosing the appropriate starting material. Since there has only been a brief amount of work reported on this reaction, furthermore quantitative studies were necessary so that most efficient use of this reaction could be made in its application to the preparation of ^{14}C-labelled materials. Such studies have now been made and are the subject of this presentation.

On the basis of this work one can predict with confidence not only the yields of ^{14}C-labelled compounds in these non-random insertions but the distribution of labelled products from each insertion. Not the least benefit is the fact that one has exact knowledge of the ocation of label in each product.

J. W. WOODS and H. D. JORDAN (Nuclear Research Chemicals, Inc., Orlando, U.S.A.):

The synthesis of ^{14}C trifluoroacetic acid

The necessity for a convenient method for the preparation of ^{14}C TFA led to an evaluation of the available methods for adaptation to small-scale operations.

The classical method of Swarts, with modifications by Watcher, Haven, as well as Haworth and Stacy, utilizing benzoic acid-7-^{14}C, would yield TFA-2-^{14}C, or employing benzoic acid (ring-UL-^{14}C) would produce TFA-1-^{14}C. However, yields are not good, and in the case of TFA-1-^{14}C considerable Carbon-14 is lost.

The report by Haszeldine that trans-1,1,1,4,4,4-hexafluoro-2-butene is oxidized by alkaline permanganate to TFA forms the basis for a convenient synthesis of the ^{14}C-labelled compound.

^{14}C fumaric acid (prepared from acetylene through the dilithioacetylene, which is readily prepared in THF) is converted by SF_4 to trans-1,1,1,4,4,4-hexafluoro-2-butene, which is oxidized with $KMnO_4$ to ^{14}C-trifluoroacetic acid.

W. E. SPROTT (Unilever Research Laboratory, Colworth, Sharnbrook, Bds., England):

Synthesis of isotopically labelled 1-amino acid-1-deoxy-2-ketohexoses.

Kinetic studies of the formation of Amadori-rearrangement products from amino acids and aldoses led to the formulation of methods for the synthesis of isotopically labelled 1-amino acid-1-deoxy-2-ketohexoses on a millimolar scale. Condensation at 80 °C of D-glucose with the appropriate amino acid in glacial acetic acid containing dimethyl sulphoxide was followed by the Amadori rearrangement to give the amino-ketose in up to 75 percent yield.

After removal of the solvent the chromatographically pure products were obtained by a combination of dextran ion exchange—and cellulose column chromatography.

J. W. WOODS, M. J. PEACOCK and J. D. JOHNSON (Nuclear Research Chemicals, Inc., Orlando, U.S.A. and the Department of Biochemistry, the University of Alabama Medical Center, U.S.A.):

The synthesis of alpha-hydroxyacids from carboxylic acids

Classical methods for the synthesis of alpha-hydroxyacids are somewhat difficult to carry out on a small scale, and the yields are somewhat variable, particularly for the longer chain acids of biochemical interest.

The method developed in these laboratories involves conversion of the acid to a thiol ester, which is treated with t-butyl perbenzoate to yield an alpha-benzoyloxythiol ester (in contrast to the results obtained when an O-ester is treated with the same reagent under the

same conditions), which is readily hydrolyzed to either the alpha-benzoyloxy acid or the alpha-hydroxy acid. Over-all yields of 75%-85% from the carboxylic acid have been obtained at the 5-10 millimole level.

Since alpha-hydroxy acids can be readily degraded to aldehydes of one less carbon, this method also represents a degradative method for carboxylic acid.

Some implications concerning the properties of the thiol ester linkage will be discussed.

R. F. NYSTROM and D. GURNE (Department of Chemistry and Chemical Engineering and Radiocarbon Laboratory, University of Illinois, Urbana, U.S.A.):

Synthesis of carbon-14 labelled formic acid

A simple process has been developed for the preparation of formic acid-^{14}C. It involves reaction between carbon dioxide-^{14}C and sodium trimethoxyborohydride in a sealed tube under appropriate experimental reactions. Separation of the major products is accomplished by vacuum distilling the methyl borate from the sodium formate-^{14}C, followed first by careful acidification of the reaction mixture, then by steam distillation which gives formic acid-^{14}C in yields ranging from 80-90% based on carbon dioxide-^{14}C.

Isotope dilution assay and degradation of a known weight of sodium formate-^{14}C by mercuric chloride have been employed to establish the radiochemical purity of the product. A trace of methanol-^{14}C is formed in the reaction but is removed when the aqueous sodium formate-^{14}C solution is evaporated to dryness. No formaldehyde-^{14}C could be detected.

D. BELLA (Istituto Superiore di Sanità, Laboratorio di Chimica, Biologica, Roma, Italia):

Microscale preparation of volatile, high specific activity labelled alcohols by direct reduction with $LiAlH_4$ from the corresponding acids

The direct closed system reduction of carboxy acids, especially their alkali salts, with $LiAlH_4$, using high boiling solvent and alcoholytic agent, is examined for the microscale preparation of high specific activity labelled alcohols.

The conditions of reduction (time, temperature) and alcoholysis, the selection of solvent-decomposer pair, the impurities arising by scission of solvents, and the mode of isolation of labelled alcohols are studied experimentally to obtain essentially quantitative yields of highly pure products in amounts as small as 0.2 m/mole.

The preparation of carbon-14 labelled n-Butanol, Isohexanol and 2-ammino-I-Propanol is described in detail as well as the apparatus used.

P. A. HARTHOORN (Woodstock Agricultural Research Centre, Sittingbourne, England):

Chemical synthesis of labelled pesticides

The growing interest in the mode of action of pesticides and the ralationship between structure and activity has brought an increase in the demand for radioactively labelled compounds.

Specific requirements for the labelling of pesticides by chemical synthesis are discussed briefly and are illustrated with examples. Details of the synthesis of the herbicide Prefix-C -14 (2,6-dichlorothiobenzamide), the molluscicide '8008'-C-14 and H-3 (N-tritylmorpholine) and the biochemical intermediate DCCD-C-14 (dicyclohexyl carbodiimide), are given.

M. J. DUNN, R. J. MARTIN, N. ROBERTS, P. SLADE, H. STANDON and P. WALKER (Plant Protection Limited, I.C.I. Agricultural Division, Bracknell, Berks., England):

The synthesis of ^{14}C-labelled bipyridylium salts

Several diquaternary 22′- and 44′-bipyridylium salts possess biological activity as systemic herbicides. NN′-ethylene-22′-bipyridylium dibromide and NN′-dimethy-44′-bipyridylium dichloride and dimethyl sulphate are important examples. We have synthesised these salts, labelled with ^{14}C in the heterocyclic ring, for use in biochemical studies. (1,-2-$^{14}C_2$)-acetylene was converted into (2,3,-$^{14}C_2$)-pyridine, which was then used to make the dipyridyls. Quaternisation was the final step. The reactions which were used in this work are well known. We wish to describe the special techniques which were devised to carry out the synthesis with material of high specific activity on a semi-micro scale.

R. RAPUN and J. A. DE LA CERDA (Isotopes Section, Junta de Energia Nuclear, Madrid, Spain):

Preparation and purification of labelled menazon-^{32}P. (0,0-dimethyl-S (4,6-diamine-S-(triazin-2-methyl) phosphorodithioate)

The Menazon-^{32}P is prepared by reaction between the ammonium salt of the 0,0-dimethyl phosphorothionthioic acid, synthesized by the reaction, and the 2-chloromethil-4,6-diamine -1,3,5-triazine.

Preparation of pure phosphorus pentasulphide is very difficult. Melting point determination of a purified product, by several methods and from two different producers, shows the presence of a remarkable impurification. Preparation and purification of ammonium salt of the free acid has been studied. The ammonium salt has been purified by thin layer chromatography, and its radiochemical analysis made by autoradiography. The Menazon has been firstly purified by consecutive extractions, and afterwards, by formation of an easily soluble derivative, which is also purified by preparative thin layer chromatography. This derivative, purified in such a way, is descomposed getting a product, which cochromatographied against a pure sample of inactive Menazon, shows the same value as Rf. Besides, the I.R. and U.V. spectrum of the radioactive Menazon and the reference one exactly alike.

R. RAPUN (Isotopes Section, Junta de Energia Nuclear, Madrid, Spain):

Preparation and purification of labelled parathion-^{32}P

Parathion-^{32}P labelled with phosphorus-32 has been prepared. The preparation and purification methods are also valid for other similar compounds labelled with phosphorus-32 and/or sulphur-35.

This product has been prepared starting from phosphorus thiochloride-^{32}P, following Jensen and Pearce method.

Against several authors' opinions, it is not possible to purify the 0,0-diethyl phosphorochloridothionate-^{32}P by distillation. Preparative thin layer chromatography has been employed instead of. The best operation conditions have been studied too.

Similarly, the high vacuum distillation does not permit either to obtain the pure Parathion; by the contrary, this operation leads to a remarkable descomposition.

Selective evaporation followed by a preparative thin layer chromatography conducts to a product with a radiochemical purity higher than 99%

A solution of Parathion-^{32}P in methanol, stored at room temperature suffers a noticeable decomposition in 20 days.

L. PICHAT, J. P. GUERMONT and J. C. LEVRON (Service des Molécules Marquées, C. E. N. de Saclay, Gif-sur-Yvette, France):

Use of the Wittig reaction for the synthesis of ^{14}C-labelled monoethylenic fatty acids: oleic acid-10-^{14}C, palmitoleic acid-10-^{14}C

The action of heptanoic acid-1-^{14}C and nonanoic acid-1-^{14}C on carbonyldiimidazole leads respectively to non-isolated N-heptanocyl-1-^{14}C-imidazole, and non-isolated N-nonanoyl-1-^{14}C-imidazole, which, on reduction with Li ACH_4, gives heptyl aldehyde-1-^{14}C and pelargonic aldehyde-1-^{14}C in yields of 70-75%. Under the action of ω-carbomethoxyoctylidenetriphenylphosphorane in dimethylformamide, these aldehydes yield the methyl esters of oleic acid-10-^{14}C and palmitoleic acid-10-^{14}C contaminated with the corresponding trans isomers.

After removal of the latter by chromatography on silica gel impregnated with $AgNO_3$, followed by saponification, oleic and palmitoleic acids of over 99% purity are obtained. Total radioactive yields 16 and 20% respectively.

C. COLOMBINI, M. VIDALI and G. DEGANELLO (Università di Padovà, Padovà, Italia):

The radiochemical synthesis of 14-C specifically labeled-3-hydroxypalmitic acid and alpha-beta-unsaturated palmitic acid.

These two unusual fatty acids were necessary for a metabolic study that will be reported elsewhere.

The starting material was Myristaldehyde-1-^{14}C, prepared by fusion of Ba-Myristate-1-^{14}C, Ba Acetate and $ZnCl_2$. Reformatsky condensation of Myristaldehyde-1-^{14}C and Ethylbromoacetate, and basic hydrolysis of the crude product gave Beta-Hydroxypalmitic acid-3-^{14}C. Fusion of this product with Boric Anhydride and vacuum distillation of the obtained borate ester yielded Alpha-Beta-unsaturated Palmitic acid 3-^{14}C.

H. SORANTIN (Institut für Chemie des Reaktorzentrum, Wien, Österreich):

Direct-labelling of oils with ^{131}I

Direct transfer of radioactive iodine from aqueous solutions into oils without using solvents or interhalogen compounds is described.

The amount of incorporated ^{131}I as function of temperature, time and amount of carrier was studied. The yield of permanently fixed radioiodine was determined after leaching the organic substances with $Na_2S_2O_3$-KI-solutions.

The highest values were found in vegetable oils, where 90% of the original present ^{131}I could be introduced.

The labelled compounds were examined by paper electrophoresis for free iodine.

It was also possible to extract ^{131}I directly from a solution of irradiated telluric acid into oils and fix the ^{131}I permanently.

An apparatus for performing all necessary operations by remote handling is described.

N. P. BUU HOI, N. D. XUONG and N. V. BAC (Institut de Chimie des Substances Naturelles du Centre National de la Recherche Scientifique, Gif-sur-Yvette, France):

Synthesis of linear fatty acids, totally deuterated or uniformly labelled with tritium

The desulphurising hydrogenolysis of fully halogenated thenoic acids by means of Raney nickel alloy in alkaline medium is a particularly simple method for the preparation of totally deuterated linear fatty acids when the reaction is performed in deuterium oxide, or of uniformly tritium-labelled linear fatty acids when the reaction is carried out in tritiated water. As an example of this method, the synthesis is described of perdeuterio-*n*-valerianic

acid and of uniformly tritium-labelled *n*-valerianic acid, starting from 3,4,5-tribromothiophen -2-carboxylic acid. This method can also be applied to the synthesis of totally deuterated or uniformly tritium-labelled linear aliphatic diacids; for example, starting from 3,4-dibromothiophen-2,5-dicarboxylic acid, totally deuterated or uniformly tritium-labelled adipic acid can be prepared.

J. C. STRINI and J. METZGER (Faculté des Sciences, Marseille, France):

Use of ^{13}C in the study of a mechanism for the oxidation of propene to acroleine

The complexes obtained by the reaction of mercuric ions with propene in aqueous solution decompose, in certain conditions, giving high yield of acrolein, accompanied with minor quantities of acetone and propanal.

A choice between various plausible mechanisms was made possible by the use of ^{13}C-labelled propene. The positions occupied by the isotopic carbon atoms in the products and the recovered propene were determined by mass spectrometry and by proton NMR (^{13}CH coupling).

A synthese of labelled propene ^{13}C-1 will be described and the mechanism of the reaction will be discussed according to the results of the isotopic analyses.

L. OTVOS, L. NOSZKO and J. SZAMMER (Central Research Institute for Chemistry of the Hungarian Academy of Sciences, Budapest, Hungary):

Synthesis of α-substituted dicarboxylic acids labelled with ^{14}C on one of the carboxyl groups and its use for the synthesis of ^{14}C-labelled cyclic ketones.

The preparation of cyclic ketones generally and also in labelled form is frequently carried out by the cyclization of dicarboxylic acid salts or by the cyclization of free acid catalyzed by barium oxide, thorium oxide, barium carbonate, etc...

Three methods were employed for the synthesis of α-substituted dicarboxylic acids labelled with ^{14}C on one of the carboxyl groups:

A/ the opening of lactone ring systems,

B/ reaction of the ω-halocarboxylic acids with ^{14}C-carboxyl-labelled alkyl malonester or ω-halocarboxylic-^{14}C acids with alkyl malonester,

C/ reaction of ω-haloalkyl-alkylmalonesters with potassium cyanide-^{14}C or ω-haloalkyl-alkylmalonesters-/carboxyl-^{14}C/ with potassium cyanide.

