Journal of Labelled Compounds — October-December 1967 — Vol. III, Supt. No. 2

International conference on the use of radioactive isotopes in pharmacology

Conférence internationale sur l'utilisation des isotopes radioactifs en pharmacologie

Summaries of communications
Résumés des communications

Metabolism of 9α, 11β-dichloro-17α, 21-dihydroxy-16α-methyl Δ1, 4-pregnadiene-3, 21-dione (I).

B. KATCHEN and P. FRASSA (Schering Corporation Bloomfield, New Jersey, U. S. A.).

I is an interesting exception to the rule that oxygen substitution at C_{11} is required for corticoid activity. In non-enzyme catalyzed systems, II, the 21-disodium phosphate of I, slowly loses both of its chlorine atoms in 25 per cent aqueous propylene glycol solutions. The half-life for total dechlorination of II at pH 7 is about 25 hours at 65°. At 60° in aqueous solutions adjusted to pH 8.5, II is transformed to its 11β-hydroxyl derivative (N. Murrill *et al.*, Steroids, **8** : 233, 1966). These non-enzyme catalyzed reactions raise the possibility that III (9α-chloro-16α-methylprednisolone) is a metabolite of I.

To study this possibility, we incubated IV (the ^{36}Cl labelled 21-acetate of I) with rat liver slices. IV was extensively metabolized. Paper chromatography showed a major metabolite (V) with the same R_f as III. We eluted V from the chromatogram, acetylated it and III with acetic anhydride in pyridine and co-chromatographed both derivatives. The two compounds had different R_f values showing IV is not converted to III by rat liver slices. Thin layer chromatography of the incubation medium or a homogenate of the liver slices failed to reveal $^{36}Cl^-$, which shows rat liver slice enzymes do not catalyze the hydrolysis of the C_9 and C_{11} chlorines of I.

Synthesis of tritiated erythro and threo α-methyl-noradrenaline, metaraminol and their β-desoxy derivatives.

S. G. HALLHAGEN (Biochemical Research Laboratories, AB Hässle, Göteborg, Sweden).

In our studies on the liberation and the enzymatic conversion of the biogenic amines we needed the highly interesting ^{3}H-α-methyl-noradrenaline (^{3}H-α-Me-NA) and ^{3}H-metaraminol (^{3}H-MA).

One simple way to obtain these substances labelled in the last reaction step, was the use of the corresponding α-oximino ketones as precursors. These unsaturated compounds were treated with tritium gas in a modified Birkhofer-Hempel apparatus with 10% Pd/C catalyst suspended in the solution.

The crude products were purified on a cation-exchange column and the eluates were simultaneously scanned for radioactivity by an anthracene flow-cell technique.

From the α-Me-NA synthesis we immediately could identify α-ME-NA and α-methyldopamine (α-Me-DA) and from the MA synthesis we obtained MA and α-methyl-*m*-tyramine (α-Me-*m*-TA). From the first synthesis we also found an unknown substance with its peak between α-Me-NA and α-Me-DA. After evaporation of the hydrochloric acid at 30° C under reduced pressure, the residue from the α-Me-NA batch showed altered paper chromatographic data compared with those before the evaporation. It was later confirmed that the unknown peak corresponded to threo-α-Me-NA and the first found α-Me-NA peak to the erythro compound.

From the MA elution curve we could detect a very small peak at the "threo" position. The evaporation of the MA batch however did not change the purity of the substance.

The approximate specific activities were 60 mC/mmole for erythro or threo ^{3}H-α-Me-NA and 200 mC/mmole for ^{3}H-α-Me-DA, while ^{3}H-MA had 120 mC/mmole and ^{3}H-α-Me-*m*-TA 500 mC/mmole.

The use of labelled precursors in pharmacological studies on the mucopolysaccharide metabolism of fibroblast cell cultures.

D. A. KALBHEN, K. KARZEL and R. DOMENJOZ (Institute of Pharmacology, University of Bonn, Germany).

In contrast to cell cultures of permanent strains, freshly prepared monolayer cultures of fibroblast cells deriving from embryonic connective tissue do not lose their ability to produce and secrete mucopolysaccharides. In order to study the synthesis of mucopolysaccharides in fibroblast cells and its alterations induced by antirheumatic drugs a method was developed using radioactive precursors (glucosamine, galactosamine, sulfate, proline and serin) and the molecular sieving technique which will be described in detail. By these procedures it is not only possible to determine quantitatively the effect of the investigated drugs on mucopolysaccharide synthesis but also (using the double labelling technique) the correlations between the polysaccharide and the peptide part within the mucopolysaccharide-peptide complex produced by connective tissue. The results of our investigations indicate that phenylbutazone, oxyphenbutazone, indometacin, Na-salicylate and mephenamic acid induce a marked inhibitory effect on mucopolysaccharide synthesis even at low concentrations which do not influence the glucosamine-6-phosphate-transaminase.

Technique d'autohistoradiographie en microscopie électronique.

S. GOTHIE (Ecole Pratique des Hautes Etudes, Hôpital Broca, Paris, France)

Les coupes sont colorées à l'oxyde de plomb et carbonées. La couche monogranulaire est obtenue par extension d'une goutte d'émulsion Ilford L4 prise dans une anse de fil de cuivre au bérylium de 3/10 mm dont un brin est fixe et l'autre mobile dans une glissière.

Cette méthode permet l'étude des ultrastructures sans avoir à diluer ou digérer l'émulsion qui est transparente aux rayons.

Simultaneous liquid scintillation determination of tritium and sulphur-35 in biological low-level samples using the oxygen flask method.

R. RONCUCCI, G. LAMBELIN, M.-J. SIMON and W. SOUDYN (Continental Pharma, Brussels and Janssen Pharmaceutica, Beerse, Belgium).

The combustion of biological samples for the simultaneous determination of ^{3}H and ^{35}S requires two trapping systems. Tritium is generally trapped by simple cooling, while Sulphur-35 has to be fixed by a chemical trapping agent (e.g. 2-phenylethylamine). When biological low-level samples have to be assayed by the oxygen flask method, as it occurs very often in human tracer studies, large amounts of material have to be burnt and consequently large amounts of the chemical trapping agent are used which leads to an important increase of the quenching in the final counting mixture. This increase can be avoided by adequate dilutions but, in the case of low activities, the counting rate must be easily distinguished from the back-ground rate. In this last case a compromise between counting rate and quenching level (i.e. the dilution) has to be found.

We tried to find the most accurate and reliable experimental conditions which may be expected with the described method. Experiments were carried out on total blood. Five radioactive levels (ranging from 9,600 ^{3}H-dpm and 3,200 ^{35}S-dpm.ml^{-1} to 600 ^{3}H-dpm and 200 ^{35}S-dpm.ml^{-1}) at 4 different quenchings (i.e. at different dilutions) for each level were assayed.

The results obtained are discussed on a statistical basis.

Inhibition by lysine of nicotinic acid formation from tryptophane. Study of the effect of high doses of L-lysine in the diet on the tryptophane-niacine metabolism in rats by radiocarbon labelled tryptophane-[methylene-^{14}C].

Dr. J. R. PĚKNICE (Medical Faculty of Hygiene, Charles University, Prague, Czechoslovakia).

In the course of investigations of aminoacid imbalance, the effect of high doses of lysine administered in the diet on the tryptophane-niacine metabolism has been observed. In the first series, white rat males, weighing 150 g, have been given a standard niacine free diet, containing 22.5% casein and supplemented with 0.11% L-tryptophane, labelled with carbon-14 in the methylene group. The latter was administered intraperitonealy in a dose of 2.2 mg per day and animal what represented an activity of 5.8 μCi. The use of radioactive labelled tryptophane enabled us to investigate in the urine of rats the excretion of niacine and other metabolites of the tryptophane-niacine metabolism in very low concentrations.

The excreted metabolites in the urine of rats were isolated and identified by paper chromatography. In the subsequent part of the experiment, the same animals were given a standard diet, supplemented by intraperitoneal administration of 0.11% of radioactive labelled tryptophane together with 2 g lysine pro kg weight (i.e. 0.327 mg pro animal daily). The paper chromatography of urine revealed a significant decrease of niacine level, due to the inhibition of tryptophane-niacine metabolism by the high doses of lysine. The excess of lysine in the diet blocks the metabolic pathway leading to niacine formation and leads probably to an increased metabolic transformation of tryptophane to 5-hydroxy-tryptamine and its oxidation product serotonin. This results in rats in marked clinico-toxicological symptoms. Immediately after the intraperitoneal administration of tryptophane with lysine, the rats manifest tremor, unrest, stupefaction, loss of orientation, staggering with loss of stability (the animals lied down on their side), tachypnoe, dejection proceeding into somnolence and loss of appetite.

These symptoms indicate the abundance of serotonin in the brain and the overstressing of the natural metabolic pathways of the enzymatic degradation of monoamino-oxidase. In addition, the intraperitoneal administration of lysine led in rats to a strong sensation of prurigo in the vicinity of the puncture which in some animals lead finally to laceration of the skin by their teeth. All these manifestations were completely absent in the case of peritoneal application of tryptophane alone or with a low level addition of lysine. In repeated intraperitoneal injections of high doses of lysine, the duration of manifest clinical and toxicological symptoms decreased, suggesting a probable adaptation of the organism.

These experiments proved the evidence in rats of the inhibition of nicotinic acid formation from tryptophane by high doses of lysine in the diet, accompanied by clinico-toxicological symptoms.

Stereospecific enzymatic hydrolysis of sarin in blood plasma.

E. M. COHEN and P. J. CHRISTEN (Medical Biological Laboratory of the National Defence Research Organization TNO, Rijswijk Z. H., The Netherlands).

Sarin (isopropyl methylphosphonofluoridate) is a potent irreversible cholinesterase inhibitor. The high toxicity of this organophosphorus compound is caused by the inability of inhibited cholinesterase to hydrolyze acetylcholine.

Hydrolysis of sarin yields the non toxic product isopropyl hydrogen methylphosphonate (hydroxysarin). It has been shown earlier that blood plasma from different animal species contains a sarin hydrolyzing enzyme, the so called sarinase; in these investigations sarinase activity was measured with the conventional Warburg and titration techniques. The sarin concentrations used were comparatively high (10^{-2} M and higher).

In order to study the hydrolysis of sarin in plasma in the small concentrations in which this compound exerts its toxic action *in vivo* a more sensitive method had to be designed.

Our technique was as follows : blood plasma was incubated *in vitro* with ^{32}P labelled sarin in a final concentration of 10^{-4} and 10^{-5} M. In samples at short intervals taken from the mixture the reaction was stopped and the sarin and the hydroxysarin were separated by lyophylization, the former compound being volatile and the later not. The non-volatile residue was dissolved in ammonia and its radioactivity measured in a liquid counter. The hydrolysis curves obtained on incubation of sarin with plasma from different sources appeared to be biphasic : about 50% of the sarin was hydrolyzed at a very high rate and the remaining 50% much slower. It could be shown that this was caused by a preference of the enzyme for one of the stereo-isomeric forms of sarin (+ sarin).

During hydrolysis of sarin fluoride ions are released in the medium. These ions catalyse the racemisation of sarin and this reaction is dependent on their concentration. This is the reason why in the earlier experiments, where higher concentrations of sarin had to be used to demonstrate sarinase activity, no indication for a biphasic hydrolysis had been found.

Etude de l'utilisation des triglycérides et acides gras par le rat en fonction de la longueur des chaînes acylées.

P. METAIS et A. BACH (Strasbourg).

Après ingestion de triglycérides ou d'acides gras marqués sur le carbone 1 (graisses en C_8, C_{12} et C_{16}) on détermine en continu pendant les 7-8 heures qui suivent l'ingestion, l'activité spécifique du CO_2 expiré par l'animal. On peut alors calculer :

1° la quantité d'acides gras dégradés : près de 85% des acides gras en C_8 contre environ 50% des acides en C_{16}. Les acides gras courts ne sont donc pas stockés, contrairement aux acides plus longs.

2° leur vitesse d'absorption : les acides gras en C_8 passent aussi vite que le glucose, l'absorption des chaînes longues est plus lente.

3° leur vitesse d'utilisation : elle est aussi beaucoup plus rapide pour les acides gras courts que pour les acides gras longs.

Des différences, surtout au stade d'absorption, se manifestent pour les acides gras par rapport aux triglycérides. Ces premiers résultats montrent l'intérêt métabolique et diététique des graisses à acides gras à chaînes courtes.

Etude de l'autodécomposition des molécules marquées conservées en solutions aqueuses.

S. APELGOT et M. FRILLEY.

L'autodécomposition est un phénomène de radiolyse par effet indirect du rayonnement β émis. Elle est influencée par les agents qui modifient les phénomènes de radiolyse : température, radioprotecteurs, etc. L'expérience montre qu'en solutions aqueuses liquides, il existe une bonne corrélation entre les phénomènes d'autodécomposition et ceux de radiolyse par irradiation X. En revanche, cette corrélation disparaît en solutions aqueuses congelées. En particulier, si la vitesse de radiolyse diminue notablement lorsque la température baisse, la vitesse d'autodécomposition ne diminue notablement que si le traceur est un émetteur β d'énergie supérieure à une quinzaine de keV. C'est l'hétérogénéité des solutions congelées qui est responsable de ces phénomènes parce qu'elle conduit à des concentrations locales en soluté, et par conséquent, en énergie dans celui-ci (dose locale) dans le seul cas des particules β peu énergétiques, telles celles de ^3H. C'est pourquoi les composés marqués par le ^{14}C ont une vitesse d'autodécomposition très basse dès -75 °C, alors que ceux marqués par le ^3H gardent une vitesse d'autodécomposition élevée, même à -196 °C.

Etude du métabolisme d'un barbiturique N-substitué. Le N-benzoyl-luminal, par marquage au carbone 14.

A. UZAN, B. GAUTHIER, C. DUCAMP et L. LE BARBU (Laboratoire de Recherches de l'Industrie Biologique Française, S. A., Gennevilliers, France).

Le N-benzoyl-luminal, dérivé acylé du phénobarbital, antiépileptique comparable au luminal, diffère cependant de ce dernier par un effet hypnotique et une toxicité moindres et une plus longue durée d'action. Dans le but de déterminer si ces différences sont attribuables à la molécule elle-même ou bien à sa transformation dans l'organisme en un métabolite actif (par exemple phénobarbital), nous avons synthétisé le N-benzoyl-luminal marqué soit

sur le carbone carboxylique du radical benzoïque (I), soit sur le carbone 2 du noyau barbiturique (II), et comparé les deux produits obtenus au phénobarbital $2^{14}C$ (III).

La distribution de ces molécules marquées a été étudiée, après injection I. P., en fonction du temps, soit qualitativement par autohistoradiographie chez la souris, soit quantitativement chez le rat, par mesure de la radio-activité des organes, du sang et de l'urine.

Les résultats obtenus mettent en évidence la transformation du N-benzoyl-luminal en phénobarbital *in vivo*, la radio-activité correspondant à I étant presque totalement localisée au niveau des organes d'excrétion et de l'urine, alors que II présente une distribution du même type que celle de III et conduit aux mêmes métabolites.

Toutefois, il apparaît, deux heures après l'injection, une différence d'ordre quantitatif (plasma et cerveau), III donnant les concentrations les plus élevées. Mais au cours des heures suivantes, cette différence s'annule : la concentration en barbiturique augmente progressivement avec le benzoyl-luminal alors qu'elle régresse avec le phénobarbital. Des dosages chimiques confirment cette observation.

Les résultats obtenus tendent à montrer que l'activité du N-benzoyl-luminal est liée à sa transformation dans l'organisme en phénobarbital.

A novel method for the determination of tritium in freshly dissected biological tissues.

H. E. DOBBS and G. N. LAND (Research and Development Laboratories, Reckitt and Sons Ltd., Hull, England).

Numerous techniques utilising liquid scintillation counting for the assay of tritium in biological tissues have been described. Severe quenching limits the quantities of most tissues that can be assayed by direct dissolution in a liquid scintillator, or "scintillation cocktail". Large samples can be assayed by drying the tissue, burning the residue, and measuring the radioactivity in the tritiated water formed as the result of oxidation. This method is time consuming in that the tissues must be dried before combustion. It is precluded if the radioactivity is present in a volatile form.