C. COLOMBINI, G. DEGANELLO and M. VIDALI (Università di Padovà, Padovà, Italia):

The synthesis of adrenaline, uniformly labeled with C-14 in the benzene ring.

The chemical synthesis of Adrenaline, uniformly labeled in the benzene ring at a high specific activity, has been investigated starting from benzene-UL-14-C. Two methods have been studied.

First method. By a modified synthesis described by R. W. Schayer, Adrenaline was obtained from Pyrocatechol-UL-14-C which was prepared by the following steps: Benzene ⟶ Phenol ⟶ o-Salicylaldehyde, and a Dakin reaction.

Second method. Adrenaline, together with Sympathol, was obtained by Ascorbic acid hydroxylation of N-Methylphenyl-ethanolamine, obtained from Benzene-UL-14-C via 2-Chloroacetophenone and N-Methylamino-acetophenone.

The practicity of these two syntheses will be discussed and compared with other possible methods.

R. GWOZDZ and B. SADOWSKA (Institute of Nuclear Research, SWIERK, Otwock, Poland):

The production of the ^{131}I-labeled thyroxine with a high specific activity

The method of isotope exchange is used for preparation of the ^{131}I-labeled thyroxine. The exchange is carried in an alkine water-organic phase medium. After changing the pH in water solution, what causes the precipitation, and subsequently washing, a pure thyroxine is obtained. The specific activity of ^{131}I-thyroxine is about 30-50 mCi/mg.

The ^{131}-I-thyroxine is controlled for radiochemical purity with use of reversed phase partition chromatography. Butanol is stabilized on the siliconized silica, and alkine water is used as mobile phase. The usefullness of this method for purification of 0.2 to 1 mg quantities of ^{131}I-thyroxine was controlled. The preparations of precipitated and washed ^{131}I-thyroxine are 98% radiochemical pure.

The stability of ^{131}I-thyroxine was also controled in various media: in water, in propylen glycol solution. The influence of various amounts of benzyl alkohol on the stability of the ^{131}I-thyroxine was also investigated.

R. MICHEL, R. TRUCHOT and M. DIDEY (Laboratoire de Chimie Pharmaceutique, École de Médecine et de Pharmacie, Dijon, France):

Synthesis of 5-iodometanephrine labelled with radioiodine and of metanephrine tritiated in the 5 position.

Synthesis of metanephrine (MN), the principal metabolite of adrenalin, tritiated in the 5 position is effected by catalytic dehalogenation of 5-iodometanephrine. (IMN).

IMN is prepared by the action of I_2 (1.25 mM) in C_2H_5OH on an ethanolic-ammoniacal solution of MN[2] (0.50 mM) chilled to $-10°C$, the reaction being carried out under H_2.

IMN is labelled by cold halogenation of a solution of this substance (0.5-5μgM) with an ^{125}I or ^{131}I-labelled iodine solution (10 mC) under hydrogen.

^{3}H-labelled MN is prepared by reduction of a methanolic solution of IMN (0.2 mM) with H_2 tritiated to 5%, in the presence of Raney nickel.

L. OTVOS, S. ELEKES, A. SZABOLCS and L. GRUBER (Stereochemical Research Laboratory and Central Research Institute for Chemistry, Hungarian Academy of Sciences, Budapest, Hungary):

Synthesis of some ^{14}C labelled anticarcinogenic drugs

Several ^{14}C-labelled biological alkylating agents were produced for the purpose of pharmacological investigations.

Cytostatics, in which β-chloroethylamino or methanesulfonyloxy groups are incorporated in polyhydroxy compounds have an important role among anticarcinogenic drugs. Such species are Degranol/1,6-dideoxy-1,6-bis-β-chloroethylamino-D-mannitol dihydrochloride/ and 1,4-bis-methanesulfonyloxy-meso-butane-2,3-diol /1,4-mesylmesoerythrite/. The forms of the compounds labelled with ^{14}C in the methylene group were prepared by opening the epoxide rings with ethylene $^{14}C_1$-imine in a hydrochloric acid medium from the corresponding bisepoxide 1,2-5,6-dianhydro-3,4-isopropylidene-D-mannitol and 1,2-3,4-dianhydromesoerythritol, respectively.

To get close evidence of the stereoselectivity and the biochemical effect of biological alkylating agents, radiocarbon-labelled d- and 1-2,5-dimethanesulfonyloxypentane/ ^{14}C-labelled methylmyleran/ were synthesized:

d- and 1-2,3-dichloropropane were reacted with potassium cyanide ^{14}C and the dinitrile hydrolyzed to dicarboxylic acid/ α-methylsuccinic acid-1,4-$^{14}C_2$/. After esterification and reduction the diols/ d and 1/ formed were transformed to the corresponding mesyl derivatives.

W. MEHRHOF and H. LETTRE (Institut für Experimentelle Krebsforschung der Universität Heidelberg, Deutschland):

Preparation of 6-azacholesterol containing ^{14}C at C-4 or C-26

The method for the preparation of 6-azacholesterol from cholesterol was revised. A new route was found for the preparation of the intermediate product 3β-acetoxy-5,6-seco-cholestane-5-one acid II. With the use of cholesterol labelled with ^{14}C in the C-4 position, the required labelled 6-azacholesterol could only be obtained with difficulty. No trouble was encountered in the production of 6-azacholesterol labelled with ^{14}C in the C-26 position. Other substances produced were tritiated 6-azacholesterol, 5,6-seco-cholestane-3β, 5α, 6-triol. Some of the compounds were tested in order to ascertain their effect on animal and plant cells and their distribution in the animal organism. Some radiochemical problems not previously encountered have still to be clarified.

J. LEVISALLES and I. TKATCHENKO (Laboratoire de Chimie Organique, Faculté des Sciences, Nancy, France):

Preparation of 2-(^{14}C) (5α) cholestane 3-one and of 3-(^{14}C) (5α) cholestane 2-one

A study of the stereochemistry of the benzilic acid rearrangement of (5α) cholestane 2,3-dione made it necessary to prepare this diketone with a label at C-2 or C-3.

The synthesis starts from A-nor (5α) cholestane 2-one **1** ^{14}C-methylmagnesium iodide (1,2 mole/mole of **1**) gives quantitatively the tertiary alcohol **2a** that can be converted to acetate **2b**. Thionyl chloride dehydration of **2a** in cold pyridine affords the three possible hydrocarbons **3** (50%), **4** (30%) and **5** (20%). Pyrolysis of acetate **2b** gives also **3** (2%), **4** (38%) and **5** (60%). These hydrocarbons can be easily separated by thin layer chromatography on silica gel containing silver nitrate. Hydrocarbon **4**, identified by IR and NMR spectra, is partially converted to hydrocarbons **3** and **5** by treatment with acid.

Osmium tetroxyde hydroxylation of **3** and **5** affords the *cis* diols **6** and **10**. Periodic acid cleavage of diols **6** and **10** gives ketoaldehydes **7** and **11**, which are cyclized to **8** and **12** by heating with ethanolic potassium hydroxyde. The structures of ketones **8** and **12** are proved by comparison with authentic samples, and, in turn, prove the location of the double bond in hydrocarbons **3** and **5**. Catalytic hydrogenation of **8** and **12** gives respectively 2-(^{14}C) (5α) cholestane-3-one **9** and 3-(^{14}C) (5α) cholestane-2-one **13**. These are obtained in 90% yield *from the hydrocarbons.*

F. SCHLENK (Division of Biological and Medical Research, Argonne National Laboratory, Argonne, U.S.A.):

Biosynthesis and isolation of labelled S-adenosylmethionine

The exceptional capacity of yeast cells to produce and store S-adenosylmethionine has aided greatly in the study of various group transfer reactions in which this sulfonium compound plays a role. Not less important has been the development of chromatographic procedures for its isolation under lenient conditions.

Two routes of cellular biosynthesis play a major role in the production of the sulfonium compound:

1. $SO_4^{--} \rightarrow \rightarrow$ L-methionine
   Precursors $\rightarrow \rightarrow$
   $\Big\} \rightarrow$ S-adenosyl-L-methionine + P + PP

2. a) Adenosine + L-homocysteine → S-adenosyl-L-homocysteine;
   b) S-Adenosyl-L-homocysteine + CH_3-precursor → S-adenosyl-L-methionine.

In both cases the cell transfers the end product into the vacuole where it is stored.

Numerous labelled forms of the sulfonium compound have been obtained by supplementation of the yeast cultures with labelled precursors: L-Methionine-$^{14}CH_3$, L-Methionine-C^3H_3, L-Methionine-$^{14}COOH$, L-Methionine-α^{14}C, L-Methionine-^{35}S, $^{35}SO_4^{--}$, adenine-8-^{14}C, adenine-^3H, adenosine^{14}C, $^{15}NH_4^+$, ^{14}C-glucose, and H_2O-^3H.

J. H. SCHMITT and M. H. ZENK (Botanisches Institut der Universität München, München, Deutschland):

Resolution of labelled DL-amino acids into their optical antipods by enzymatic acetylation

An enzyme from yeast has been isolated and highly purified which acetylates stereospecifically the D-form of the 43 amino acids investigated. This enzyme was named acetyl-CoA: D-amino acid α-N-acetyl-transferase (belonging to the group EC 2.3.1.) and catalyzes the following reaction even in the presence of L-amino acids:
DL-amino acid + acetyl-CoA → acetyl-D-amino acid + L-amino acid + CoA.

The acetyl-CoA needed in this reaction can be generated enzymatically by the use of acetylphosphate, CoA and phosphotransacetylase which are commercially available.

This reaction can be used for the separation of the optical isomers of isotopically labelled DL-amino acid mixtures.

The acetyl amino acid can easily be separated from the L-form which is optically completely pure. The acetyl D-amino acid can now again be razemised by the azlactone method, hydrolyzed and the enzymatic resolution repeated. By additional repetition of this process, it was possible to transform a ^{14}C-labelled DL-amino acid mixture into the pure L-form in a yield of 80-90% of the total radioactivity. The experimental procedures will be given in detail.

L. OTVOS, H. TUDOS, J. MEISEL and A. SZABOLCS (Central Research Institute of Chemistry, Hungarian Academy of Sciences, Budapest, Hungary):

Synthesis of ^{14}C labelled "radioenantiomer" compounds of biological importance

Several polyfunctional compounds of biological importance /glycerol, citric acid, etc./ have a plane of symmetry. In enzymatic reactions compounds consisting of molecules of such type are of asymmetric character. The stereospecifity of these enzymic reactions can be investigated with the aid of ^{14}C-labelled compounds. There are two ways to synthesize "radioenantiomers" which are needed for these examinations:

1. Synthesis of specifically labelled D- and L-modifications of compounds transformable to the same chemical form /called radioenantiomer form/, the enantiomer character of which is given by the positions of labelling. In such way D-glycerol-1-^{14}C and L-glycerol-1-^{14}C can be obtained from D-glyceraldehyde-3-^{14}C and L-glyceraldehyde-3-^{14}C. Another variation of this method is the transformation of D-glyceraldehyde-1-^{14}C and D-glyceraldehyde-3-^{14}C to the radioenantiomer forms of glycerol.

2. Preparation of a derivative of the ^{14}C-labelled compound in which the position of labelling and the position of the removable incorporated group is known; resolution of these derivatives; removing of the incorporated group, which caused the chemical asymmetry. The "radioenantiomer" form of citric acid can be synthesized in this way.

B. GLASSON, A. BENAKIS, and G. MEDWED (Laboratoire du Métabolisme des Médicaments, École de Médecine, Genève, Suisse):

Double labelling in the metabolic study of diphenylhydantoin-C^{14}

In previous studies the authors outlined the part played by diphenylhydantoin in antiepileptic medication. The present report gives an account of the metabolism of this substance by means of 2-C^{14} and 4-C^{14} double labelling. Whereas the synthesis of diphenylhydantoin-4-C^{14} gives a pure product, the 2-C^{14} synthesis results in a product which is contaminated with radioactive impurities. The purification conditions are described. The report presents the results of the metabolism of this substance in animals and than discusses the usefulness of double labelling in the study of the metabolism of drugs.

K. ROTH, A. ZELLER and H. E. OBERLANDER (Landwirtschaftlich — Chemische Bundesversuchsanstalt, Wien, Österreich):

Preparation of ^{14}C-labelled humic acids formed biosynthetically in the soil

In our investigations on the gradual formation of humic acids from ^{14}C-labelled plant matter in soil, the biosynthesis, isolation and purification of ^{14}C-labelled humic acids constitutes an essential step.

Therefore, in order to obtain uniformly labelled plants, maize was grown for 8 weeks in a fully automatic growth-chamber and was continuously supplied with $^{14}CO_2$ of constant specific activities of soluble substances, hemi-celluloses, cellulose and lignin have been determined.

30 gr of dry matter of the harvested ^{14}C-labelled plants were mixed with 10 kg of soil and were kept in pots in the open for transformation into humic substances. After 1, 6 and 12 months resp., samples of the soil-plant mixture were taken. Soluble non-humic substances were removed by ethanol-water extraction. Humic acids were extracted by sodium pyrophosphate solution and precipitated with HCl. Impurities were removed by repeated reprecipitations with HCl and by ethanol-benzene extraction.

The specific activity of the humic acids prepared in this way had increased during the year of incubation to 3μC ^{14}C /gC. The humic acids were characterized by determination of their C/N-ratio as well as by the slope of their curve of light absorption.

B. TANACS, L. BARANYAI, L. BURSICS and J. MARTON (N. A. E. C. Institute of Isotopes, Budapest, Hungary):

The preparation of vincamine labelled with carbon-14 and tritium.

Vincamine, the major alkaloid of *Vinca-minor L*, is well known as an antihypertensive and sedative. It was, therefore, of interest, to investigate the mechanism of its action by using isotopic tracer method.

Vincamine, labelled with carbon-14 has been prepared by the esterification of vincaminic acid with diazomethane-^{14}C. By this method high specific activity, excellent radiochemical and chemical purity could be obtained without any change in natural constitution of the alkaloid. The overall radiochemical yield based on $K^{14}CN$ was about 15-20%.

Some preliminary results of the biosynthesis of labelled vincamine by *Vinca minor L*, from tryptophane-^{14}C will also be reported.

Tritium labelling has been investigated by the method of Wilzbach and by esterification with diazomethane-^{3}H.

P. D. KLEIN and R. LESTER (Division of Biological and Medical Research, Argonne National Laboratory, Argonne, U. S. A.):

The introduction of tritium into bile pigments

Most work on bile pigment metabolism utilizing labeled forms of bilirubin has depended upon ^{14}C bilirubin prepared biosynthetically from deltexamino levulinic acid-4-^{14}C or upon bilirubin which has been treated by the Wilzbach procedure to introduce tritium. Comparative studies of excretion rates of free and conjugated bile pigments have required a variety of bile pigments specifically labeled with ^{3}H. We have developed procedures for the introduction of tritium by exchange-labeling into delta aminolevulinic acid and have utilized this material to prepare bilirubin biosynthetically.