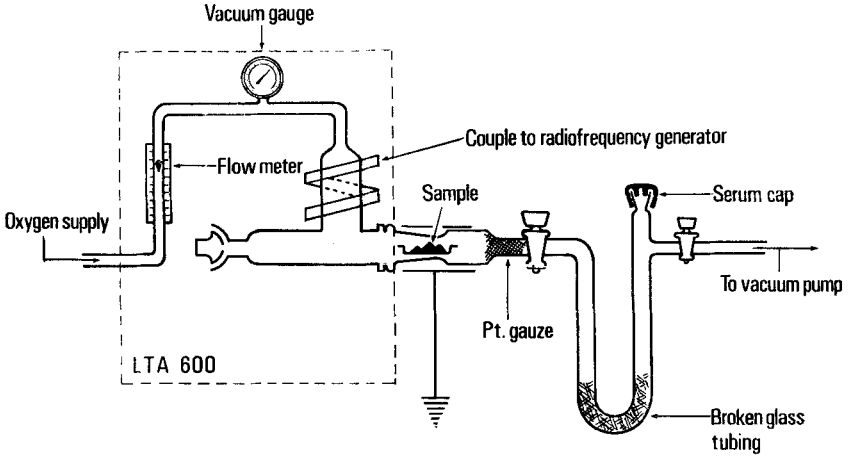


FIG. 1. Apparatus for the assay of tritium in freshly dissected animal tissues.

A method has now been developed in which the total tritium content of relatively large tissues can be determined within 30 minutes of their removal from an animal.

The freshly dissected tissue is chopped and placed in silica boat. A stream of oxygen at a low pressure, that has been passed through a radiofrequency field, is streamed over the sample. A Tracerlab LTA 600 instrument is used to generate the radiofrequency field. Volatile components of the tissue are rapidly removed. At the same time the excited oxygen reacts with the organic material of the tissue leaving an inorganic residue. Volatile compounds and oxidation products containing the tritium are condensed from the gas stream in a trap cooled with liquid nitrogen. They are subsequently dissolved in a dioxan-based liquid scintillator injected into the trap. The speed of ashing varies with the size and composition of the tissue. Thirty minutes is sufficient for most tissues weighing up to 0.5 g. The recovery of activity from "spiked" tissues is virtually quantitative. The counting efficiency of the scintillator varies from 3-9%.

Autoradiographic studies on the distribution of ^{14}C-meprobamate, solubilized in drug additives in mice.

E. VAN DER KLEIJN (Department of Pharmacology, University of Nijmegen, Netherlands).

Ullberg's (1954) macroautoradiographic technique has been used previously by Ewaldsson (1963) for distribution studies of ^{3}H-meprobamate after intravenous and oral application.

The present examination was undertaken to compare the general picture of the distribution of ^{3}H-meprobamate and metabolites (Ewaldsson, 1963) with that of the ^{14}C-labelled substance and to visualize differences in absorbtion velocity and distribution under the influence of drug additives like polyethyleneglycol 400 (P.E.G.) and polysorbate 80 (Tween) and of the elimination velocity under influence of barbital pretreatment which is supposed to enhance the metabolism of meprobamate ([3-4]).

Distribution of ^{14}C-meprobamate after oral application agrees well with the findings with ^{3}H-meprobamate. After five minutes rather high activity could already be detected in all organs especially in the liver and the kidneys. Obviously high levels of activity were present in the stomach and the proximal part of the intestines. Hardly no activity could be found yet in the fatty tissues of the body.

Of particular interest was the very high activity in the salivary glands, harderian glands and in the nasal cavity, even after four hours. Differences in activity between cortex and medulla of the adrenals as well as the relative low concentrations in the central nervous system and relative high concentrations in the hypophysis could be confirmed.

After one and a half hours all differences were leveled out. Accumulation in the bile then was evident.

By comparing organ levels and stomach-intestine passage as function of the time it may be concluded that the absorption of the drug in presence of P.E.G. (50 pCt) is slower than in the presence of Tween (10 pCt); whereas the general picture of distribution was the same in both cases.

Pretreatment of barbital enhances the rate of elimination of meprobamate as could be observed after one and a half hours.

The autoradiographic experiments have been performed with the aid of Mr. H. Prins of the Tracer Chemistry Department of the Research Laboratory of Brocades, Stheeman and Pharmacia, Haarlem.

BIBLIOGRAPHY

1. ULLBERG, S. — *Acta Radiol.*, 1954, suppl. 118.
2. EWALDSSON, B. — *Arch. Int. Pharmacodyn. Ther.*, **142** : 163-169 (1963).
3. REMMER, H. — *Arch. exp. Path. Pharmak.*, **237** : 296-307 (1959).
4. KATO, R. — *Med. Exp.*, **3** : 95-100 (1960).

The use of radioactive chromium in studying the absorption of intrinsic factor and bacterial endotoxin by small animals.

C. ROSENBLUM and R. F. GEOFFROY (Merck Sharp and Dohme Research Laboratories Rahway, N. J., U. S. A.).

Chromium-51 was used to label bacterial endotoxin and intrinsic factor (IF) derived from hog stomach concentrate. The endotoxin (polysaccharide) was labelled by incubation with $^{51}CrCl_3$ in pH 7 phosphate buffer at 37° C for 48 hours. The IF (mucopolysaccharide) was labelled by incubation with aqueous $^{51}CrCl_3$ for 24 hours at 37° C. The endotoxin was purified by successive precipitation with alcohol, with or without chromatography on SE Sephadex C-50. Labelled IF was purified on either SE Sephadex C-50 or CM Sephadex C-50, which retained excess ^{51}Cr.

The oral absorption of 10 mg of endotoxin-^{51}Cr by the rat was compared with that of an equivalent amount ($\simeq 1$ μg) of $CrCl_3$. In neither case was a significant quantity of ^{51}Cr detected in urine, plasma or tissue, suggesting that the polysaccharide was not absorbed through the gut wall.

Similar absorption experiments were performed with IF-^{51}Cr in guinea pigs with and without vitamin B_{12}-^{57}Co. Oral doses were 0.04-2.0 mg IF-^{51}Cr, 0.05-0.1 μg vitamin B_{12} and 0.02-1 μg $^{51}CrCl_3$. In no instance was more than 1 % of ^{51}Cr observed in the urine. Thus, despite the involvement of IF in vitamin B_{12} absorption, labelled IF does not appear to accompany vitamin B_{12} across the gut wall.

The non-metabolite residue — A limitation to the tracer method.

C. ROSENBLUM and C. R. HIREMATH (Merck Sharp and Dohme Research Laboratories Rahway, N. J., U. S. A.).

Basic to radioactive tracer methodology is the assumption that the radioactive atom remains an integral part of the molecule. This requirement is often ignored. Errors introduced with tritiated drugs are revealed by the radioactivity of tissue water. Changes occurring with ^{14}C-labelled drugs may be more subtle. Formation of $^{14}CO_2$ is always possible. Less profound biodegradations can yield small molecular fragments which are incorporated into pools of normal tissue constituents. Such residual radioacivity implies the presence of drug-related metabolites which, in fact, have no functional or structural relationship to the parent compound. Conclusions based exclusively on radioactivity, determined either by counting or by radioautography, may thus be questioned.

Experiments with small molecules reveal in part the pattern characterizing the non-metabolite residue. Thus $NaH^{14}CO_3$, which is almost completely eliminated by animals as $^{14}CO_2$ in 24 hrs, still leaves detectable ^{14}C in tissue 15 days after oral administration to rats. This activity occurs in all tissues primarily in lipid and protein fractions, but still measurable in nucleic acids and glycogen. A similar behavior was noted with $H^{14}COONa$ and with several complicated organic compounds. The appearance of non-metabolite residues constitutes a real limitation to the usefulness of tracer studies with labelled drugs.

A biosynthetic method for labelling snake venoms.

M. G. LOMBA, O. V. BRAZIL (University of Campinas, Campinas, São Paulo, Brazil), J. KIEFFER and J. C. BARBERIO (Atomic Energy Institute, São Paulo, Brazil).

Snake venom components are as a rule proteins rich in sulfur. Crotoxin, for instance, the main component of *Crotalus durissus terrificus* venom contains nearly 4 per cent of this element, most of which in the form of cystine (87.6 $\pm$ 2.3 per cent of the total sulfur). This suggested us to try the labelling of ophidic venom by injecting venomous snakes with ^{35}S labelled compounds. In the present experiments, South American rattle snakes (*C. d. terrificus*) and sodium sulfate with radiosulfur were used. A Na_2SO_4 ^{35}S solution, pH 8, was intraperitoneally administered to seven snakes after extracting their venom. The quantity given to each snake corresponded approximately to an activity of 1 mCi or, at the time the readings of the venoms were made, 0.16 mCi. After twenty days from the first venom extraction, another was done. The venoms were submitted to paper electrophoresis, and autoradiograms from the electrophoretic strips were made. They were also counted in a liquid scintillator (mod. 720 of Nuclear Chicago). The mean of the specific activities was 0.91 nCi per mg. Two months after the sodium sulfate injection, a new venom extraction was done from two surviving snakes. The specific activities of these venoms were even less than those of the venoms from the precedent extraction (first extraction after the sodium sulfate injection : 0.73 and 0.15 nCi per mg; second extraction : 0.15 and 0.023 nCi per mg). The ^{35}S incorporation to the molecules of the venom components was proved by the trichloroacetic acid test and the autoradiographs of the electrophoretic strips.

The specific activities obtained in this preliminary experiment were very small. Nevertheless the viability of the method seemed to be proved. In a more recent experiment, five injections of the sodium sulfate solution were given to a rattle snake at 15 to 20 days intervals. This snake was also allowed to swallow at two different times a mouse injected with the sodium sulfate solution. The venom obtained from this snake after two and half months has specific activity much higher (3.100 nCi per mg) than those of the venoms in the precedent experiment. Now, a larger scale experiment with repeated injection of sodium sulfate solution at shorter intervals (at 3 days intervals) is being carried out. The use of ^{35}S-labelled cystine instead of sodium sulfate will also be tried.

Modèles mathématiques des procédés de transport par le foie et/ou les reins de quelques radiopharmaceutiques.

P. JIROUNEK (Faculté de Médecine générale, Université Charles, Prague 2, Tchécoslovaquie).

Les tests de fonction des reins et du foie à l'aide des radiopharmaceutiques (hippuran ^{131}I, rose bengale ^{131}I, 2-6 diiodosulfanilic acid ^{131}I, etc.) sont parmi les plus répandus dans la médecine nucléaire, mais les avis sont assez différents en ce qui concerne les méthodes d'évaluation d'information représentées par les courbes néphrographiques ou hépatologiques.

Les difficultés d'évaluation sont causées par l'insuffisance de connaissances des processus physiologiques qui influencent la forme des courbes obtenues.

C'est pourquoi en établissant les modèles mathématiques des procédés de passage il n'a pu être pris en considération comme base que les courbes expérimentales.

Le fonctionnement du système traité (transport de radiopharmaceutiques à travers les reins, resp. à travers le foie) est caractérisé par la fonction d'entrée et par la fonction de sortie du système considéré.

Dans les cas que nous avons considérés, la fonction d'entrée a été représentée par la courbe d'activité du sang, et celle de sortie par l'activité mesurée au dessus du foie, resp. des reins. Toutes les valeurs ont été mesurées par un détecteur à scintillations et enregistrées

automatiquement. En établissant le modèle mathématique, il a été considéré que le système était linéaire.

Deux méthodes ont été appliquées pour établir les équations différentielles correspondant aux systèmes traités.

La première méthode consiste en l'optimalisation des paramètres à l'aide d'un ordinateur analogue.

La deuxième méthode consiste à établir le diagramme de Nyquist sur la base des courbes néphrographiques et hépatologiques. D'après ce diagramme on détermine la fonction de transfert du système.

Une série de courbes a été considérée et les résultats ont prouvé :

1) que les équations sont de troisième ordre (ou plus haut encore);
2) que très souvent les coefficients des équations ne sont pas constants dans le temps.

On peut en déduire :

1) que les méthodes d'évaluation basées sur les suppositions que les équations sont de premier ordre et que les coefficients sont constants, sont très incertaines;
2) que des méthodes mathématiques plus approfondies basées sur l'emploi d'ordinateurs, doivent être employées.

Synthesis of 2-iodosulfanilic-^{131}I and 2,6-diiodosulfalinic-^{131}I acid and their use in examination of renal function.

P. RABAN, V. GREGORA, J. BROUSIL, P. JIROUNEK and T. BLAŽEK (Faculty of General Medicine, Charles University, Prague, Czechoslovakia).

2,6-Diiodosulfanilic acid labelled with ^{131}I (DISA-^{131}I) was tested some years ago by Winter and Myers as a possible agent for isotope renography. Based on slow renal clearance in man the authors considered this compound less convenient than labelled *o*-iodohippurate. With respect to the increasing interest in new types of radiopharmaceuticals especially for measurement of glomerular filtration rate we reinvestigated the biological behaviour of DISA-^{131}I and compared it with the behaviour of 2-iodosulfanilic-^{131}I acid (ISA-^{131}I). As the synthesis of DISA-^{131}I has not been described we used iodination of sulfanilic acid as radioactive iodine monochloride and investigated the reaction conditions so that it was possible to obtain DISA-^{131}I of good radiochemical purity in high radiochemical yield (84%). For the preparation of ISA-^{131}I we used the same reaction. This compound, however, is in any case accompanied with DISA-^{131}I and sulfanilic acid and therefore a purification on ion-exchange resin had to be employed. The final radiochemical yield of pure ISA-^{131}I is usually about 82%. Both these compounds were injected to the rabbits and the time-course of radioactivity in kidney, liver, and blood was registered by means of scintillation counter with automatic recorder. The experimental curves were analyzed by means of analog computer and compared with the results obtained with radioactive sodium *o*-iodohippurate.

Utilisation de deux marquages, au ^{14}C et au ^{71}Ge, sur le tétraéthylgermanium, à des fins d'études métaboliques.

J. OUSTRIN (Centre de Recherches sur les Toxicités, C. N. R. S., Toulouse, France).

Les techniques de dosage chimique, aussi bien que l'utilisation de molécules marquées au seul ^{71}Ge, n'ont pas permis de savoir si le tétraéthylgermanium, molécule très stable, était métabolisé sans coupure ou était détruit dans l'organisme.

Les deux marquages distincts par ^{14}C et par ^{71}Ge, font appel, pour le ^{14}C à la synthèse à partir de bromure d'éthyle-^{14}C, et, pour le ^{71}Ge, à l'activation neutronique. Si elles posent des problèmes de synthèse et de purification, ces molécules marquées permettent de suivre le destin des deux parties, minérale et organique, du composé étudié.

L'utilisation de ces deux marquages permet de déceler une coupure de la molécule, qui est suivie de l'élimination rapide et importante par voie pulmonaire (CO_2) des radicaux carbonés.

Les glycuro-conjugués urinaires se révèlent marqués au ^{14}C mais aussi, pour une plus faible proportion, au ^{71}Ge.

L'élimination urinaire totale est beaucoup plus importante pour ^{14}C que pour ^{71}Ge. Le ^{71}Ge, par contre, se fixe de façon durable dans le poumon, la rate, et le foie des rats d'expérience.

Dilution isotopique inverse. Application à l'étude métabolique du facteur M_1 de la staphylomycine.

M. ROBERFROID et P. DUMONT (Université Catholique de Louvain, Louvain, Belgique).

Le facteur M_1 est le constituant le plus important de l'antibiotique staphylomycine tan par son abondance relative que par son activité antibactérienne. L'étude de son métabolisme chez le rat a été entreprise à l'aide du produit marqué. La tritiation a été effectuée par réaction d'échange avec le tritium gazeux selon la technique de Wilzbach. Après administration par voie intra-veineuse du facteur M_1, la radioactivité disparaît du sang selon une cinétique de premier ordre (demi-vie : 290 minutes) à l'intervention d'un double processus d'excrétion et de fixation tissulaire. Dans l'excrétion, le rôle principal est tenu par la bile. Durant son séjour dans le sang, le facteur M_1 se retrouve en majeure partie dans la phase plasmatique où il apparaît lié aux protéines.

Les techniques de dilution isotopique inverse simple et double ont été utilisées en vue de déterminer la proportion de facteur M_1 libre, présente dans le plasma et la bile ainsi que l'évolution de son catabolisme et d'étudier la réversibilité de la liaison antibiotique-protéinese

Metabolic formation of methane from N-methyl-substituted hydrazine compounds and carcinostatic activity.

D. E. SCHWARTZ, G. B. BRUBACHER and M. VECCHI (Research Department, F. Hoffmann-La Roche et Co., A. G., Basle, Switzerland).