Two other bile pigments representing the addition of four and eight atoms of hydrogen to the bilirubin molecule (mesobilirubin and urobilinogen) have been prepared by alternative procedures of direct hydrogenation of unlabeled bilirubin. Paladium in sodium hydroxide catalyses the tritium gas reduction of the vinyl side chains to ethyl groups, while sodium amalgam in tritiated water accomplishes the same reduction and also reduces the methine bridges linking the pyrrole rings. Side reactions are minimal; the over-all yields are 60-70% and activities of 5-50 mc/mM have been obtained. The use of tritiated water either as the direct

reductant or as the source of tritium gas in a small scale electrolysis unit enables the radiochemical syntheses to be carried out at low cost without special equipment. Identification and purification techniques as well as isotope localization procedures using deuterium syntheses and NMR spectroscopy have been developed and will be described.

S. BOROCHOWSKI and P. CZERNIAK (Department of Radiotherapy & Isotopes, Tel-Hashomer Hospital, Israel):

Synthesis of radioactive sodium P-iodo-hippuran ^{131}I

Sodium para-iodo hippuran can be used as a tracer for renal study. Injected intravenously is quickly taken up by the kidney parenchyma and excreted by the tubuli. These dynamic changes can be examined by a renogram if the compound is labelled by a gamma emitter.

We performed a method for labelling p-iodo hippuran with radioactive iodine. The method is based on the exchange of the amino group of *p*-amino hippuric acid into radioactive iodine by diazotization. (Sandmeyer's Reaction). The product is examined for purity by chromatography in *n*-butanol-acetic acid-water solvent and its melting point investigated.

The yield, calculated for radioactive iodine used, is 40-50 percent. The procedure is very simple and can be performed in a small clinical laboratory.

R. F. NYSTROM, N. S. S. RAJAN, N. H. NAM and D. GURNE (Department of Chemistry and Chemical Engineering and Radiocarbon Laboratory, University of Illinois, Urbana, U.S.A.):

Preparation of tritium labelled cyclohexenes and degradation by pyrolytic radio-gas chromatography

This communication reports the synthesis of cyclohexene-1-^{3}H cyclohexene-3-^{3}H and cyclohexene-4-^{3}H. The position of labelling with tritium has been established by both pyrolytic radio-gas chromatography and chemical degradations.

The vinylic carbon of cyclohexene has been labelled with tritium to give cyclohexene-1-^{3}H by decomposition of cyclohexyl-1-^{3}H S-methylxanthate and by protonolysis of 1-N-piperidylcyclohexyl-1-^{3}H borane-2. Tagging of the allylic carbon of cyclohexene with tritium producing cyclohexene-3-^{3}H has been accomplished by reducing 3-bromocyclohexene with a mixture of lithium aluminium tritide and lithium tritide. Finally, cyclohexene-4-^{3}H has been synthesized by monohydroboration of cyclohexadiene-1,4 followed by protonolysis with tritiated propionic acid.

The use of other procedures to prepare radioactive cyclohexene labelled in one of the desired positions with tritium will also be described.

Isotopically reliable degradations used to determine the position of labelling with tritium in the various cyclohexenes will be discussed; these include pyrolytic radio-gas chromatography and chemical methods.

L. PICHAT, M. HERBERT and F. AUBERT (Service des Molécules Marquées, C.N.E. de Saclay, Gif-sur-Yvette, France):

Use of vinylmagnesium bromide for the synthesis of ^{14}C-labelled compounds. Synthesis of DL-hydroxyproline 3,5-^{14}C and DL-allohydroxyproline-3,5-^{14}C

Acrylic acid-1-^{14}C is prepared by carbonation of vinylmagnesium bromide. The 2,3-dibromo-1-propanol-1-^{14}C obtained from the acrylic acid-1-^{14}C is converted by the action of zinc into allyl alcohol-1-^{14}C.

The latter, when treated with PBr_3, yields allyl bromide. This is condensed on the sodium derivative of ethyl acetamidomalonate. The brominated derivative obtained by adding bromine

to ethyl allyl acetamidomalonate is hydrolysed to a mixture of cis and trans DL-hydroxyproline-3,5-^{14}C.

Purification by chromatography over Dowex 50-W-12 ion-exchange resin enables partial separation of these two forms to be effected. Separation is then completed by means of electrophoresis on paper. The overall yield, referred to $Ba^{14}CO_3$ is 20%, half of which is in the DL form and half in the allo form. The specific activity is 10 mCi per millimole.

L. PICHAT, M. HERBERT and C. FABIGNON (Service des Molécules Marquées, C.E.N. de Saclay, Gif-sur-Yvette, France):

Amino acids specifically labelled with ^{14}C: DL-aspartic acid-4-^{14}C, DL-2,4-diaminobutyric acid-4-^{14}C and DL-homoserine-4-^{14}C

Condensation of potassium cyanide-^{14}C on the iodomethylate of ethyl dimethylaminomethylacetamidomalonate gives ethyl-β-cyano-^{14}C-α-acetamidopropionate in a yield of 65% on KCN.

From this intermediate product several labelled amino acids can be synthesized. Hydrolysis gives aspartic acid-4-^{14}C. Catalytic reduction in the presence of Raney nickel, followed by hydrolysis, yields 2,4-diaminolbutyric acid-^{14}C. The reduction product on treatment with nitrosyl chloride in 6N. HCl, followed by acid hydrolysis and treatment with concentrated NH_4OH, gives DL-homoserine-4-^{14}C. Paper chromatography and electrophoresis show that the homoserine thus obtained does not contain isohomoserine. The overall yield of these three amino acids, referred to KCN-^{14}C, is 55%.

W. J. LEQUESNE and R. D. CLARK (The Radiochemical Centre, Amersham, England):

The preparation of L-asparagine-^{14}C(U) and L-glutamine-^{14}C(U)

L-Aspartic acid-^{14}C(U) and L-glutamic acid-^{14}C(U) are obtained by acid hydrolysis of Chlorella protein. L-Aspartic acid is converted into β-methyl L-asparate, which gives L-asparagine directly with aqueous ammonia.

L-Glutamic acid is converted to γ-ethyl (or γ-methyl) L-glutamate; these esters with aqueous ammonia give only L-pyrrolidone 2-carboxylic acid. γ-ethyl L-glutamate is converted into the N-carbobenzyloxy derivative, which with aqueous ammonia gives carbobenzyloxy-L-glutamine. The protecting group is removed by catalytic hydrogenation.

I. MEZO, I. TEPLAN and J. MARTON (N.A.E.C. Institute of Isotopes, Budapest, Hungary):

Studies on preparation of glutamic acid-1-^{14}C, ornithine-1-^{14}C and arginine-1-^{14}C by using the cyanhydrine-synthesis

A convenient method has been elaborated for the preparation of glutamic acid-1-^{14}C by extending the Strecker synthesis for α-oxo-carboxylic acids.

It was of interest, to develop another synthesis for the preparation of DL-glutamic acid-1-^{14}C, permitting the preparation of DL-ornithine-1-^{14}C and DL-arginine-1-^{14}C as well. To achieve this by using the cyanhydrine synthesis in cases of β-cyano-propionic aldehyde and γ-benzoylamino butyric aldehyde DL-glutamic acid-1-^{14}C and DL-ornithine-1-^{14}C was formed, respectively, in good chemical and radiochemical yields. Ornithine-1-^{14}C was then converted to arginine-1-^{14}C by using S-methyl isothiouronium iodide according to the standard inactive method.

W. SULLIVAN and P. PEYSER (Biochemistry Department, New England Nuclear Corporation, Boston, U.S.A.):

The semi-micro scale synthesis of L-asparagine^{14}-C, U.L.

An unambiguous and stereochemically specific method of synthesis of L-asparagine-^{14}C, u.l., starting with 200 micromoles of L-aspartic acid-^{14}C, u.l., has been devised. The sequence of reactions is as follows:

L-aspartic acid —(dry HCl, absolute ethanol)→ diethyl L-aspartate hydrochloride (100% yield)

—(dry NH_3, absolute methanol, 0 °C, 6 days)→ L-aspartic diamide (98% yield)

—(leucine aminopeptidase, tris Buffer pH 8.5, Mg^{++}, 3 hours, 25 °C.)→ L-asparagine (83% yield)

The L-asparagine obtained was purified on a weekly acidic cation exchange resin (Amberlite CG-50) column; overall yield, 60-70%. The final product was found to be better than 98.0% pure in two paper chromatographic systems. After incubation of a sample in the presence of L-asparagine (*E. Coli*) for two hours, less than 0.4% remained unhydrolyzed.

J. CIFKA, V. KACENA and L. KRONAD (The Nuclear Research Institute of Czechoslovak Academy of Sciences, Prague, Czechoslovakia):

Preparation of some organomercurials labelled with mercury isotopes ^{197}Hg and ^{203}Hg

Exchange reactions between radioactive mercuric bromide or chloride and some organic mercury compounds were used for labelling of the respective compounds.

The exchange rate in various media, was studied using paper chromatography as the separation method. The ion exchange colums were used for the preparative purpose.

1-bromo-mercuri-2-hydroxy propane and 3-chloro-mercuri-2-methoxypropyl urea, used for diagnostic purposes were among the compounds studied.

B. TANACS, J. MARTON and L. BURSICS (N.A.E.C. Institute of Isotopes, Budapest, Hungary.

A simple one-step method for the preparation of carbon-14 and tritium labelled purine and tiazolo-/5,4-d/-pyramidine derivatives

For the preparation of purine-8-^{14}C derivatives /e.g. adenine-8-^{14}C, guanine-8-^{14}C, hypoxanthine-8-^{14}C, 6-mercaptopurine-8-^{14}C etc../ mainly two-steps syntheses are employed by using formate-^{14}C or thiourea-^{14}C as carbon-14 source.

In our laboratory these reactions were carefully investigated and the formate method was improved. As a result, a simple one-step microsynthesis has been developed through dry fusion of a mixture of 4,5-diamino-pyrimidine derivatives and labelled formate. By this method purine derivatives labelled with carbon-14, tritium or both of these isotopes have been prepared with high specific activity on a 0,1-1,0 millimole scale by using formate-^{14}C, formate-^{3}H-^{14}C, respectively as starting material.

H.W. VAN MEETEREN and H.C. VAN DER PLAS (Laboratory of organic Chemistry of the Agricultural University, Wageningen, Netherlands):

Synthesis of 4-chloro-2-phenylpyrimidine-4-^{14}C and 4-methyl-2-phenyl-*s*-triazine-4-^{14}C

Since the investigation describing the transformation of 4-chloro-2-phenylpyrimidine into 2-methyl-4-phenyl-*s*-triazine by treatment with potassium amide in liquid ammonia, many analogous ring conversions have been found.

In order to investigate the mechanism of these reactions 4-chloro-2-phenylpyrimidine-4-^{14}C has been prepared. The following method was used: the sodiums alt of ethyl formyl-acetate-1-^{14}C, obtained by formylation of ethyl acetate-1-^{14}C with ethyl formate and sodium ethoxide in dry ether was coupled with benzamidine to give 4-hydroxy-2-phenylpyrimidine-4-^{14}C. This compound was treated with phosphorus pentachloride yielding the desired labelled chloro compound. After the reaction of 4-chloro-2-phenylpyrimidine-4-^{14}C with potassium amide in liquid ammonia, labelled 4-methyl-2-phenyl-*s*-triazine was isolated in a 40% yield.

Degradation of this triazine by acid hydrolysis showed the ^{14}C-atom to be exclusively present on the 4-position.

E. PETREANU, J.S. COHEN and D. SAMUEL (Weizmann Institute of Science, Isotope Department, Rehovoth, Israel):

The preparation of purines, pyrimidines, nucleotides and related compounds labelled with ^{17}O, ^{18}O and other stable isotopes

The shape and structure of nucleic acids in solution is one of the most important problems in biology. One method of attacking this problem is by means of I.R., Raman or N.M.R. spectroscopy of aqueous solutions. In orderto obtain more precise information on base pairing, coiling and changes in configuration, detailed spectra of both model compounds and nucleic acids themselves must be studied. Specific interreactions between these molecules can be obtained by specific labelling of given atoms with stable isotopes (D, ^{13}C, ^{15}N, ^{17}O and ^{18}O).

Methods of preparing labelled purines, pyrimidines, nucleotides and nucleosides in our laboratory and elsewhere will be outlined and the use of these molecules both as tracers and for the investigation of more fundamental aspects of the structure of nucleic acids will be discussed.

Z. NEJEDLY, J. FILIP and D. GRUNBERGER (Research Institute for Production and Uses of Radioisotopes, Prague, Czechoslovakia):

The preparation of ^{14}C nucleic acids components, amino-acids and sugars of high specific activity by algae chlorella pyrenoidosa

A method is described for the preparation of nucleic acids components, aminoacids and sugars randomly labelled with ^{14}C of high specific activity from algae chlorella pyrenoidosa grown in $^{14}CO_2$ atmosphere.

The high specific activity of radioactive algae (1 mCi/1 mg of dry weight) was achieved by photosynthesis of the algae in a special apparatus, in a nutrient solution containing inorganic salts and a trace amount of beef extract and by using $Ba^{14}CO_3$ of isotopic ratio higher than 50%. The total amount (500-600 mCi) of $^{14}CO_2$ was envolved and fed to the system in portions, so that at the beginning of the photosynthesis the concentration of $^{14}CO_2$ in air was 3-5% at the pressure of 600 torr.

Various procedures of fractionation of the so-called insoluble material of radioactive algae have been investigated.

The specific activity of ^{14}C purine and pyrimidine bases of nucleic acids obtained was about 60 mCi/mMole, of nucleosides and nucleoside-5′-monophosphates about 250 mCi/mMole and of aminoacids and sugars about 150 mCi/mMole.

J.M. SAUCIER, D. TOUTAIN and C. PAOLETTI (Unité de Biochimie et d'Enzymologie, Institut Gustave-Roussy, Villejuif, France):

Isolation and purification of tritiated high specific activity E. Coli deoxyribonucleic acid

A simple method is described to extract deoxyribonucleic acid (DNA) from e. Coli using sodium dodecyl-sulfate, papain and phenol as denaturing agents of proteins. The recovery of the extraction, followed by radioactivity measures of (^{3}H) labelled DNA, is about

65%. The average molecular weight is 10^7 Daltons and there is less than 1% proteins and ribonucleic acid. This method is particularly suitable to prepare labelled DNA obtained by thymine-methyl (^3H) incorporation by thymineless bacterial mutants. With tritiated thymine presently available, a specific activity of 10 to 2000 mC/mM (expressed in-P-DNA) can be obtained. This activity can be fixed at will, and if we assume a counting efficiency of 20%, will give from 14×10^3 cpm/μg DNA to 28×10^6 cpm/μg DNA.

The radiochemical purity has been checked by distribution in a gradient of CsCl and by chromatographic fractionation on methylated albumin column.

J. MORAVEK and J. SKODA (Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague, Czechoslovakia):

A simple method for preparation of nucleotides and oligonucleotides labelled with ^{14}C, ^3H and/or ^{32}P by thermic reaction of nucleosides with inorganic phosphate

Simple heating of an equimolar mixture of ^{14}C or ^3H labelled uridine and inactive or radioactive inorganic phosphate results in an easily separable mixture of labelled nucleotides and oligonucleotides in 60% yield. Several products, e.g. uridylyl (3′-5′) uridine, and uridine 2′3′ cyclic phosphate, are valuable also as a source for preparation of oligonucleotides. The thermic phosphorylation is not limited to naturally occuring nucleosides and many anomalous nucleosides have been found to react, some of them in higher yield than the natural ones.

The dependence of the course of reaction on time, on the concentration of inorganic phosphate and on the presence of magnesium sulphate in the reaction mixture has been established.

The method described is suitable especially for work with small quantities of highly radioactive nucleosides or inorganic phosphate where the general methods of organic synthesis often fail.

O. L. KLAMERTH (Institut für Virusforschung, Heidelberg, Deutschland, und Institut für Biologie, Reaktorzentrum, Seibersdorf, Österreich).

Separation of DNA and RNA in high molecular state.

On the basis of the selective adsorbtion of single stranded DNA on nitrocellulose fibres of a certain nitration degree a method has been developed for the quantitative separation of DNA from RNA leaving unchanged the high molecular state of both the nucleic acids isolated by common technique from cells or tissue. The efficiency of separation amounted to more than 97%. By stepwise elution with increasing pH of a labelled DNA isolated from HeLa cells several fractions were obtained which differ markedly in specific activity and molecular size but not in the G + C content.

L. DIMITRIJEWICZ, P. LAUNAY, and C. PAOLETTI (Unité de Biochimie et d'Enzymologie, Institut Gustave-Roussy, Villejuif, France):

Deoxyribonucleasic activities measured with tritiated DNA

The present method, uses a tritiated thymine labelled DNA, for the assay of neutral and acid DNases. It is based upon the hydrolysis of an acid-insoluble substrate, into acid-soluble oligonucleotides, which are measured by counting of the radioactivity.

The kinetic characteristics of pancreatic and splenic DNases, have been tested with this method in various conditions of environment, and found to be identical with those previously reported.

The interest of the use of a radioactive substrate, lies in the requirement of small quantities of DNA and in a great sensibility. In our hands, this method made it possible to detect and measure DNases activities 30 times lower than those measured with usual methods.

This method can be used for the assay of DNase activities in biological mediums and organ extracts.

J. LEONIS, A.G. SCHNEK and J. VAN PEBORGH (Laboratoire de Chimie Générale I, Université de Buxelles, Belgique):

Tritium labelling of proteins

A comparative study was undertaken to ascertain the value of biological and of chemical methods for labelling proteins with tritium atoms.

Biosynthetic labelling of the main proteins of hen egg white was carried out by using as precursor each one of the following amino acids arginine, glycine, lysine, tryptophan or tyrosine. Our results favour the assumption that, by using an essential amino acid, it is possible to introduce the label at specific sites of the macromolecular structure.

Chemical exchange with tritium gas, according to Wilzbach's procedure, was shown to be adequate in the case of rather small proteins (M around 15,000). Exchange tritiation does not result in a uniform distribution of the label among the constituent amino acids.

Catalytic exchange, between tritiated water and simple organic compounds, has been extensively used by Garnett; we are presently investigating its usefulness for macromolecules. Conditions have been worked out which damage neither the structure of lysozyme nor its biological activity.

J. M. MORETTI, L. CLOAREC and E. NUNEZ (Laboratoire de Biochimie, Faculté de Médecine, Paris, France):

A comparative study of tritiated protein by two different methods.

Human haptoglobin Hp 2-1 was labelled with tritium by the Wilzbach method and by the catalytic dehalogenation method (according to Roche and Nunez). We sought to determine where the tritium atom attached itself.

In spite of filtration through Sephadex, chromatography on DEAE-cellulose or prolonged dialysis, some tritium labile always remained. Only evaporation in a vacuum succeeded in eliminating the major part.

We submitted the glycoprotein to a series of hydrolyses to detach the glucides and the amino-acids. We then separated these by chromatography in order to determine their specific activity. Ala, Tyr, Phe, His, Lys, Pro were especially labelled by the Wilzbach method. Leu, Ileu, Gly, Val, Met, Ser were unlabelled. All the sugars were labelled, including the sialic acid and the glucosamine.

By the second method, tyrosine was strongly labelled. Ala, His, Phe received light labelling. The glucides were not labelled at all, only in certain conditions. To the method of labelling by catalytic deshalogenation therefore, there is surimposed in the course of agitation, a slight exchange which appears in the Wilzbach method.

J. M. MORETTI, E. NUNEZ and J. POMMIER (Laboratoire de Biochimie, Faculté de Médecine, Paris, France):

A comparison of the Wilzbach method and the catalytic dehydrogenation method of labelling proteins by tritiation.

To label a protein with tritium gas, one can use the exchange method (Wilzbach) or the method of substitution of tritium for an atom of iodine fixed in the tyrosine residue of the protein (Roche and Nunez). We have made a comparison of these two methods from the point of view of specific activity and biological activity of the protein.

We worked with the haptoglobin Hp, which together with the hemoglobin Hb gives a combination Hp-Hb that has a peroxydasic activity which can be measured with precision.

With the Wilzbach method, the optimal conditions are: 2 curies for 3 days per 100 mg of protein. Maximal specific activity: 5.900 dpm/μg. Denaturation: 10 % of the initial product is destroyed by radiolysis.

With the catalytic dehalogenation method, the best conditions are: 20 curies for 1 day per 100 mg of protein and 5% catalyst. Maximal specific activity: 600 dmp/μg. Denaturation:

twice 10% by freezing, plus an amount which varies from 10 to 30% in direct ratio to the suds, produced in the course of agitation.

The Wilzbach method yields a product containing much more tritium labile, very easily detached in vivo. With the catalytic method, this disadvantage is less marked, but the specific activity of the final product is 10 time weaker. The respective advantage and disadvantage of the two methods are discussed in function of the end of the tritiation.

S. CHRISTENSEN (Department of Physiology, University of Aarhus, Aarhus, Denmark):

A plasma macromolecule labelled with 32-P in phospholipid and phosphoprotein moieties

Twenty-four hours after the intramuscular administration of one to two mC of 32-P labelled inorganic phosphate per kg body weight to oestrogenized cockerels, the birds were bled into siliconized cooled centrifuge tubes and plasma in this way obtained without use of anticoagulants. The plasma trichloroacetic acid soluble phosphate, the lipid phosphate and the protein phosphate were of almost the same specific activity (about 0.5 to 1μC per mg of P). Separations by paper electrophoresis in veronal buffer at pH 8.6 showed about one third of the phosphoprotein phosphate activity to move slightly ahead of the major peak of activity which contained more than 95% of the lipid phosphate activity and the remaining two thirds, approximately, of the phosphoprotein phosphate activity.

The uptake in vivo at the aortic endothelial surface of the plasma lipid and protein phosphate activity, following the intravenous administration of labelled plasma, was consistent with the uptake primarily of one double labelled unit. This feature may perhaps prove of value in other studies of macromolecular uptake.

On a rough average the plasma used contained about 1μC per ml (spec. act. of the P being about 1μC per mg.) From these data, a dose rate of about 30 rad per day can be calculated for our plasma pools. We have no suspicion that this caused any troubles in our experiments concerned with plasma samples stored maximally 6 days following bleeding of the birds (i.e. 7 days if the day of the biosynthesis is included).

H.C. HEINRICH, E. E. GABBE and W. P. NASS (Physiologisch-Chemisches Institut der Universität Hamburg, Deutschland) :

Chromatographic and metabolic criteria for non-denaturated human-serumalbumin

It was demonstrated by dextrangel-filtration and ionexchange-chromatography on diethylaminoethyl- and carboxymethyl-loaded dextrangel that commercial ^{131}I- and ^{51}Cr-labelled human-serum-albumin and also their unlabelled starting material (HSA) have chromatographic properties which are significantly different from the native human-serumalbumin. In addition these labelled HSA-preparations can be distinguished by their completely anormal metabolic properties. From the chromatographic and metabolic properties of such ^{131}I- or ^{51}Cr-labelled human-serumalbumen preparations we have concluded that the carrier protein is denaturated.

A non-denaturated ^{131}I-human-serumalbumen (=^{131}I-HSA-H) was prepared in our laboratory by labelling non-denaturated human-serumalbumen which was isolated from the plasma of a blood donor by using only dextrangel-filtration. This ^{131}I-labelled human-serumalbumin was purified and demonstrated to be undistinguishable from unlabelled native human-serumalbumin by dextrangel filtration and ionexchange-chromatography on DEAE- and CM-loaded dextrangels.

The relative and absolute whole body turnover rates of non-denaturated ^{131}I-human-serumalbumen-H were calculated and will be discussed as metabolic criteria in comparison with the chromatographic criteria of non-denaturated ^{131}I-human-serumalbumin.

M. F. ABDEL WAHAB and S. A. EL-KINAWY (Middle Eastern Regional Radioisotope Centre for Arab Countries in Cooperation with I. A. E. A. Dokki, Cairo. UAR, and Drug Research and Control Centre, G. O. P. C. A. Giza, UAR).

Preparation of labelled proteins, unsaturated esters, and hormones. Using ^{131}I and ^{99m}Tc

In a previous work we separated unsaturated fatty acids, by reverse phase paper chromatography. The localization of these acids on the paper and hence their R_F-values have been determined by the help of radioiodine entering the double bonds. The iodination went quantitatively and could be used for the determination of the unsaturation and consequently the iodine value in different fats and oils.

This work is now extended to introduce radioiodine or pertechnetate in other molecules rendering them labelled with different specific activities. The labelling depends on the interaction of freshly liberated ^{131}I or ^{99m}Tc, with these molecules at a favourable pH. Due to the ease of the process, it was applied on egg white, human serum albumin, human serum globulins, insulin, iodotyrosines, thyroxine and drugs containing double bonds as sitosterol, vitamin F, progestrone, methylestosterone and ethisterone.

Purification of the labelled molecules from non-reacting inorganic impurities and separation in pure condition have been achieved by means of gel filtration on Sephadex.

Confirmation of the homogeneity and purity has been done by paper chromatography, paper electrophoresis, ultra-violet or infra-red spectra. Proteins labelled with ^{131}I or ^{99m}Tc have been prepared undenatured, insulin was obtained in biologically active form and other preparations were identical with authentic references. The stability and the yield of labelled compounds have been investigated and proved to be convincing.

J. C. CLARK, H. I. GLASS and D. J. SILVESTER (Medical Research Council Cyclotron Unit, Department of Medical Physics, London, England):

In vitro labelling of red cells with carbon-11

The ready availability of Carbon-11 labelled carbon monoxide from the Medical Research Council's cyclotron has led to the development of a routine technique for labelling red cells with this isotope.

The method of producing the labelled gas and of labelling the cells will be described, and preliminary results of a comparison with the conventional chromium-51 labelling procedure will be presented.

J. C. LAVALLEY, A. JANIN and R. ROMANET (Faculté des Sciences, Université de Caen, Caen, France):

Preparation of alkynes deuterated in predetermined positions.

The preparation of alkynes containing the D—C≡C—group has already been described.

The other deuterated alkynes are prepared by the interaction of an alkyl bromide (deuterated or non-deuterated) with the sodium derivative of an alk-1-yne (deuterated or non-deuterated).

Alkynes with the groups $CD_3—C\equiv C—$
" " " " $R—CD_2—C\equiv C—$
" " " " $CD_3—CH_2—C\equiv C—$
" " " " $CD_3—CD_2—C\equiv C—$
" " " " $CD_2H—CDH—C\equiv C—$
" " " " $CDH_2—CDH—C\equiv C—$
" " " " $CDH_2—CH_2—C\equiv C—$

Under the experimental conditions employed (condensation in liquid ammonia):

— no isotope exchange was observed; in particular, the α-position or positions of the triple bond do not undergo exchange.

— the reactivity of the deuterated alkyl halides used is practically the same as that of the corresponding non-deuterated halides.

P. DIZABO and C. VIVES (Laboratoire de Spectrochimie Moléculaire, Faculté des Sciences, Paris, France):

Synthesis of a series of orthosubstituted D_3 α-toluenes.

In order to study the free rotation of the methyl group by vibration spectroscopy, we prepared a series of orthosubstituted D_3 α toluenes. The labelling had to be specific and the isotopic ratio high.

The only feasible general method is reduction of the corresponding orthosubstituted benzoic ester by means of lithium aluminium deuteride, followed by halogenation of the alcohol thus obtained and dehalogenating reduction of the orthosubstituted α-chlorodideuterio-toluene.

This method has been applied in the case of the substituents X = F, Cl, Br, I, CH_3, and CD_3, but it does not enable orthotoluidine to be obtained directly; it is for this reason that orthotoluic acid and orthocresol were prepared from orthobromotoluene using magnesium orthotolyl bromide.

E. GARD, A. VASILESCU, A. BARABAS and A. T. BALABAN (Institute of Atomic Physics, Laboratory of Labelled Compounds, Bucharest, Roumania):

Preparation of methyl-deuterated aromatic and heterocyclic compounds

As shown previously, benzylic hydrogen atoms of α- and γ-standing, side-chains of pyrylium salts undergo facile deuterium exchange in D_2O or CH_3COOD. The kinetics of this process and the relative reactivity of various pyrylium salts are reported.

Partial deuterations and dedeuterations of 2, 4, 6-trimethylpyrylium perchlorate allow a comparison between the relative reaction rates of α- and γ-standing methyl groups.

Starting from 2,4,6-tri-d_3-methylpyrylium salts, the following methyl-deuterated aromatic and heterocyclic compounds were prepared: 2,4,6-tri-d_3-methylpyridine (withNH_3) 2, 4,6-tri-d_3-methylpyridinium salts and 3, 5,-di-d_3-methylxylidines (with primary amines), 3,5-di-d_3-methylphenol, 1,3,-di-d_3-methylnaphtalene, 4,6,8-tri-d_3-methylazulene, 2,4,6-tri-d_3-methylbenzonitrile, 2,4,6-tri-d_3-methylacetophenone, 2,4,6-tri-d_3-methylnitrobenzene and 3,5-di-d_3-methylfuran. Reaction conditions, IR and NMR spectra are reported and the mechanism of the deuteration of methylpyrylium and methyltropylium salts is discussed.