Compounds labelled with ^{14}C in the N-methyl group were investigated. Male Wistar rats weighing 200 g received 100 μmole/kg of the labelled compounds intraperitoneally. The animals were placed in a metabolism chamber provided with an air flow of 300 ml/min. The chamber exhaust was refrigerated in 3 traps at —183° C (liquid oxygen) to retain $^{14}CO_2$ quantitatively and the remaining radioactivity of the uncondensed gases was continuously registered by a gas flow ion chamber and vibrating reed electrometer. Simultaneously samples of this gas were drawn with a syringe and analyzed in a Mikrotek 2000 gas chromatograph. Quantitative correlation between radioactivity and height of peaks was regarded as evidence of methane formation.

Methane formation was observed as soon as 3 minutes after injection. In the case of monomethylhydrazine the production of methane reached a maximum after 10 minutes :

it first declined rapidly, then more slowly; in the case of procarbazine (1) a similar curve was observed although the initial peak was much less pronounced. Methane formation within one hour amounted to 20% and 5% of the dose for monoethylhydrazine and procarbazine respectively.

Methane is indicative of free methyl radical formation. Other examples suggest that azo derivatives may serve as intermediates in the formation of radicals from hydrazine compounds, the molecule splitting into radicals possibly with liberation of nitrogen. In the case of monomethylhydrazine, formation of methane from a methyl radical could be favoured by the simultaneous liberation of a highly reactive hydrogen atom. In the case of procarbazine, a more stable radical would be formed. This may leave the methyl radical free to react with other substrates such as purine bases and bear some relation to the carcinostatic activity of the drug.

---

(1) 1-Methyl-2-*p*-(isopropylcarbamoyl)benzylhydrazine-hydrochloride, Trade mark = Natulan (R).

Pharmacological effects on contraction force and simultaneously measured decarboxylation of ^{14}C labelled glucose and hexanoate by isolated atria.

M. SIESS (Marburg/Lahn).

It will be reported results of a method which allows the continuous measurement of the rate of $^{14}CO_2$ production by isolated heart auricles incubated with ^{14}C-labelled substrates for a period of hours. Simultaneously was recorded the mechanical work of contraction under defined conditions to compare quantitatively changes in substrate consumption and efficiency by drug influence.

Primary effects on metabolism were studied in resting auricles. The turnover of either substrate increased with the external concentration with saturation being reached at 2 mM hexanoate and 15 mM glucose. The consumption of either substrate by beating atria was at this point approximately 0.5 µMol/h and 100 mg wet weight (30° C). The efficiency of the energy production was under optimal conditions 15%. The ratio of the basic metabolic rate to the functional rate of either substrate determined in resting and beating atria depended on the mechanical work and was about 1 : 1.

Addition of unlabelled hexanoate (up to 2 mM) and β-hydroxybutyrate (up to 15 mM) inhibited the decarboxylation of ^{14}C-glucose (15 mM) by 50% and 30% respectively, determined from a Lineweaver-Burk plot. This effect can be shown in the same way if the work of contraction is intensified by g-strophanthin or by epinephrin. In contrast, low concentrations of glucose (5 mM) enhanced the decarboxylation of ^{14}C-hexanoate or ^{14}C-β-hydroxybutyrate. Intensified force of contraction caused by g-strophanthin increased the efficiency of the energy production by glucose and hexanoate, while acceleration of frequency by stimulation or by epinephrin or histamin diminished it. The effect of depletion of catecholamines in atria of reserpinized guinea pigs and effects of triiodothyronine on the metabolism of glucose and hexanoate will be reported.

Oestradiol a reducing agent for androgens. An application of "Stoffwechsellabil" labelled steroids and radio-thin-layer-chromatography.

M. WENZEL und K. POLLOW (Radioisotopenabteilung. Physiologisch-Chemisches Institut der Freien Universität Berlin, Germany).

Δ^4-Androstendion-3,17 is reduced by liver tissue at the keto-groups and at the double-bond, yielding Androstandiol. The reducing agent within the cell is DPNH or TPNH. By

incubating liver-tissue with certain Tritium-labelled substrates, which are oxydized by DPN or TPN intracellar DPN-T or TPN-T is generated. For example :

$$\text{Laktat-2-T} + \text{DPN} \rightarrow \text{Pyruvat} + \text{DPN-T}$$

or

$$\text{Oestradiol-17}\alpha\text{-T} + \text{TPN} \rightarrow \text{Oestron} + \text{TPN-T}$$

These Tritium-labelled reduced Coenzymes are oxydized to water or can transfer their Tritium to other substances, which are reducable.

In this paper the question is answered : which DPN-T or TPN-T generating systems effects the transfer of Tritium to the reduction products of Androstandion ?

Comparing the Tritium-incorporation into reduced androgens from

Laktat-2-T
Glycerol-2-T
Glutamat-2-T
Glucose-1-T
Oestradiol-17α-T

it can be shown by direct measurement of thin-layer-chromatograms that although the yield of Tritium-water is with all substrates in the same order of magnitude, Tritium from Oestradiol-17α-T is incorporated into the reduction products of Androstendion (for example Androstandiol) 1,000 times stronger than by other DPN-T or TPN-T generating systems.

Promotion of radiomercury excretion.

W. H. STRAIN, R. M. PEER, M. R. THOMAS, A. P. GASS and W. J. PORIES (School of Medicine and Dentistry, University of Rochester, Rochester, N.Y., U.S.A.).

The excretion of chlormerodrin-Hg-203 from rats is greatly increased by the intramuscular administration of chlormerodrin, the corresponding stable mercury compound. If chlormerodrin is administered immediately after injection of the radiopharmaceutical, 90 percent of the radio-activity compared to controls is eliminated in 10 days. A delay of one day in the administration of chlormerodrin is less effective, and only 84 percent of the radioactivity is eliminated in 10 days. Studies on the retention of mercury-203 by various tissues indicate that the kidney binding sites are primarily affected.

(This research was supported in part by Research Grant RH 00042, National Center of Radiological Health, U.S. Public Health Service.)

Gonadal retention of radioselenium.

W. H. STRAIN, R. M. PEER, R. C. CHILDERS, R. L. WORLAND, S. N. ZARESKY and W. J. PORIES (School of Medicine and Dentistry, University of Rochester, Rochester, N.Y., U.S.A.).

Radioselenium is retained to a high degree by the gonads of both male and female rats after intravenous administration of sodium selenite Se-75. Retention by the ovaries begins to decrease on the second day after injection but that by the testes increases with time untill on the tenth day gonadal tissue has the highest specific activity. The high retention of radioselenium suggests that the gonads have specific receptors for selenium, especially since Mason *et al.*, *Anat. Rec.*, in press, have shown that selenium like zinc prevents cadmium from sterilizing males.

(This research was supported in part by Research Grant RH 00042, National Center of Radiological Health, U.S. Public Health Service.)

Absorption of etorphine (M99 Reckitt) and dihydromorphine from the buccal cavity.

G. F. BLANE, A. L. A. BOURA and H. E. DOBBS.

Narcotic analgesics are usually administered clinically by parenteral routes, but it is known that these drugs can also enter the body via the buccal mucosa. The recent finding of the extremely potent morphine-like drug etorphine (Blane, Boura, Fitzgerald and Lister, 1967) which has a much higher lipid solubility than morphine and would therefore be expected to pass more readily across cellular barriers, prompted examination of its rate of absorption after administration by the sub-lingual route in a number of laboratory species.

Observations in conscious rats, dogs and baboons, details of which will be presented in summarized form, show that etorphine is extremely efficaceous by the sub-lingual route. By contrast, etorphine is much less active when placed directly in the stomach by means of a catheter. Morphine or its closely related analogue dihydromorphine is relatively ineffective when placed below the tongue.

The radioisotope phase of this investigation involved a comparison between the absorption of tritiated etorphine and tritiated dihydromorphine, using buccal and intramuscular sites in the lightly anaesthetised dog. Samples of arterial and venous blood from the relevant vascular beds were obtained at intervals, via appropriately placed cannulae, and assayed after drying by an oxygen flask combustion technique (Dobbs, 1966). The results confirm that etorphine crosses the buccal mucosa and appears in the systemic circulation with a rapidity exceeding that seen after sub-lingual administration of a dose of dihydromorphine causing an equivalent degree of analgesia when administered intramuscularly.

After its intramuscular administration, blood levels of etorphine were maximal within 2 minutes, which is consistent with the established finding that it has a very rapid onset of action by this route.