R. N. RENAUD and L. C. LEITCH (National Research Council, Ottawa, Canada):

Synthesis of deuterated trimethylamine

Contrary to the predictions of von Doering and Hoffman but in agreement with the results of Weygand, Daniel and Simon with tritium, a convenient method of preparing deuterated trimethylamine has been developed which is based on exchange of tetramethylammonium, hydroxide in deuterium oxide between 135 and 150°C. A small amount of quaternary salt decomposes simultaneously to trimethylamine and dimethyl ether, especially at 150°C. By repeating the exchange with fresh deuterium oxide a number of times trimethylamine deuterated in excess of 97% is obtained.

Under the same conditions, phenyltrimethyl ammonium hydroxide gives deuterated trimethylamine, deuterated dibenzyl ether and deuterated benzyl methyl ether. The rates of decomposition and exchange in the compound are too similar to make this a convenient method of preparing these deuterated ethers.

R. N. RENAUD and L. C. LEITCH (National Research Council, Ottawa, Canada):

Synthesis of 3,4,5,6-tetradeuteriobenzene-1,2,-$^{13}C_2$

The synthesis of the title compound employed hexadeuteriobutadiene and maleic-1, 2-$^{13}C_2$ anhydride as starting materials. The Diels-Alder synthesis afforded cis-Δ^4-3,4,5,6-tetradeuteriotetrahydrophtalic-1, 2-$^{13}C_2$ anhydride in good yield. Several methods of converting this compound into the doubly-labelled benzene without randomization of deuterium were tried. Dehydrogenation and decarboxylation in one step by heating the anhydride with phosphorus pentoxide to 200 °C, as reported by Skavorchenko, Levina and Belyavskaya, gave a 40% yield of benzene which contained not only 4 but also 6,5-3- and 2 deuterium atoms and was quite useless for NMR measurements. With lead tetraacetate in dimethyl sulphoxide in the absence of pyridine (which was prohibitive in our case) a mixture of the labelled benzene and dihydrobenzene was obtained under the conditions used by van Tamelen and Pappas or Anet.

This difficulty was overcome by heating the reactants in a sealed tube at 55 °C with excess lead tetraacetate for 2 days. VPC showed no trace of dihydrobenzene in the product and mass analysis indicated 91.5% tetradeuteriobenzene and only 6.4% trideuteriobenzene. There was therefore no randomization.

J. G. ATKINSON, J. J. CSAKVARY, A. T. MORSE and R. S. STUART (Isotopic Laboratories of Merck Sharpe & Dohme of Canada, Ltd., Montreal, Canada):

Base catalyzed deuterium exchange reactions. A simple synthesis of α-deutero carboxylic acids.

A convenient preparative technique has been developed for the synthesis of salts of carboxylic acids deuterated on the carbon *alpha* to the carboxyl group.

It was found that the refluxing of concentrated basic solutions of the acid salts in deuterium oxide brings about ready exchange of the α-protons. The exchange is repeated until the desired extent of deuteration has been reached.

The reaction has been applied successfully to the salts of the following acids:—acetic, propionic, hexanoic, phenylacetic, succinic and glutaric acids. The extent of deuteration in final products is over 98 atom % deuterium.

The same conditions have also been applied to the exchange of amino acids. The exchange is slower in this case, but proceeds satisfactorily. Because of the slower exchange of amino acid alkali metal salts, the copper (II) chelate salts of amino acids have been investigated and found to undergo ready exchange in basic deuterium oxide.

J. G. ATKINSON, D. W. CILLIS, A. T. MORSE and R. S. STUART (Isotopic Laboratories of Merck Sharpe & Dohme of Canada, Ltd., Montreal, Canada):

Base catalyzed deuterium exchange reactions. A new synthesis of formaldehyde-d_2

Because of the usefulness of formaldehyde-d_2 as an intermediate in further syntheses, several new approaches to its preparation in molar quantities have been explored.

Dimethyl-d_6-sulphoxide is readily available, and its intramolecular oxidation reduction has been investigated.

Reaction with hydrogen chloride gas in benzene solution gave rise to formaldehyde-d_2 in about 10% yield, accompanied by considerable amounts of dimethyl-d_6-sulphide.

An alternative two-step synthesis from dimethyl-d_6-sulphoxide proved more successful. Reaction DMSO-d_6 with acetic anhydride gave α-acetoxydimethyl-d_5-sulphide in 80% yield, which was readily hydrolyzed to formaldehyde-d_2 (40%) formaldehyde dithioacetal-d_8 and acetic acid.

Because of the difficulties encountered in obtaining reproducible good yields from DMSO-d_6, an alternative synthesis has been developed as the method of choice for formal-

dehyde-d_2. Methylene bromide and methylene iodide undergo ready exchange when refluxed with basic deuterium oxide to provide the corresponding deuterated materials of high isotopic purity. Reaction of either halide with potassium acetate in refluxing acetic acid followed by hydrolysis, yields pure formaldehyde-d_2 in 50% yield from the deuterated halide.

G. E. CALF, B. D. FISHER and J. L. GARNETT (Department of Physical Chemistry, The University of New South Wales, Sydney, Australia):

Catalytic deuterium exchange reactions with organics. The isotopic hydrogen labelling of cycloalkenes using a homogeneous technique.

A novel homogeneous technique for labelling cycloalkenes and some substituted aromatic compounds with deuterium and/or tritium is discussed. The procedure utilizes the addition-elimination reaction of hydrogen halides to olefins in the presence of isotopic water at temperatures of approximately 100-130 °C. The reactivity of a representative number of cycloolefins is reported to illustrate total isotope incorporation and orientation of isotope within the molecule by the homogeneous technique. For substituted aromatics, the system is an acid catalysed exchange and is therefore suitable for aromatic hydrogens in compounds such as m-xylene and mesitylene which possess hydrogens slow to exchange by heterogeneous methods.

I. KISS, G. JANCSO, G. JAKLI, H. ILLY and K. POROS (Central Research Institute for Physics, Budapest, Hungary):

Application of isotopic exchange in gaschromatographic column for labelling of organic compounds.

Isotopic exchange processes used for the production of labelled compounds can be conveniently, rapidly, and highly efficiently performed in a gaschromatographic column, as shown by several attempts reported in recent literature.

Investigations of the gaschromatographic deuteration of organic compounds using a preparative column are described. The column contains as a stationary phase 25 p.c. diglycerol or 35 p.c. polyethylenglycol on a suitable supporting solid. The hydrogens of the hydroxyl groups in the packing are changed to deuterium by injecting heavy water into the column. Subsequently passing compounds with exchangeable hydrogen atoms through the column, their mobile hydrogens exchange to deuterium of the packing. Adding to the stationary phase alkaline catalyst even the less mobile hydrogen atoms (e.g. those of acetone) can be exchanged with high efficiency.

Some features of the isotopic exchange processes in the gaschromatographic column, analogous to those occuring on ion exchange columns have been studied as well.

J. C. BOUHET and R. CARDINAUD (C.E.N. de Saclay, Gif-sur-Yvette, France):

Stereospecific effect in self-induced tritium-labelling (Wilzbach-Method)

Whereas the exchange induced by recoil tritons occurs at the double bond of maleic acid without configuration inversion, the exchange on this same compound in the solid state in the presence of a tritium atmosphere, or under the action of a high-frequency electrical discharge, is accompanied by considerable formation of labelled trans isomer. Under the same conditions the formation of an adduct, namely succinic acid, was observed. When the proportion of trans isomer formed is reduced by adsorption on an adsorbent carrier, it is found that the quantity of adduct formed is also reduced, which indicates that the adduct is due to a trans addition mechanism. The existence of this mechanism was verified by comparing the reaction results of acetylene-dicarboxamide on various types of carrier and in the absence of any carrier.

The bond formed by the compound with an adsorbent carrier has the effect of giving a predominantly (80%) cis addition compound (maleic acid) while at the same time appre-

ciably decreasing the total quantity of adduct formed, in comparison with the amount of product formed in the absence of carrier. Use was also made of other carriers (catalytic surfaces) which besides increasing the cis percentage also increased the total quantity of products formed. It was therefore shown that the radio-induced addition of tritium takes place via a trans addition mechanism and that by careful choice of the carrier it is possible to obtain the preferential formation of a labelled stereo-isomer.

C. MANTESCU, A. GENUNCHE and A. T. BALABAN (Institute of Atomic Physics, Laboratory of Labelled Compounds, Bucharest, Roumania):

Isotopic exchange of aliphatic and aromatic hydrocarbons and of nitrogen heterocyclics by HTO + $AlCl_3$

In continuation of previous work, the tritiation of n-heptane and of cyclohexane by means of tritiated water in the presence of anhydrous aluminium chloride was investigated, by following the specific activity as function of the ratio HTO: $AlCl_3$ of the temperature and of the competing isomerization.

The tritiation of uracil and adenine by the same reagents was followed as function of the ratios substrate: HTO: $AlCl_3$, of the temperature and the solvent.

Tritiations by means of HTO + $AlCl_3$ allow the labelling of aliphatics (hydrocarbons, detergents, simple polypeptides), aromatics (hydrocarbons, nitro and halo derivatives) and heterocyclics (pyridines, pyrimidines, purines), by electrophilic hydrogen exchange even in the presence of deactivating groups, because $T^+ (AlCl_3OH)^-$ is one of the strongest Brønsted acids known.

G. KASANG, M. WENZEL and P. E. SCHULZE (Physiologisch-Chemisches Institut der Freien Universität Berlin, Deutschland):

Wilzbach-tritiation of a high-specific-activity acetylenic acid.

Whereas in the tritiation of olefins by the Wilzbach method the tritium is predominantly added to double bonds, we have now been able to show that during direct Wilzbach labelling of an alkine-(2) acid hardly any hydration products arise and extraordinarily high specific activities (22 mc/mg) can be attained.

T. GOSZTONYI, A. KOVACS and J. MARTON (N. A. E. C. Institute of Isotopes, Budapest, Hungary):

Tritiation of organic compounds with acid-complexes

The tritiated phosphoric acid-boron trifluoride complex described by Yavorsky and Gorin was found in our experiments to be applicable in tritiation of a variety of organic compounds. In some cases, however, considerable or total damage of the organic compound could be observed in the strongly acidic medium. Therefore we tried the application of a less acidic tritiating agent, i.e. the boron trifluoride complex of acetic labelled with tritium in the carboxylic group. This complex offered milder conditions in tritiation and in some cases the reaction was homogeneous. Specific activities are lower than those obtained by using the phosphoric acid reagent.

Some of our results in tritiation various organic compounds by the phosphoric acid and acetic acid reagent are reported in this paper. Successfull tritiation of cholesterol and proteins by the acetic-boron trifluoride complex are also reported. Comparison of these results with those obtained by using other carboxylic acid-boron trifluoride complexes are given too. The effect of various solvents, reaction time and temperature was also investigated and will be reported.

T. GOSZTONYI, A. KOVACS and J. MARTON (N. A. E. C. Institute of Isotopes, Budapest, Hungary):

Studies on the Wilzbach tritiation technique

In this paper results of a systematic investigation of the Wilzbach technique are reported. The aim of these investigations was to find any correlation between the nature and reactivity of various-CH bonds without performing any degradation or subsequent substitution reactions. Amino acids were chosen as model compounds. Labelling experiments were performed with a series of aliphatic, aromatic and heterocyclic amino acids and substituted derivatives. Specific activities and percentual values of tritium incorporation were determined.

These experiments were performed by using the original Wilzbach technique. Some of the results, however, were compared with those obtained by using microwave discharge as an external energy source.

C. ROSENBLUM, A. M. GERBER, H. T. MERIWETHER and H. E. MERTEL (Merck Sharp & Dohme Research Laboratories, Rahway, U. S. A.):

Experiences with tritium labelling of steroids

Seven steroids with different physiological activities have been labelled with tritium by a variety of methods. Dexamethasone, prednisolone, cholic acid and lithocholic were labelled by exposure of solids to tritium gas. Dexamethasone and prednisolone were labelled by catalytic exchange with tritiated aqueous acetic acid. Hydrocortisone and allodihydrocortisone were tritiated by catalytic hydrogenation of suitable precursors with tritium gas. Dexamethasone, prednisolone and spiroxasone were labelled by chemical treatment following catalytic hydrogenation of suitable precursors. Products were isolated and purified by a variety of crystallization, chromatographic and solvent extraction procedures. Of special use was a partition system for the purification of labelled dexamethasone. Identification was achieved by carrier and chromatographic techniques. Catalytic exchange was found to be more desirable than contact with tritium gas because of higher yields, greater purity and higher specific activities than exposure to comparable quantities of tritium gas. Greatest success was achieved by direct chemical labelling or by chemical processing of catalytically tritiated unsaturated intermediates. Maximum specific activities produced under our conditions were dexamethasone-^{3}H 790 μc/mg; prednisolone 368 μc/mg; hydrocortisone 368 μc/mg; allodihydrocortisone 750 μc/mg; cholic acid 1,530 μc/mg; lithocholic acid 1,450 μc/mg; spiroxasone 643 μc/mg.

A. M. GERBER and C. ROSENBLUM (Merck Sharp & Dohme Research Laboratories, Rahway, U. S. A.):

Tritium labeling of alphamethyldopa and of alphamethyltyrosine analogs by catalytic exchange in aqueous media

Catalytic exchange, normally performed in strong acid solution has shown considerable utility even when carried out in neutral or ammoniacal solution. This was observed during the labeling of alphamethyltyrosine derivatives and of the related L-alphamethyldopa ("Aldomet"). The conventional procedure, i.e., exchange labeling by platinum in concentrated acid such as 70% aqueous acetic acid was applied successfully to L-alphamethyldopa and to L and DL-alphamethyltyrosine. These conditions were too inconvenient for alphamethylmetatyrosine because of isolation difficulties and chemical degradation. This compounds was, however, successfully labeled by catalytic exchange in tritiated water without addition of extraneous electrolyte. Unsuccessful attempts were made to exchange - label 3-iodo-alphamethyltyrosine in acid and in ammoniacal solution. In 18N ammonia solution, the iodo derivative deiodinated quantitatively to produce tritiated alphamethyltyrosine of higher specific activity than was obtained by exchange in acid medium. Accordingly, the 3-iodo derivative has to be prepared synthetically by re-iodination of tritiated methyltyrosine. Specific activities obtained were "Aldomet" 9.3 μC/mg; DL-alphamethyltyrosine 42.5 μC/mg;

L-alphamethyltyrosine 67.1 μC/mg; L-alphamethylmetatyrosine 40.0 μC/mg L-3-iodo-alpha-methyltyrosine 22.5 μC/mg.

"Aldomet" labeled by direct exposure to tritium gas had a lower specific activity (3.9 μC /mg) than was attained by catalytic exchange. Details of the above procedures, as well as systems employed for isolation and purification of labeled products, will be described in detail.

W. MENDELSON, D. BLACKBURN and V. SPAZIANO (Smith Kline & French Laboratories, Philadelphia, U.S.A.):

Synthesis of high specific-activity tritiated phenoxybenzamine

Phenoxybenzamine, an adrenergic blocking agent was labelled with tritium by direct synthesis. Catalytic tritiation of N-(phenoxyisopropyl)-N-(bromobenzyl) ethanolamine gave N-(phenoxyisopropyl)-N-(benzyl-3-^{3}H) ethanolamine which was purified by absorption chromatography. The tritiated ethanolamine was converted to phenoxybenzamine-^{3}H (6.2 C/ mM). Stability studies on various storage forms are presented.

M. BRUHMULLER and M. WENZEL (Physiologisch-Chemisches Institut der Freien Universität Berlin, Deutschland):

Enzymatic tritiation of DPNH-reducible substances over glutamate-2-T with HTO as starting product.

Glutamate-2-T is formed by means of the glutamate-pyruvate-transaminase in HTO. The further transfer of tritium from glutamate-2-T to various DPNH-reducible substrates is effected via a coupled enzyme system with the appropriate dehydrogenase. The oxidative deamination of the glutamic acid, catalysed by glutamate-DH, always constitutes a part of the coupled enzyme system. This yields the DPN-T, which can then serve for the enzymatic reduction of numerous biological substrates:

$$\text{Glutamate-2-H} + \text{HTO} \xrightarrow[\text{transaminase}]{} \text{glutamate-2-T} + H_2O$$

$$\text{Glutamate-2-T} + \text{DPN} + H_2O \xrightarrow[\text{glu-DH}]{} \alpha\text{-ketoglutarate} + \text{DPN-T} + NH_4^+$$

$$\text{DPN-T} + \text{substrate}_{0\alpha} \xrightarrow[\text{substrate-DH}]{} \text{DPN} + \text{substrate-T}_{red}$$

Although only DPN-T of the B type is formed from glutamate-2-T, it is in any case possible, through suitable choice of the DPN concentration, to transfer the tritium from glutamate-2-T to DPNH-reducible substrates, regardless of the DPN-stereospecificity of the dehydrogenases involved.

T. HESSELBO and R. F. LONG (Smith Kline & French Laboratories, Mundells, Herts. England):

The synthesis of tritiated l-noradrenaline of high specific activity

Demethylation of 2-bromo-4,5-dimethoxyphenylethylamine was followed by tritiation in a strongly alkaline solution under N_2 using Pd/C catalyst. The resultant tritiated dihydroxyphenylethylamine (dopamine) after purification, was β-hydroxylated using enzyme from bovine adrenal medulla. The l-noradrenaline formed was separated from unchanged dopamine on a sulphonic acid resin (AG 50 W-x4), purified by adsorption on to alumina and elution with HCl. Determination of noradrenaline fluorimetrically and by bioassay in the pithed rat indicated a specific activity of 26-30 curies/millimole.

A sample of the l-noradrenaline was converted biologically to normetanephrine which was oxidised with periodate to vanillin and diluted with carrier. Chemical substitutions and transformations were carried out and from the specific activities of the compounds obtained it was deduced that tritium had been present in the 2-, 5- and 6-positions of the l-noradrenaline.

F. MOSETTI (Osservatorio Geofisico, Trieste, Italia):

Tritium labelling in hydrological problems

At present time, the tritiated water is probably the most suitable tracer for hydrological researches. Physical and chemical properties of the tritiated water are similar to the normal water itself and do not affect the circulation with significative perturbations, so that it is possible to have quantitative results instead of the qualitative ones as it is the case when using other tracers as colours or other radioisotopes. Modern techniques of measure can, on the contrary, rend significant these small differences of physical properties between tritium and hydrogen to allow precise quantitative measurements starting from a very low limit of dilution.

The quantitative determination derive from the interpretation of the c (t) curve which gives the concentration of the tracer as function of the time. The c (t) curve can be expressed mathematically depending from the injection type and the geometry of the problem. The analysis of the c (t) curve forwards the run velocity, the eddy coefficients, the amount and the distribution of the water in superficial or in subterranean flows. Clear informations in hydrological balance can be therefore obtained by using these methods.

The application of tritium injection in various points simultaneously labelled is possible if different forms versus time (i.e. different c (t) curves) are performed: such a method is equivalent to the employ of different isotopes as the mathematical analysis of the resulting cumulative c (t) curve is capable to separate the individual contributions due to each point of injection.

C. MANTESCU, A. GENUNCHE, E. ROMAS and C. BRATU (Laboratory of Labeled Compounds, Institute of Atomic Physics, Bucharest, Roumania):

Reduction of steroidal ketones with recoil tritiated lithium aluminium hydride

Steroidal carbonyl groups in the androgen and gestogen series were reduced by means of tritiated lithium aluminium hydride (LAH-t) obtained by neutron irradiation of $LiAlH_4$ recoil labeling).

The comparative reducibility of the 3-keto, 17-keto, 20-keto, Δ^4-3-keto, Δ^4-3, 20-diketo, and $\Delta^{5,16}$-20-keto groups with different molar ratios of LAH-t has been established as well as the radiochemical yield as a function of the irradiation conditions of $LiAlH_4$. The behaviour of the α, β- unsaturated keto steroid in the presence of LAH-t and $AlCl_3$, has been studied in order to reduce the carbonyl group to metylene. The occurence of 1, 4- addition in the reduction of the above α, β- unsaturated ketones, with tritiated tetrakis-(N-dihydropyridyl)-aluminate has also been investigated.

The reduced products have been separated and purified by thin layer chromatography and analysed by I.R. and absorption spectra and by polarography. The radiochemical analysis of the spots visualized on the chromatoplates has been performed by burning the eluated compounds and by measuring the activity of tritiated water in gaseous phase in two compensated internal gas counters.

L. KRONRAD and J. CIFKA (The Nuclear Research Institute of Czechoslovak Academy of Sciences, Prague, Czechoslovakia):

Recoil labelling of organic mercury compounds of medical significance

The possibility of preparation of labelled organomercuals ^{197}Hg by direct neutron irradiation was studied. Irradiated material was analyzed by paper chromatography. The activity of ^{197}Hg was found mostly in inorganic fraction and in that of parent molecule. The yield of activity in the lastly mentioned fraction was studied as a function of irradiation conditions. Simultaneously, the radiation decomposition of the target material was established. The influence of postirradiation heating of the target was determined. Optimum conditions for preparation of 3-chloro-mercuri-2-methoxy propyl urea were found.

I. KISS, K. BEREI and L. VASAROS (Central Research Institute for Physics, Budapest, Hungary):

Some possibilities in hot synthesis of radio-halogen labelled organic compounds

Radionuclides formed in nuclear transformation lend themselves well for the production of labelled compounds because of their high chemical reactivity. The method utilizing this fact is called recoil-labelling. The advantages of this technique over the conventional chemical synthesis are that the production of the radionuclide and its incorporation into the molecule of the compound to be labelled takes place in one step and that it offers the possibility of producing compounds of high specific activity.

At our laboratory the investigation of the chemical effects of $^{37}Cl/n\gamma/^{38}Cl$ reaction led to the production of several ^{38}Cl labelled aliphatic and aromatic compounds in carrier free from which could be successfully separated by gas chromatography.

The irradiations were performed in the 2,5 MW VVRS-type reactor using a pneumatic facility developed for fast irradiations. As ^{38}Cl source CCl_4 was used, irradiating always a binary system choosing the additional component to CCl_4 with respect to the compound to be labelled.

The results suggest the method to be useful also for the labelling of similar compounds with other halogens.

H. ELIAS (Technische Hochschule, Darmstadt, Deutschland):

Radiation-induced exchange labelling of aromatic halides.

The main effect of radiation on organic halides is to break the carbon-halogen bond to give an organic free radical and halogen atom. It has been shown by tracer experiments that the G-value for recombination of the radicals is high as compared with the G-values for the formation of radiolysis products.

This opens the possibility of radiation-induced exchange labelling of aromatic halide.

The results obtained with iodobenzene are very good. The application of this method to the labelling of *o*-, *m*- and *p*-iodobenzoic acid and of *o*-iodohippuric acid is discussed.

J. RATUSKY, R. TYKVA and F. SORM (Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Science, Prague, Czekoslovakia):

Carbon-14 labelling of carboxyl groups in aromatic acids by exchange reaction in presence of $^{14}CO_2$

On the basis of a study of intermolecular transcarboxylation reactions occurring during the catalysed, thermal (at 350-450 °C) isomerisation and disproportionation of the salts of aromatic acids, a method of labelling these acids with ^{14}C in all their carboxylic groups proportionally, was elaborated.

By a suitable choice of the quality and specific radioactivity of the starting $^{14}CO_2$, it is possible to obtain the product (for example benzoic, benzene-dicarboxylic, 2,6-naphtalene-dicarboxylic, 2,5-pyridine-dicarboxylic acids etc...) in highly radioactive form. Moreover the specific radioactivity (per millimole) of the resulting acids often may even approach that of the starting $^{14}CO_2$.

The method consists in carrying out these reactions (which take place with ionic cleavage of the C-C bond between the carboxylic group and the aromatic nucleus) under an $^{14}CO_2$ atmosphere. Closer examination of this reaction showed that there was an extremely fast exchange between the released carbon dioxide and the labelled carbon dioxide of the reaction atmosphere.

On the basis of these $^{14}CO_2$ incorporation studies, and on the basis of the compositions of the reaction mixtures formed during these transcarboxylation reactions, a probable mechanism could be constructed to explain the experimental data.

D. S. URCH, M. J. WELCH and A. JOHNSTON (Queen Mary College, London, England):

The recoil tritium labelling reaction as a function of tritium energy and molecular size

Hydrocarbons can easily be labelled by recoil tritium atoms. Near the end of the recoil track, after the triton has been neutralized and whilst it still retains some excess translational energy, a tritium atom can replace hydrogen atoms in CH bonds with high collision efficiency. Results of studies of this reaction in mixtures of hydrocarbons (ethane, n-butane-ethane, neo-pentane-athane, trans-butane-2-propane, ethylène, trans-butane-2) are analysed using the Estrup-Wolfgang method. It is found that the reactivity integral for the labelling reaction increases with the size of the molecule labelled and that it is larger for an alkene than for the corresponding alkanes. Furthermore, it is shown that the labelling reaction commences at relatively higher tritium atom energies as the size of the molecule is increased. The implications of these results for the formation of tracer amounts of tritium labelled hydrocarbons are discussed.

I. C. GEDDES (Liverpool University, Liverpool, England):

Preparation of ^{82}brominelabelled halothane

Samples of halothane of varying degrees of purity have been encapsulated in 10 ml silica ampoules and subjected to irradiation by neutrons in either the BEPO. reactor at Harwell or in the University Research Reactor at Risley. Following decay of ^{80}Br it was established that the isotope present was ^{82}Br by its half life and the gamma spectrum.

Analysis of samples for chemical purity was by means of gas chromatography using a flame ionisation detector. Samples were also analysed for radioactive purity by a combination of gas chromatography and scintillation counting.

In early experiments impure halothane was irradiated and details of the impurities detected will be presented. Preparative gas chromatography has been used to purify the halothane before irradiation and also to remove impurities found to be present following activation.

Evidence will be presented to demonstrate that ^{82}Br labelled halothane can be obtained with chemical impurities less than 5 parts per million and that all the radioactivity is associated with the halothane peak on the gas chromatograph with different stationary phases.

S. APELGOT and M. FRILLEY (Institut du Radium, Laboratoire Curie, Paris, France):

Comparison of the self-decomposition processes of ^{3}H-thymidine, kept as aqueous solutions containing 10% or no glycerol

In aqueous solutions (no glycerol), the velocity of the ^{3}H-thymidine self-decomposition is maximum at $-20°$C, minimum at -196 °C intermediary and the same at 0 and -75 °C. If the solutions are frozen at -196 °C and stored at -20 °C, the velocity of self-decomposition decreases. These results and those obtained by X-ray radiolysis have led us to assume that the frozen solutions have a heterogeneous structure.

Autoradiography has confirmed our previous results showing the heterogeneous distribution of the solute molecules in the frozen aqueous solution (0% glycerol), from a macroscopic point of view and also a melocular scale. In the case of aqueous solutions containing 10% of glycerol, the macroscopic heterogeneity is diminished.

The study of the velocity of the self-decomposition of ^{3}H-thymidine in aqueous solutions containing 10% glycerol has given unexpected results, different from these obtained in the absence of glycerol: this velocity is maximum at -75 °C, minimum and nearly the same at $-196°$ and 0 °C, intermediary at -20 °C. If freezing is carried out at -196 °C and storage at -20 °C, this does not change the results.

All these results will be fully discussed in the paper.

Y. COHEN, J. BRALET and P. FRAPART (C.E.N. de Saclay, Gif-sur-Yvette, France):

Radiochemical purity and storage of thyroxine labelled with iodine-125 or iodine-131

The importance of radiochemical purity in thyroxine for biological applications is emphasized; a review is given of the paper or thin-layer chromatographic analysis methods that have so far been described.

An original method of thin-layer chromatography is proposed and descriptions are given of the impurities detected, whether or not they were identified. This method is used to investigate the factors that can cause degradation of the compound, namely:

1. The influence of the chromatographic analysis technique itself, which is liable to give rise to deiodination of the molecule;
2. The effect of storage: study of the radiochemical purity of ^{125}I or ^{131}I-labelled thyroxine as a function of time.

Finally, radioiodinated thyroxine preparations of various origins are compared in respect of their radiochemical purity.

E. A. EVANS, W. R. WATERFIELD and R. H. GREEN (The Radiochemical Centre, Amersham, England):

Preparation and stability of generally and specifically tritiated amino-acids and their behaviour with biological oxidases

The methods available for preparing tritiated amino-acids are briefly reviewed. The preparation is generally labelled DL-, D- and L-amino-acids by platinum catalyzed exchange in tritiated water is described and the extent of racemization of the L- and D-isomers under the exchange reaction conditions determined by reverse isotope dilution analysis. The results are compared with the labelling of amino-acids by exposure to tritium gas (Wilzbach method).

The preparation of some specifically tritiated amino-acids by catalytic hydrogenation and by halogen-tritium exchange, are also described.

The labilization of the tritium atoms during the reaction of the tritiated DL-amino-acids with biological oxidases is discussed.