REFERENCES

BLANE, G. F., BOURA, A. L. A., FITZGERALD, A. E. and LISTER, R. E. — *Brit. J. Pharmac. Chemother.*, **30** : 11-22 (1967).

DOBBS, H. E. — *Int. J. appl. Radiat. Isotopes*, **17** : 363-364 (1966).

Some aspects of the distribution of orphenadrine and structurally related drugs in mice, using the macroautoradiographic technique according to Ullberg.

W. HESPE and H. PRINS (Research Department, N.V. Koninklijke Pharmaceutisch Fabrieken v/h Brocades, Stheeman en Pharmacia, Amsterdam, The Netherlands).

The distribution of the radioactivity, as obtained by Ullberg's macroautoradiographic method, after administration of tritiated orphenadrine (labelled in the ethylamine moiety) and structurally related compounds to female mice, shows many common aspects.

We mention :

1. The large volume of distribution of the radioactivity; nearly all organs show a definite level of radioactivity exceeding that of the blood.
2. The high uptake of radioactivity in different glands and glandular tissues.
3. The high uptake of radioactivity in the lungs.
4. A concentration pattern in the brain in which cortex and especially hippocampus show a relatively higher concentration than other brain areas.
5. A high concentration of radioactivity, both in the kidneys and in the adrenals, in a specified zone near the cortico-medullar border.

Similar distribution patterns, also obtained by Ullberg's method were described for chlorpromazine-^{35}S [2], promethazine-^{35}S [3] and amitriptyline-^{14}C [4]. The obvious independence of the distribution from the position of the label, indicates that the intact compound and the metabolites wherein the original structure is still intact to a large extent, e.g. the *N*-demethyl congeners and substituted derivatives, are mainly involved.

When compounds with a pharmacologically different spectrum of actions, are distributed in animals according to patterns which are similar in details, one has to recognize the existence of a large amount of non specific bindingplaces in the various organs for this type of compounds.

A notable exception to the observed independency of the distribution pattern on the position of the label is found in the case of a ^{14}C-labelled *N*-methyl group in some of the substances mentioned.

The liver shows a level of radioactivity that is much higher and longer lasting than in the corresponding distribution pictures obtained with the same compound labelled in other positions.

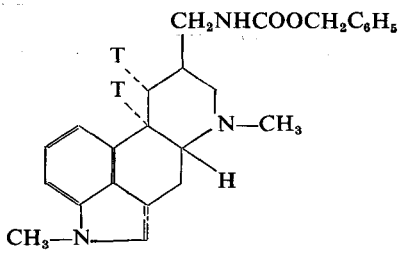
REFERENCES

1. ULLBERG, S. — *Acta Radiol.*, Suppl. **118** (1954).
2. SJÖSTRAND, S. E., CASSANO, G. B. and HANSSON, E. — *Arch. intern. Pharmacodyn.*, **156** : 34-37 (1965).
3. HANSSON, E. and SCHMITERLÖW, C. G. — *Arch. intern. Pharmacodyn.*, **81** : 309-324 (1961).
4. CASSANO, G. B., SJÖSTRAND, S. E. and HANSSON, E. — *Psychopharmacologia*, **8** : 12-22 (1965).

Synthesis of 1,6-dimethyl-8β-carbobenzyloxyaminomethyl-10α-ergoline-9,10-^{3}H (^{3}H-MCE) and method for the analysis of its metabolites in the excreta of rat, dog and man.

A. MINGHETTI, F. ARCAMONE, NICOLELLA (Istituto Ricerche FARMITALIA, Milan, Italy), M. DUBINI and G. P. VICARIO (Laboratori di chimica Organica esplorativa della Soc. MONTECATINI-EDISON, Novara, Italy).

The new drug 1,6-dimethyl-8β-carbobenzyloxy-aminomethyl-10α-ergoline (MCE) [1] shows an highly specific and long acting anti-5-hydroxytryptamine activity [2-5]. Clinical trials revealed its preventive effect in humans affected by vascular migraine.



^{3}H-MCE

The synthesis of tritiated MCE has been carried out starting from dihydrolysergic acid 9,10 T [6] as already reported for the non tritiated compound [1].

The radioactive compounds excreted by rats, dogs and men after ^{3}H-MCE administration have been divided in two groups, on the basis of their paper chromatographic behaviour with the solvent system *n*-butanol, pyridine, water (4 : 1 : 5). The compounds of the first group (hydrosoluble fraction) showed R_f values lower than 0.5; those of the second group (liposoluble fraction) showed a single peak covering the R_f range 0.7-0.9. The resolution of the first group metabolites was possible with the above mentioned chromatographic system. Separation of the second group metabolites was afforded by TLC on Kieselgel G with the solvent system chloroform-methanol 80 : 20 (v/v).

The drug appears to be metabolized for more than 95% in all the species examined. The chromatographic patterns shown by the excreta of the said species was qualitatively similar. The main differences are observed in the relative ratios of the different radioactive metabolites.

BIBLIOGRAPHY

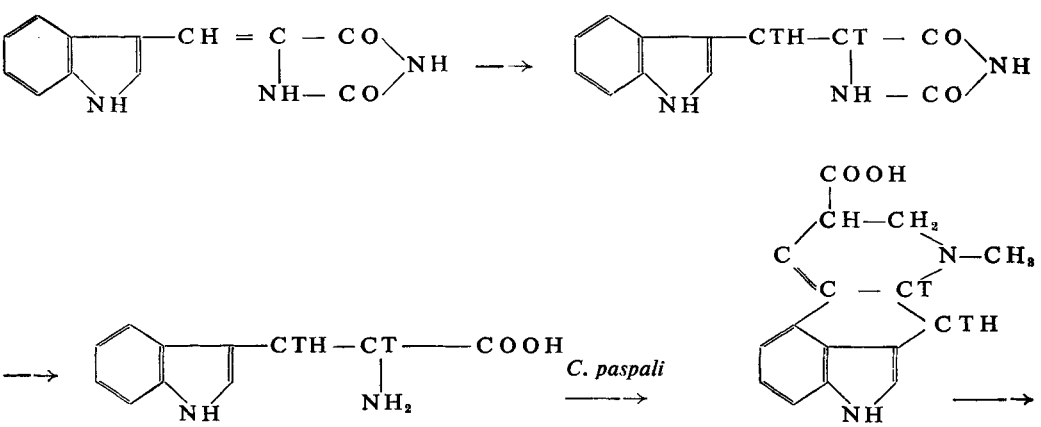
1. BERNARDI, L., CAMARINO, B., PATELLI, B. and REDAELLI S. — *Gazz. Chim. Ital.*, **94** : 936 (1964).
2. BERETTA, C., FERRINI, R. and GLÄSSER, A. H. — *Nature*, **207** : 421 (1965).
3. C. BERETTA, A. H. GLASSER, M. B. NOBILI and R. SILVESTRI: *J. Pharm. and Pharmacol.* **17** : 423 (1965).
4. FREGNAN, G. B. and GLÄSSER, A. H. — *Med. Pharmacol. Exp.*, **13** : 189 (1965).
5. BALDRATTI, G. ARCARI, G. and SUCHOWSKY, G. K. — *Experientia*, **21** : 396 (1965).
6. DUBINI, M. — *Proceedings of the Conference on Methods of Preparing and Storing Marked Molecules.* Brussels, Nov. 13-16, 1963, p. 911.

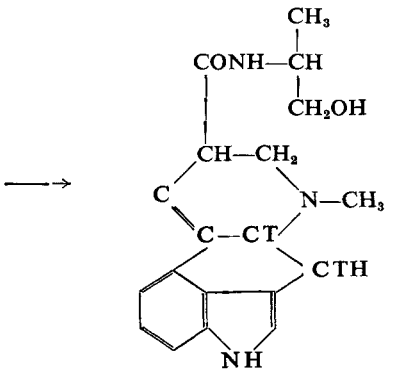
Synthesis of ^{3}H-ergometrine and its use for biosynthetic studies in *Claviceps paspali* and *Claviceps purpurea*.

G. P. VICARIO, M. DUBINI (Laboratori di Chimica Organica Esplorativa della Soc. MONTECATINI-EDISON, Novara, Italy), A. MINGHETTI and F. ARCAMONE (Istituto Ricerche FARMITALIA, Italy).