H. C. HEINRICH (Physiologisch-Chemisches Institut der Universität Hamburg, Deutschland):

Purity and stability of radioactive vitamin B_{12}

Commercial and research preparations of Co-labelled cyanocobalamin, aquocobalamin and vitamin B_{12}-coenzyme are normally contaminated with other labelled vitamin B_{12}-compounds and contain sometimes between 10-95% of such occasionally bioinert or even inhibitory vitamin B_{12}-analogues. This contamination is caused:

1. By the application of insufficient purification procedures;
2. By the light sensitivity of the vitamin B_{12}-coenzyme and also of very dilute cyanocobalamin solutions;
3. By the reaction of aquocobalamin or vitamin B_{12}-coenzymes with CN, SO_2, etc;
4. By self-irridiation degradation products if the stored solutions contain much more than 1 μC/ml.

Suitable analytical systems for the examination of the purity of labelled cyanocobalamin, aquocobalamin and vitamin B_{12}-coenzyme solutions are the paperelectrophoretic separation on highly acetylated and carboxyl paper.

At least two different pH values (2.7 and 8.9). Microcolumns made of carboxymethylcellulose or -dextrangel were used for the chromatographic examination and also the microscale purification of Co-labelled cyanocobalamin, aquocobalamin and vitamin B_{12}-coenzym.

F. BERTHOLD (Wildbad, Deutschland):

New methods for the assay of radioactivity in chromatography

When working with the radioactively marked substances the control of the radiochemical purity is indispensable. Depending on the method of labelling, undesired by-products are formed. Likewise, a substance pure when produced may show impurities caused, for instance, by auto-radiolysis, when the experiments are performed.

Fractionating procedures, as paper, thin-layer, gas and column chromatography, have proven to be important tools. It is, therefore, important to make available efficient radioactivity measuring methods. Not only accuracy and sensitivity but also simplicity, rapidity and, if possible, the automatic procedure of the measurement is of importance. Different new developments will be discussed under this aspect.

In connection with the thin-layer scanner an improved recording device is described, providing a quantitive record of both radioactivity and its location in a two-dimensional paper or thin-layer chromatogram.

There is a lack of satisfactory methods for measuring soft β-emitters (^{3}H, ^{14}C) in column eluates. Some results obtained with a new equipment are reported. The liquid is dropped continuously unto a moving belt, the solvent evaporated and the radioactive residue measured under the 2π counter tube of the thin-layer scanner. High counting yields are obtained of up to 50% memory effects do not occur as with scintillation counter flow cells.

P. D. KLEIN, D. W. SIMBORG and B. A. KUNZE-FALKNER (Division of Biological and Medical Research, Argonne National Laboratory, Argonne, U.S.A.):

The establishment of radiochemical purity by chromatographic means

Chromatographic purity is judged by several criteria: as the appearance of a single homogeneous peak or spot on the chromatogram and (in more sophisticated fashion) as a constant specific activity in successive fractions across the chromatographic peak. Both of these criteria *may* be indicative of radiochemical purity, but it is important to recognize the assumptions on which they are based and the circumstances in which the assumptions may not be fulfilled. These assumptions are based upon the "mesh size" of the chromatogram and upon the belief that in all regards the radiochemically labelled molecules and the unlabelled molecule are equivalent. Recent work in our laboratory has shown these beliefs to be fallible. A variety of instances in which the radioactivity and mass did not behave in an identical fashion in a chromatographic system have now been observed and studied. From these instances and from a mathematical description of the chromatographic process, equations describing the specific activity of successive fractions have been derived for the following situations: simple isotope fractionation without contamination, isotope fractionation with differences in dispersion, simple contamination, and contamination in the presence of isotope fractionation with and without differences in dispersion.

H. C. HEINRICH, E. E. GABBE and B. MEINEKE (Physiologisch-Chemisches Institut der Universität Hamburg, Deutschland):

Organometallic Hg-compounds, their purity and metabolism in man

The purity and whole body metabolism of ^{203}Hg-mersalyl and ^{203}Hg-Chlormerodrin was studied by paperchromatography and whole body counting. Commercial ^{203}Hg-chlormerodrin preparations were found to contain 2-3 fractions in ascending paperchromatography with five different solvent systems. The main ^{203}Hg-chlormerodrin fraction was not identical with the main fraction of a commercial preparation of unlabelled chlormerodrin.

The whole body retention, excretion and plasma clearance of ^{203}Hg was measured in normal human volunteers following the intravenous injection $^{203}Hg^{++}Cl_2$, ^{203}Hg-mersalyl and ^{203}Hg-chlormerodrin. Considerable differences in the elimination rate as well as different whole body retention were observed with the three ^{203}Hg-compounds during the first week. The biological half life of the ^{203}Hg in the human whole body was however finally identical for all three compounds.

P. PEYSER (Biochemistry Department, New England Nuclear Corporation, Boston, U.S.A.):

The design of criteria of purity for labelled compounds.

A major parameter in the valid application of tracer methodology employing isotopically labelled compounds is the purity of the tracer employed. The ultimate requirement of purity rests upon the design of the experiment, which must allow for the nature of the impurities that might be present, and the levels that are permissible and yet yield meaningful data from which valid conclusions may be drawn.

The situation which exists today, where most investigators no longer prepare the labelled compounds they employ, but obtain them from commercial laboratories, the criteria of purity that are used cannot always satisfy each and every investigator's requirements.

Our own experiences with the design of criteria of purity for biosynthetically prepared labelled compounds, with particular emphasis on carbon-14, uniformly labelled amino acids, will be discussed. Briefly, the techniques we employ are designed to detect and quantitate the impurities that are likely to occur. A "likely impurity" would depend upon (1) the nature of the precursor(s) employed; (2) the side reactions they may undergo, due to such factors as contaminating enzymes; (3) the simultaneous formation of a variety of products as in the case of microbial syntheses; (4) the methods of isolation and purification employed; (5) consideration of possible breakdown products that may arise due to chemical instability.

S. BOROCHOWSKI and P. CZERNIAK (Department of Radiotherapy & Isotopes, Tel-Hashomer Hospital, Israel):

Organization and activity of a labeling unit in a clinical isotope laboratory.

The establishment of a small labeling Unit in our Radiotherapy & Isotopes Department of a General Hospital will be presented.

The possibilities and methodology of labeling of small amounts of Radioactive Rose Bengal, $^{131/125}$I Hippuran, $^{131/125}$I Triolein and $^{131/125}$I Oleic acid, ^{203}Hg Neohydrine, RHISA will be detailed including the purification and control devices. The clinical and other advantages will be stressed out.

The establishment of a small unit is especially valuable for a medical centre, far situated from a radiochemical centre which usually supply labeled substances.

T. SZARVAS, Cs. OMBOLY and G. VECH (N.A.E.C. Institute of Isotopes, Budapest, Hungary):

Activity measurements of tritium in form of ammonia

A new method is developed for activity measurement of tritium in form of ammonia. The basic process is the conversion of HTO on barium cyanamide to ammonia.

By using this reaction the tritiated ammonia can be measured in proportional gas counters, in ionization chambers or in liquid scintillation counters as well with an error of determination of $\pm$ 0,5% rel.

The method has the following advantages:

1. The conversion of HTO with BaNCN is quantitative and no isotop effect can be observed;
2. Ammonia has good counting properties in proportional region;
3. The reaction between HTO and BaNCN is very fast;
4. No memory effect in the bombe tube reaction can be observed.

S. MLINKO, I. GACS and T. SZARVAS (Central Research Institute for Chemistry of the Hungarian Academy of Sciences, and Isotope Research Institute of the National Atomic Energy Commission, Budapest, Hungary):

Production of carbon dioxide counting gas for the gas analysis of ^{14}C-labelled organic compounds in the proportional region

Experiments performed by the authors aimed at producing for Carbon-14 analysis carbon dioxide counting gas with the use of manganous oxide. The very great affinity between the latter and oxygen is intense enough to satisfy the most stringent requirements in respect of gas purity.

^{14}C-labelled organic compounds are combusted in air flow in presence of a Co^{II-III} oxide catalyst and the oxygen content of the air removed by a copper filling placed into the combustion tube. The carbon dioxide leaving the combustion tube is passed—with the nitrogen carrier gas—through a manganous oxide filled reactor, and is meanwhile transformed into counting gas.

Subsequently the active carbon dioxide gas is frozen out in the trap of an atmospheric counting tube, the latter is subjected to reduced pressure, the carbon dioxide is evaporated, the gas deficiency arising in the counting tube, being replaced from an outside carbon dioxide source up to one atmospheric pressure.

Analytical results—yielding activity values for weighed sample—proved to be similary reproducible with microanalytical accuracy, as those obtained by our former methods.

A. PASTERNAK and K. ZELENAY (Institute of Nuclear Research, Swierk, Otwock, Poland):

On some difficulties arising during the elaboration of methods for the routine radiochemical purity control of preparations labelled with short-lived isotopes

The methods for routine control of radioactive preparations, especially these labelled with short-lived isotopes, should not include complicated manipulations, should have a good accuracy and precision. From many separation methods for routine control commonly chromatography and electrophoresis are used.

The choice of a proper method for the radiochemical control of radioactive preparations is sometimes not easy. Many parameters, difficult for taken in consideration a priori, have some influence on the results of analysis.

The examples of this kind parameters are: changes in the composition of samples after dilution, influence of kinds and amounts of carriers presented or added to the sample before analysis, stability of the analysed compounds in the time of storage.

Sometimes, it is also necessary to elaborate a new method or methodology for the activity measurements. Another difficulty arising from the formation of artefacts in the process of analysis and presence of some nonidentified chemical forms in the sample.

Some compounds labelled with ^{32}P and ^{131}I are used as illustration.

D. BLACKBURN, G. BURGHARD and A. POST (Smith Kline & French Laboratories, Philadelphia, U.S.A.):

Purification of radiochemicals by phase solubility equilibria.

Although phase solubility equilibrium is often applied to the assay and proof of purity of complex materials such as steroids and peptides, it has less frequently been applied to the purification of solid organic chemicals. It would appear that phase solubility equilibrium would be of value in the removal of high specific-activity impurities from radiochemicals. The presence of radiochemical impurities often occurs unexpectedly in the tritiation of organic compounds. Two examples of the application of solubility phase equilibrium to the purification of pharmacologically active compounds are presented:

1. A high specific-activity contaminant was found in a purified 1-^{3}H-3-one-4-ene steroid prepared by the catalytic tritiation of the precursor diene-3-one. Two successive solubility phase equilibria with 70% methanol/cyclohexane raised the radiochemical purity from 48% to 94%;

2. A dechlorinated radiocontaminant was formed in the catalytic tritiation of the unsaturated precursor to chloropheniramine. The application of solubility phase equilibrium to the maleate salt gave an acceptable chemical and radiochemical purity of 98-99%.

J. HASAN and J. PERHEENTUPA (Institute of Occupational Health and Children's Clinic, University of Helsinki, Helsinki, Finland):

Efficient, economical, and safe production of bio-organic compounds labelled with isotopes of hydrogen and/or carbon by photosynthesis in green leaves.

The production of natural compounds labelled with tritium or tritium and radiocarbon by biosynthesis in green leaves presents special problems not encountered in labelling with the latter only: (a) dilution of radioactive substrate by inactive labile hydrogen in water and organic matter in plant; (b) loss of substrate by evaporation; (c) the presence of volatile material of possibly high specific activity in the system at conclusion of the production period creates a biological radiation hazard and risk of environmental contamination; (d) for multiple labelling, an optimal amount of each substrate has to be introduced without loss or dilution of other substrates.

An apparatus overcoming these problems has been designed for the production of material labelled with isotopes of hydrogen and/or carbon (as well as oxygen), derived from respective labelled oxides. Efficient, economical, and safe operation is achieved by recirculating the substrate water in the plant, and by performing all manipulations on labelled substrates, including quantitative transfer, within a closed, evacuated miniature vacuum line equipped with suitable projections for local condensation by external chilling.

P. LERCH, A. DELAY and R. LEMP (Institute of Applied Radiophysics, University of Lausanne, Lausanne, Switzerland) :

Methods of preparing labelled microcrystalline alkaline-earth orthophosphates for the physicochemical study of calcification

The authors describe several methods by means of which they are able to prepare the alkaline-earth orthophosphates which play a part in the calcification process. These compounds are labelled with ^{32}P, ^{45}Ca or ^{89}Sr and ^{3}H and are used for the study of heterogeneous exchange reactions in systems which are intended to reproduce in vitro the calcification conditions in the bone tissue or any other part of the body. The compounds studied are distinguished by their Ca/P or Sr/P ratios and their varying degrees of hydration; since their crystal latices are very similar they form mixed compounds the composition of which can be determined only by physicochemical and radiochemical investigation methods; owing to the very small size of the microcrystals and their high adsorption capacity, the conventional chemical analyses are in fact totally inadequate. The techniques for ascertaining the microcrystalline structure are reviewed and criticized. Finally, the methods for simultaneous measurement of the β-activity of the three tracers used for the labelling are described and the selection criteria and optimum fields for the use of these methods are discussed.

N. PAWELETZ and H. LETTRE (Institut für Experimentelle Krebsforschung der Universität Heidelberg, Deutschland):

A method for localizing active particles in electronmicroscopic radioautographs

The photographic development of electronmicroscopic radioautographs with methods, frequently cited, causes the silver-grain to appear as a tangled skein. This makes it difficult to determine the relationship of the marking to the underlying structure.

In cooperation with the Institute for Applied Physics of the University of Frankfurt a method was established for a better localization of the active particles. The new developing procedure causes the silver-grain to grow in concentric layers around the primary grain. Thus the locality of the radiating particles of the underlying structure can be determined. The size of the grains depends on the temperature during development.

Using suitable experimental conditions, one obtains a grain-size that allows a distinct marking and facilitates localizing of the active particles.

2 — REVIEWS

H. LETTRE (Institut für Experimentelle Krebsforschung der Universität Heidelberg, Deutschland):

Recent results in cancer research using labelled compounds

Cancer research involves three different problems: 1) the formation of cancer cells, 2) the qualities of cancer cells, and 3) the inhibition of cancer cells. In all these fields, labelled molecules can be applied and have improved the analysis of the phenomena. Examples are given of the use of labelled carcinogenic compounds, of metabolism intermediates and of cytotoxic compounds. Synthesis of nuclear acids and of histones will be described as well as the change in these syntheses under the action of cytotoxic compounds. The use of labelled molecules for the study of the host-tumor relationship will also be dealt with.

L. PICHAT (Service des Molécules Marquées, C. E. N. de Saclay, Gif-sur-Yvette, France):

Some recent chemical synthesis of labelled compounds.

This lecture presents a bibliography of synthesis published since the end of 1963 and is confined to compounds labelled with carbon-14, sulphur 35, tritium and deuterium. The use of newer methods of organic chemistry for the preparation of labelled compounds will be evoked such as: Wittig reaction, vinylmagnesium bromide as well as exchanges on column chromatography for the obtention of deuterium or tritium labelled molecules. The new methods published for the preparation of basic molecules such as formic acid-^{14}C will be mentionned.