In order to study the behaviour of ergometrine, as intermediate in the biosynthesis of lysergic acid derived alkaloids, we have prepared a sample of the product labelled with tritium in the lysergic acid skeleton.

The synthesis of ^{3}H-ergometrine was carried out using chemical and biochemical methods according to the following reactions :





DL-tryptophane-2,3-T was prepared starting from indolylidene-hydantoin [1]. ^{3}HD-lysergic acid was obtained by incorporation of DL tryptophane-2,3-T by fermentation with Claviceps paspali [2]. From 280 mc (specific activity 650 mc/mM) of DL-tryptophane-2,3-T, 30 mc were recovered in the alkaloid fraction (lysergic and isolysergic acid α-hydroxy ethylamides). The alkaloid fraction was saponified and after purification and addition of carrier, 6 mc of ^{3}HD-lysergic acid (specific activity = 16,8 mc/mM) were obtained. ^{3}H ergometrine was prepared in 30 yield, from ^{3}HD-lysergic acid [3]. The compound was isolated as maleic acid salt.

The incorporation of ^{3}H ergometrine in D-lysergic acid α-hydroxyethylamide was tested using *Claviceps paspali* : incorporation of about 2% was found. A similar experiment was performed with *Claviceps purpurea* in order to study the incorporation in ergotamine : no radioactivity was found in this alkaloid.

These biological results are discussed.

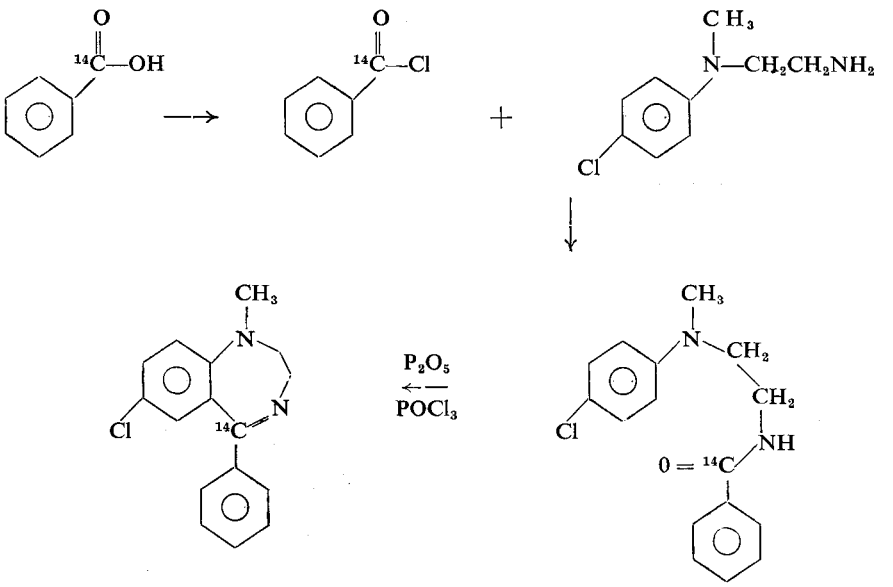
BIBLIOGRAPHY

1. ELKS, J., ELLIOT, D. F. and HEMS, B. A. — *J. Chem. Soc.*, 629 (1944).
2. ARCAMONE, F., CHAIN, E. B., FERRETTI, A., MINGHETTI, A., PENNELLA, P., TONOLO, A. and VERO, L. — *Proc. Roy. Soc. London B*, **155** : 26 (1961).
ARCAMONE, F., CHAIN, E. B., FERRETTI, A., MINGHETTI, A., PENNELLA, P. and TONOLO, A. — *Biochim. Biophys. Acta*, **57** : 174 (1962).
3. GARBRECHT, L. — *J. Org. Chem.*, **24** : 368 (1959).

Synthesis of 7-chloro-2,3-dihydro-1-methyl-5-phenyl-1H-1,4- benzodiazepine-5-^{14}C Hydrochloride.

H. H. KAEGI (Chemical Research Department, Hoffmann-La Roche, Inc., Nutley, N.J., U.S.A.).

Synthesis of the title compound, a pharmacologically active substance required for biological studies, was carried out in three steps from benzoic-7-^{14}C acid. Key step was cyclization as shown, a reaction sensitive to exact conditions.



The radiochemical yield was 60 %. The unlabelled starting material was obtained by tosylation of *p*-chloroaniline, methylation of the tosyl-sodio derivative and removal of the tosyl blocking group by strong acid hydrolysis. Aminoethylation was carried out by treatment with etyleneimine under aluminium chloride catalysis.

Autoradiographische Analysen zur Prüfung der carcinogenen Wirkung lebervergrößernder Substanzen.

G. SCHAUDE und W. KUNZ (Pharmakologisches Institut der Philipps-Universität Marburg-Lahnberge, 355 Marburg/lahn).

Bei chronischer Zufuhr vieler Fremdstoffe tritt im Tierexperiment eine erhebliche Lebervergrößerung auf. Da zu den wirksamen Substanzen zahlreiche Pharmaka und Stoffe des täglichen Anwendungsbereiches gehören, wurde geprüft, ob sich dabei im Langzeitversuch eine carcinogene oder cocarcinogene Wirkung ergibt.

Albinomäuse erhielten diese lebervergrößernden Substanzen isoliert und in Kombination mit einer sicher carcinogenen Dosis von Diäthylnitrosamin peroral im Trinkwasser.

In den initialen Stadien wurde durch Flüssigkeitsscintillationsmessungen der Einbau von ^{3}H-Thymidin in die DNS quantitativ ermittelt und durch autoradiographische Analysen die Lokalisation der Markierung in den einzelnen Gewebselementen verschiedener Organe festgestellt.

Die final beoachtete Tumorfrequenz, Tumorlokalisation und Manifestationszeit der Tumoren wurde mit diesen initialveränderungen verglichen.

In vivo studies with tritium-labelled reserpine : methodological problems and significance of drug-levels in reserpinized animals.

L. MANARA, M.D. (Head laboratory of Drug Metabolism, Istituto di Ricerche Farmacologiche « Mario Negri », via Eritrea 62, Milano, Italy).

Reserpine, a powerful therapeutic aid in psychiatric disorders and hypertensive disease, has been also extensively employed as an experimental tool in biomedical research.

Basic data which provide adequate information on the distribution and fate of this drug in different animal species have been available for several years. For some reasons, however, measurements of reserpine in body constituents appear to deserve further attention. For instance it may be recalled that reserpine, as a research tool, has often been employed in combination with other pharmacological agents including some capable of modifying its action, but no experimental evidence has so far been supplied which clarifies whether these drugs interfere with the accumulation of reserpine in the target organs.

Tritium-labelled reserpine, presently available on a commercial basis, is a valuable aid to investigators interested in measurements of reserpine in body constituents. In fact most of this drug rapidly disappears after its administration and only sensitive isotopic procedures have allowed to detect the very minute amounts of reserpine which stay in the brain throughout the duration of induced symptoms. Adequate sensitivity and specificity of the analyses of the radiochemical in biological material can be achieved through a combination of thin-layer chromatography and liquid scintillation radioassay.

Examples of the bearing of reserpine measurements to pharmacological studies include data suggesting that the antireserpine action of tricyclic antidepressants is not due to inadequate reserpinization and direct evidence that reserpine and syntbetic benzoquinolizines compete at the level of a common target.

These latter results seem also to supply more information concerning the significance of the time course of reserpine levels in the brain also in relation to amine depletion.

Untersuchungen mit « Stoffwechsel-labil » markierten Steroiden.

M. WENZEL und K. POLLOW (Berlin-Dahlem).

« Stoffwechsel-labil » markierte Steroide wie Oestradiol-17α-T verlieren bei ihrer Oxydation in der Zelle das Tritium unter Bildung von DPN-T oder TPN-T.

Bei der Inkubation von Gewebeschnitten mit Oestradiol-17α-T und Androgenen zeigt sich ein um den Faktor 1000 bevorzugter Tritium-Einbau in Reduktionsprodukte der Androgene gegenüber anderen in der Zelle ebenfalls DPN-T oder TPN-T erzeugenden Systemen, wie durch Dünnschichtchromatographie-Auftrennung der Tritium-markierten Androgenderivate bewiesen werden konnte. Die Oxydation des aus den « Stoffwechsel-labil » markierten Steroiden entstehenden TPN-T oder DPN-T zu Tritium-Wasser ermöglicht eine einfache Bestimmung der Steroid-Oxydation im lebenden Organismus durch Aktivitätsmessung des Atemwassers.