Some examples of synthesis, chosen among various compounds of biological interest will be described in the following classes: fatty acids, aminoacids, catecholamines, nucleosides, cardioglycosides, sugars, vitamines, steroides, insecticides, labelled organic reagents. Some observations published on the self radiolysis of a few tagged compounds will be pointed out. It should be stressed that this review does not pretend to be by no means exhaustive.

H. K. MANGOLD (University of Minnesota, The Hormel Institute, Austin, U.S.A.):

Synthesis and biosynthesis of labelled lipids

During the last decade, efficient new methods, particularly thin-layer chromatography and gas-liquid chromatography, have made it possible to assess the purity of labelled lipids. Analyses of commercial preparations have shown that these often were grossly contaminated with both "hot" and "cold" intermediates in their synthesis.—Typical examples of the use of chromatographic methods in the analysis and purification of labelled lipids will be presented. Significant advances have been made in the synthesis of ^{14}C-labelled unsaturated fatty acids and in the preparation of ^{3}H-labelled unsaturated fatty acids by stereospecific catalytic reduction of acetylenic acids. The use of methanesulphonates has facilitated the preparation, in good yield and high purity, of labelled long-chain hydrocarbons, halides, aldehydes, acids, and a variety of alkoxylipids. Complex ionic lipids, such as lecithins also have been synthesized in labelled form.—Improved synthetic routes will be described in detail. Uniformly labelled fatty acids, as well as phospholipids, sulpholipids, and glycolipids have been isolated chromatographically from microorganisms grown in radioactive media. Current biosynthetic methods will be discussed and possible new approaches will be outlined. Metabolic studies and clinical tests have been placed on a more sound basis through the availability of pure labelled compounds.—Examples will be given to point out erroneous conclusions that have resulted from the use of impure preparations.

R. PAOLETTI, E. GROSSI PAOLETTI, and G. GALLI (Università degli studi di Milano, Istituto di Farmacologia, Milano, Italia):

The use of drugs in biosynthetic preparation of labelled sterols and sterol precursors.

Cholesterol biosynthesis in various mammalian tissues (liver, brain) has been investigated starting from labelled precurosrs (^{14}C-Acetate, ^{14}C-Mevalonate). The techniques used for the separation, identification and purification of the sterol precursors of cholesterol are reviewed in detail.

The accumulation of some sterol precursors is obtained in mammalian tissues by using specific inhibitors acting at known enzymatic levels in the biosynthetic pathways.

For example treatment of growing rats with AY 9944 (trans-1.4-bis (2-dichlorobenzyl-aminoethyl)-cyclohexane dihydrochloride, an agent known to inhibit the Δ^7-reductase, induced a well detectable accumulation of labelled $\Delta^{5,7}$-cholestadien-3 beta-d, $\Delta^{7,24}$-cholestadien-3 beta-ol and $\Delta^{5,7,24}$ cholestadien-3 beta-ol. Treatment of adult rats with AY 9944 induces accumulation of $\Delta^{5,7}$-cholestadien-3 beta-ol. The concentration of AY 9944 with other enzymatic inhibitors active on sterol biosynthesis, gives ample possibilities to induce accumulation of a variety of labelled sterol precursors of cholesterol, which may be isolated in pure form.

The implications of this approach for obtaining pure labelled compounds in amounts sufficient for biological research are discussed.

G. JUPPE (Euratom C. C. R., Ispra, Italy):

Synthesis of ^{14}C- and T-labelled Di-and terphenyls

The reactions of phenyllithium with 2- and 4-phenylcyclohexanone yielded the two isomeric double phenyl substituted cyclohexanols. The following isomerisation gave a good yield *o*- and *p*-terphenyl respectively. Starting with phenyllithium and cyclohexanones, ^{14}C labelled at different positions of the molecules, the following terphenyls were synthesized on a 100 mg scale, each of them having a specific activity of between 2 an 20 μC/m mole: *o*-terphenyl-1-^{14}C, *p*-terphenyl-1-^{14}C, *o*-terphenyl-1′-^{14}C, *p*-terphenyl-1′-^{14}C, *o*-terphenyl-3-^{14}C, *p*-terphenyl-3-^{14}C, *o*-terphenyl-^{14}C, *p*-terphenyl-4-^{14}C, *o*-terphenyl-(1,2,3,4,5,6)-^{14}C, *p*-terphenyl-(1,2,3,4,5,6)-^{14}C, and *p*-terphenyl-(1′,2′,3′,4′,5′,6)-^{14}C.

Degradation reactions were studied in order to prove the specificity of the radioactive label.

The tritiated compounds biphenyl-2-T, -3-T, -4-T and *p*-terphenyl-2-T, -3-T, -4-T, -2′-T have been synthesized by catalytic reduction of the corresponding bromobiphenyls and bromo-*p*-terphenyls respectively. Specific activities of app. 10 mC/mg were obtained. For each of the labelled compounds the activity distribution over the various positions in the molecule was determined by degradation.

R. CARDINAUD (C.E.N. de Saclay, Gif-sur-Yvette, France):

Nucleic acids, nucleotides and nucleosides labelled by biosynthesis

Labelled nucleic acids and derivatives thereof with very high specific activities can be obtained by means of biosynthesis techniques.

1. The general labelling of DNA and RNA with ^{14}C;
2. The specific labelling of DNA and RNA bases with ^{14}C, ^{3}H or ^{32}P;
3. Obtaining 5′ and 3′ monophosphonucleotides by means of controlled degradation;
4. Obtaining RNA diphosphonucleotides by means of specific degradation;
5. The synthesis of ribosides and *d*-ribosides labelled either on the base or on the ribose or *d*-ribose;
6. The synthesis of mono-, di- and triphosphonucleotides labelled either with ^{14}C (base and ribose or *d*-ribose) or with ^{32}P in the α, β or γ positions as required.

A critical evaluation of the possible routes facilitates the choice of the best one for the purpose in view.

A. T. BALABAN and D. FARCASIU (Laboratory of Labelled Compounds, Institute of Atomic Physics, Bucharest, Roumania):

Intramolecular isotopic rearrangements of carbon atoms

The chemical and photochemical processes leading to rearrangements of carbon atoms in aliphatic or aromatic compounds, without changing their chemical nature, are reviewed. These processes may be termed "intramolecular isotopic exchange reactions" and consist in a change of places between carbon atoms within the molecule.

Examples of such processes are the isotopic isomerizations of saturated chains in alkanes, cycloalkanes or phenylalkanes proceeding by 1,2-shifts in carbonium ions under the influence of Lewis acids. In the aromatic series, the same catalysts bring about the change of the attachment site in toluene and biphenyl or, as shown in the authors' laboratory, cause shuffling of carbon atoms in polycyclic aromatic hydrocarbons. Spectacular photochemical isotopic rearrangements of the ring carbon atoms in monocyclic aromatics, proceeding by way of valence isomers, were recently reported. The mechanisms of these processes are described.

It is concluded that such intramolecular isotopic exchange reactions show that the carbon framework is more "lively" than hitherto supposed: there exist indeed systems which apparently do not change chemically but are nevertheless undergoing such exchange processes, which are unobservable in the absence of labelling.

D. SAMUEL (Weizmann Institute of Science, Isotope Department, Rehovoth, Israel):

The art of marking molecules with stable isotopes

Although many labelled compounds are commercially available, and in spite of the fact that radioisotopes are often more convenient as tracers, new compounds labelled with stable isotopes are constantly required for studies of structure and reaction mechanisms. The preparation of molecules labelled with stable isotopes such as nitrobenzene *p*-benzoquinone, tetraborane, glycine and other aliphatic molecules, each labelled in different positions, with deuterium, ^{10}B, ^{13}C, ^{15}N, ^{17}O and ^{18}O will be outlined. Problems of double labelling, symmetrical labelling and particularly stereospecific labelling will also be discussed briefly.

Future trends in the use of molecules labelled with stable isotopes appear to be in the fundamental understanding of molecular structure and in the application of isotope effects and stereochemistry to the elucidation of chemical and particularly biological processes. For this purpose, increasing emphasis is being placed on the preparation of large molecules and a start has now been made in preparing labelled biopolymers and synthetic polymers.

It appears that with the techniques and tools now available, new ways of understanding chemical and biological processes, using compounds labelled with stable isotopes, are now possible.

R. WOLFGANG (Yale University, New Haven, U.S.A.):

Mechanisms and intrinsic limitations of direct tritium labelling processes

This review seeks to correlate the achievements and potentialities of direct tritium labelling processes with their basic mechanisms.

In the recoil technique, kinetic energy causes labelling by quite specific mechanisms, particularly exchange with bound hydrogen.

Hence tritium is incorporated with high and relatively uniform efficiency. The nature and amounts of labelled impurities are predictable and they can usually be readily separated. However, since with each tritium there is associated 10^5-10^6 e.v. of radiation energy, only relatively low specific activities are obtainable without extensive decomposition. In T_2 gas exposure methods, whether driven by decay energy, electrical discharge or other means, the energy associated with each active tritium can be much smaller. Correspondingly larger specific activities should thus be possible and are usually achieved. However, the underlying chemical mechanisms appear to be very complex and are poorly understood. Labelling efficiencies vary widely, and the nature, amounts and feasibility of separation of active impurities are difficult to anticipate. In beam techniques, a low energy (1-100 e.v. tritium species,

either ionic or neutral, impinges on the material to be labelled. Such methods can, in principle, avoid the difficulties inherent to recoil and exposure methods. Because, as in the recoil method, a definite species is involved, reaction mechanisms can be readily investigated and labelling efficiencies be predicted.

J. G. BURR, M. CHER, and J. Y. YANG (North American Aviation Science Centre, Camino dos Rios. U.S.A.):

The use of labeled molecules in the study of organic radiation chemistry

Non-linear interactions between solvent and solute are observed in the radiolysis of binary organic mixtures. The use of labeled molecules to diagnose both the nature of the excited solvent species and also the nature of the interaction between these excited species and the solute molecules will be described.

The particular systems discussed in detail will be: (1) cyclohexane + benzene-^{14}C; (2) tritiated cyclohexane + benzene-^{14}C; (3) tritiated cyclohexane + benzoquinone; (4) tritiated cyclohexane + cyclopentene; (5) cyclohexane + cyclohexene-^{14}C; (6) cyclohexane or cyclohexane-d_{12} + benzene or benzene-d_6; (7) cyclohexane-d_{12} + cyclopentane; (8) various mixture of cyclohexane, cyclopentane and deuterated analogs with corresponding olefins and the deuterated olefins; (9) mixtures of light and heavy water vapor with cyclopentane, methane, cyclohexane and other hydrocarbons.

Analysis of the products obtained in the irradiation of these systems has been informative about the nature of the quenching and scavenging processes which take place and also about the nature of the reactive species involved.

A. P. WOLF (Chemistry Department Brookhaven, National Laboratory, Upton, U.S.A.):

Labelling of organic compounds by irradiation methods

The method of labelling organic compounds fall into three major classes. The most common class encompasses synthetic organic reactions and catalytic methods of atom addition or substitution. The second class involves the use of biosynthetic methods in which a particular strain of bacteria or a plant or some other organism acts as an intermediate in labelling the organic compound. The third class involves irradiation of a compound or mixture, or a decay process in bringing about labelling. Generally then, we have: I. Synthetic methods; II. Biosynthetic methods; and III. Irradiation methods.

Irradiation methods have seen considerably less use as a labelling tool than have the more conventional methods. The purpose of this paper is to review some of these methods and where applicable indicate their superiority to methods in classes I and II.

Irradiation methods can be further subdivised into:

a. Methods involving the use of an *in situ* source of radiation;
b. Methods involving the use of sources of radiation other than that from the labelling agent.
c. Methods involving nuclear recoil or fission;
d. Methods involving accelerated ion beams;
e. Methods involving molecular and atomic excitation.

A. EKSTROM and J. L. GARNETT (Department of Physical Chemistry, The University of New South Wales, and The Australian Atomic Energy Commission, Sidney, Australia):

Studies in the protection effect of aromatic compounds on the yields of radiolysis products from methanol using deuterated benzene

The radiolysis of methanol-benzene solutions has been studied with particular emphasis on the chemical fate of the added benzene. It was found possible to identify scavenging products such as cyclohexadiene-methanol, anisole, 1,4-cyclohexadiene and phenyl-cyclohexadiene. The benzene concentration dependency of the yields of these products has been

measured. Perdeuterated benzene has been used to study the isotope effects associated with the formation of these compounds and also to elucidate mechanistic aspects of the reaction. The results of the work should be of importance in determining improved methods for storing radioactive labelled compounds by minimising radiation-induced self-decomposition.

J. L. GARNETT (Department of Physical Chemistry, The University of New South Wales, Sydney, Australia):

Recent developments in heterogeneous catalytic labelling with deuterium and tritium

A very versatile method for either specific or random labelling of organic molecules with deuterium or tritium is heterogeneous exchange with isotopic water catalysed by the transition metals of Group VIII. A II-complex theory for the prediction of isotope incorporation in molecules labelled by this method has recently been developed.

A review article involving aspects of this theory pertinent to catalysis has been published, The present paper reviews the potential of the heterogeneous catalytic exchange method as a synthetic deuterium and tritium labelling tool, in particular the application of II-complex theory in predicting (a) total isotope incorporation and (b) isotope orientation within a given molecule. Organic systems treated will include aliphatics, aromatics, heterocyclics and also series of biological interest including the steroids and optically active compounds such as the amino- and hydroxy- acids. Types of catalysts used and methods of catalyst activation comprising self-activation, borohydride and hydrogen reduction of metal oxides and chlorides, will be discussed with particular reference to the efficiency of the resulting catalyst in exchange. A summary will be made of the advantages of heterogeneous catalytic labelling methods when compared with radiation-induced techniques.

P. JORDAN (Organisch-Chemisches Institut, Zürich, Switzerland):

Recent progress and present trends in the measurement of radioactive tracers

The author attempts to assess the situation on the basis of the publications that had appeared up to the end of June 1966, with particular reference to work on organic labelled substances.

The technique of proportional counting in the gas phase has made important progress as regards the accuracy and reliability of the measurements and can now be used for the simultaneous determination of ^{3}H and ^{14}C. The technique of liquid scintillation counting has been passing through a period of consolidation comprising numerous minor improvements mainly in the electronic field. As from next year, a new process, which has just been announced, for calibration by means of an auxiliary gamma source will enable this technique to overcome the problem of extinction of the fluorescence. The techniques of thin-layer and gas-phase radiochromatography have come into general use in laboratories, while semi-conductor detectors, which are only just beginning to appear in laboratory practice, offer considerable prospects for the future.