Herstellung ^{32}P-markierter Phosphorsäureester durch thermische Neutronenbestrahlung.

J. HOLZL (1) und D. NEUMEIER (Institut für Pharmazeutische Arzneimittellehre der Universität München).

Zur Markierung werden die Phosphorsäureester nach spezieller Aufbereitung einem thermischen Neutronenstrom ($4{,}2.10^{12}$ n/cm^2.sec) ausgesetzt. Durch einen n,γ-Prozeß verwandelt sich Phosphor in ^{32}P, wobei ein gewisser Gehalt der Verbindung im nativen Zustand erhalten bleibt. Die Stabilitätsabhangigkeit von Bestrahlungszeit, Begleitsubstanzen und Struktur wurde untersucht. An den Beispielen eines Zuckerphosphats, eines Nukleotids und eines Phosphatids konnte gezeigt werden, daß die spez. Radioaktivität bei einer Bestrahlungszeit von 8 Stdn. eine Höhe von 0,3 bis 1,4 mc/mM erreicht. Die markierten Abbauprodukte wurden säulenchromatographisch abgetrennt. Auf Reinheit prüfte man chromatographisch und enzymatisch.

(1) Vortragender.

Influence of diuretic agents *in vivo* and *in vitro* on the insulin-stimulated metabolism of glucose in fat tissue of the rat.

Dr. W. HEPTNER (Farbwerke Hoechst A. G.).

Comparative experiments were carried out with cell suspensions and cell-free enzyme extracts of epididymal fat tissue, as well as *in vivo* to study the question whether Lasix, acetazolamide, and ethacrynic acid have an effect on the insulin stimulated metabolism of glucose in fat tissue of the rat.

Typical inhibition curves were used to demonstrate the influence of the concentration of the diuretic agents in a protein-free medium on the oxidation of glucose-1-^{14}C and $^{14}CO_2$-fixation in cell suspensions. Independent of the addition of insulin the inhibition constants for glucose oxidation and CO_2-fixation were found to be 2×10^{-4} M for acetazolamide and 3×10^{-5} M for ethacrynic acid. Using Lasix, the maximal inhibition obtained was 30 per cent at a concentration of 6×10^{-4} M. Thus, the inhibition constant cannot be determined. In each case, addition of albumin to the medium prevented the inhibition completely, presumably because of protein binding.

On incubation with cell-free enzyme extracts none of the diuretic agents affected glucose oxidation and CO_2-fixation.

In *in vivo* experiments, the three diuretic agents, administered at doses of 1×100 mg/kg and 8×100 mg/kg p.o., had no effect on the incorporation of ^{14}C-glucose into the lipids of the fat tissue.

After intraperitoneal injection of insulin, 1 unit/kg, the incorporation of ^{14}C into the lipids is increased. The simultaneous administration of 1×100 mg of acetazolamide or ethacrynic acid caused a slight reduction in the incorporation to 80 and 70 per cent, respectively. No such effect was observed with Lasix.

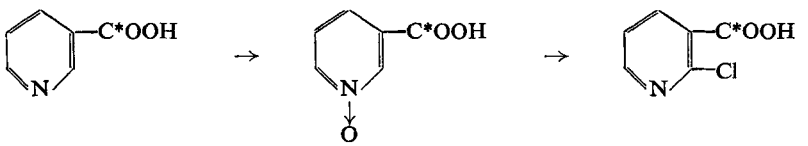
The results show that the reduction in glucose utilization, determined in isolated fat cells, is probably of no importance *in vivo*.

Synthèse et purification de l'acide 2, (m-trifluoro-methyl-anilino)-nicotinique (UPSA : UP-83) marqué par le ^{14}C dans le groupe carboxylique.

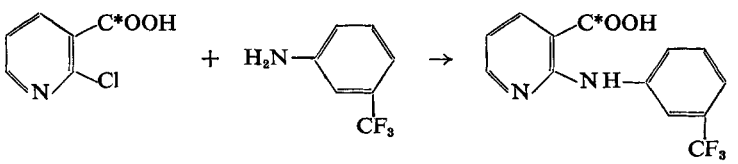
C. COLOMBINI, G. DEGANELLO, A. BENAKIS, M. STROLIN (Centro di Chimica delle Radiazioni e dei Radioelementi del C.N.R., Università di Padova, Italia; Laboratoire du Métabolisme des Médicaments, Université de Genève, Suisse).

Un nouveau anti-inflammatoire non stéroïdique, l'acide niflumique isostère pyridinique de l'acide flufénamique a été dernièrement expérimenté avec succès. Pour étudier le métabolisme de ce nouveau médicament, sa synthèse marquée a été réalisée en marquant le ^{14}C de son carboxyle.

Cette synthèse comporte 4 étapes et aboutit à un produit d'une activité spécifique de 16 mc/mM, selon le schéma suivant :



(I) (II) (III)



(IV)

L'acide nicotinique marqué au groupe carboxylique (I) a été obtenu selon la méthode décrite par A. Murray et Williams dans « Organic Synthesis with Isotopes », vol. 1, p. 392, avec une activité spécifique de 40 mc/mM et un rendement de 54,5%. Le N-oxyde de l'acide nicotinique (II) et l'acide 2-Cl-nicotinique (III) ont été préparés avec des rendements respectifs de 60% et de 20% et le produit final (UP-83) (IV) avec un rendement de 60% à partir de l'acide 2-Cl-nicotinique après une première purification par sublimation sous vide.

Le rendement final radioactif a été de 3,8%, cependant celui-ci peut être notablement augmenté dans le cas où l'on désire avoir un produit de faible activité spécifique ou encore en augmentant le nombre des opérations et la quantité produite.

La chromatographie sur papier du produit final indique la présence de 5 impuretés représentant le 2% de l'activité totale.

L'impureté principale (1,5%) est due à une benzoxazépine. Les autres impuretés sont dues à des produits marqués provenant des étapes précédentes de la synthèse, principalement l'acide 2-Cl-nicotinique-^{14}C.

Pour l'étude métabolique, le produit marqué a été purifié par chromatographie préparative sur papier avec le système *n*-butanol-pyridine-H_2O sat-NaCl avec un rendement d'environ 70%.

Une nouvelle chromatographie indique une pureté radiochimique supérieure à 99,9%. L'activité spécifique a été déterminée par scintillation liquide.

A simplified solid-state scintillation counting on glass microfiber medium in plastic bag for ^{3}H, ^{14}C, and ^{36}Cl in biological and organic materials.

Gopi N. GUPTA (The Rockefeller University, New York, N. Y. 10021).

A few years ago, the concept of solid scintillation counting was developed by Roucayrol *et al.* (1957) and Seliger and Agranoff (1959) in which dried paper strips, previously impregnated in a solution of anthracene in benzene, were placed on the surface of photomultiplier tube for counting. However, their technique was less efficient and impractical to be adapted to commercially available scintillation counters. The present paper describes a simple method of impregnating the sample on glass microfiber medium, wetting with a few drops of usual scintillation fluid, sealing in a plastic bag and counting in a lucite holder (plastic vial).

The precedure has simplified radioassay of a variety of biological and organic materials in the following ways, viz., (1) low background counts and high efficiency, (2) need of scintillation fluid, (3) glass vials not needed, (4) easier disposal of the bag and its contents, (5) even paper strips, millipore or glass-fiber discs based materials easily counted, and (6) overall low cost.

Preparation of P-32 marked Phosphate Esters by Neutron Irradiation.

J. HOELZL and D. NEUMEIER (Institut für pharmazeutische Arzneimittellehre der Universität München).

Marked phosphate esters were prepared by irradiation with thermic neutrons in a reactor. Thereby a n, γ-process takes place and P-31 is transformed to P-32. With this method a part of the compound remains in the native form and becomes radioactive. The dependance of stability from the exposure time, the accompanied substances and the structure were investigated. To prove this method, we chose a sugar phosphate, a nucleotide and a phospholipid. We obtained substances with specific activities of 0.3 to 1.4 mc/mM by an irradiation time of 8 hours and a flux of $4{,}2.10^{12}$n/sec per cm^2. The labelled products were removed from the destroyed compounds by column chromatography. The purity of the marked substances was proved with chromatographic and encymatic methods.