Paper No.56

STANDARDS OF QUALITY FOR NEW RADIOPHARMACEUTICALS

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Radiopharmaceuticals, like all pharmaceuticals, must be safe and efficacious. For some aspects of safety, such as sterility and freedom from pyrogens, general standards and methods are given in the Pharmacopoeias. For new radiopharmaceuticals any development programme must include in its major objectives:

To determine what standards of efficacy are necessary to meet the clinical needs;

To examine any special aspects of safety, for example as regards radiation dose to the patient, and to set standards;

To devise tests to ensure that these standards are met.

A critical point in the development programme is the clinical trial. At this stage, one must have not only an assurance that the product will be safe in humans but also an assurance that quality control procedures are developed to the point that future production batches will mirror those employed in the clinical trial. In its turn this means that *in vitro* analytical tests must be developed and correlated with the behaviour of the product in animals, and the behaviour in animals must be correlated with the behaviour in humans, so that *in vitro* tests, and, if necessary, animal tests, can be selected and developed as the basis for routine quality control.

Examples are drawn from a number of products recently developed, including:

Indium-111 DTPA for cisternography, where it was particularly necessary to consider radiation dose to tissues in contact with the cerebro-spinal fluid in terms of the radionuclidic and radiochemical purity of the product;

6β-methyl-[^{75}Se]-selenomethyl 19-norcholest-5(10)-en-3β-ol for adrenal scanning, where it was considered necessary to examine the possibility of radiation dose to the gonads from radiochemical impurities;

Scanning agents based on technetium-99m, where the Pharmacopoeias are presently attempting to draft monographs, but where questions arise regarding the extent to which *in vitro* and animal tests are reliable guides to efficacy.

Paper No.57

^{123}HIPPURAN: A STUDY OF RADIOIODINATION TECHNIQUES AND RADIOCHEMICAL IMPURITIES

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The increasing availability of high purity ^{123}I has prompted an investigation of published methods of radioiodination of O-iodo-hippuric acid (OIH) in order to establish the most suitable method for in hospital preparation of this potentially important radiopharmaceutical. Six methods (1-6) have been investigated with ^{125}I with particular importance being attached to labelling efficiency, simplicity, time of preparation and radiochemical purity of the final product.

The published methods were followed as closely as possible but difficulties were immediately encountered as several of the papers did not give sufficient experimental details. Important information such as quantities of materials, pH, temperature etc. were omitted. Using ultrapure OIH, all our experiments gave labelling efficiencies which were considerably less than those claimed by the authors whose methods were investigated (Table 1).

Table 1

Author	Efficiency (%)
1	44
2	26
3	2
4	57
5	2
6	24

The method which was found to be the most suitable (4) was further adapted by changing pH, temperature and reaction time in order to obtain optimal conditions to give the highest labelling efficiency. The highest value achieved was 58% using the method described by authors (4).

A survey of these published papers revealed that most authors used stock OIH which was contaminated with approximately 2% O-iodo-benzoicacid (OIB). One author (3) showed that this material produced about 50% labelled OIB and even purified material with an impurity of 1% OIB produces 16% OIB in the final product. These results suggest that preferential labelling of OIB compared with OIH occurs. For this reason it is postulated that papers claiming yields of over 90% for OIH may contain very high quantities of labelled OIB. We have shown that certain chromatographic systems used in these papers fail to differentiate OIB from OIH whilst this can be achieved using Merck Silica gel plates.

In order to show this we have carried out the following:-

(a) labelled ultrapure OIH, by the technique giving highest labelling efficiency.
(b) repeat (a) using impure commercially available material.
(c) repeat (a) adding 5% OIB to purified OIH.
(d) labelled OIB without OIH present.

The results of these investigations will be presented which show high radiolabelled contamination of OIH with OIB (30%) under certain experimental conditions. By changing experimental parameters this contamination level can be greatly reduced. Analysis of ^{123}I hippuric acid from a commercial supplier showed an OIB contamination of 18%.

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Paper No.58

CONTENT OF TECHNETIUM-99 IN TECHNETIUM-99m PREPARATIONS ITS ESTIMATION AND SIGNIFICANCE

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Practically nearly all of the great bulk of papers dealing Tc-99m tacitly neglect the influence of the content of long-living Tc-99 in the preparations of the short-lived Tc-99m. However, the different specific activity of Tc-99m may cause not only an enlargement of the absorbed radiation dose to the patient but may also change chemical yields during the labeling process and possibly even the biokinetics in vivo. Till now only in a few papers this problem has been at least touched (1-5).

In the present study a method for detailed evaluation of the Tc-99 amount in Tc-99m preparations has been worked out. Not only the actual characteristics of the generator (e.g. time between elutions, their total number, elution yield etc.), but also the generator prehistory (e.g.conditions of producing Mo-99, waiting time, processing etc.) have been taken into account. Possibilities of experimental estimation of the specific activity of Tc-99m preparations have been mutually compared.

Practical evaluations of Tc-99m specific activity from commercial absorption generators as well of instant technetium from an Institute´s in-house methylethylketone extraction generator have been performed. (Ratios of Tc-99 to Tc-99m are expressed in values: RN-number of atoms, RA-activity in pCi/mCi, RD-activity in dpm/mCi). Our values of Tc-99 in instant Tc-99m (RN: 5-40, RA: 15-130, RD: 40-250) are in general lower than those given by Srivastava and coll.(5) (RN: 10-360, RA: 30-1200, RD: 80-2700) and comparable with values for the absorption generator.

On the basis of the estimated Tc-99 content in Tc-99m preparations its influence on the chemistry of the labeling pro-

cess and the absorbed dose during application to man has been evaluated and discussed.

An example of Tc-99 to Tc-99m ratios in different elutions of the same generator is reproduced in Fig.1; values of Tc-99 contribution to the absorbed dose in man during application of Tc-99m are given in Table 1.

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Table 1

Absorbed doses from Tc-99 present in Tc-99m preparations (a)

Organ	Dose /mrad/from 1 mCi				% dose Tc-99/Tc-99m (e)			
	Tc-99m		Tc-99		I		II	
Whole body(b)	1,1	(1)	7,4	(1)	1,1	(-5)	8,6	(-4)
Whole body (c)	1,1	(1)	1,3	(6)	1,6	(-1)	1,2	(1)
Intestines	1,2	(2)	2,9	(3)	3,9	(-5)	2,8	(-3)
Thyriod	1,3	(2)	7,3	(2)	9,1	(-4)	6,5	(-2)
Bones (d)	3,8	(1)	4,0	(5)	1,7	(-2)	1,2	(0)

Notes: (a)-the number in paranthesis gives the exponent of the decimal order; (b)-calculated under the assumption of identical biokinetics of Tc-99 and Tc-99m; (c)-calculated under the assumption of Tc-99 deposition for 50 years; (d)-calculated on biokinetic data of the Tc-99m-EHDP bone diagnostic agent; (e)-I: lowest value of Tc-99 content following our estimations corresponding RN=5; RA=16; RD=40; II:highest value of Tc-99 content following estimations of Srivastava (5) corresponding to RN=360; RA=1200; RD=2700.

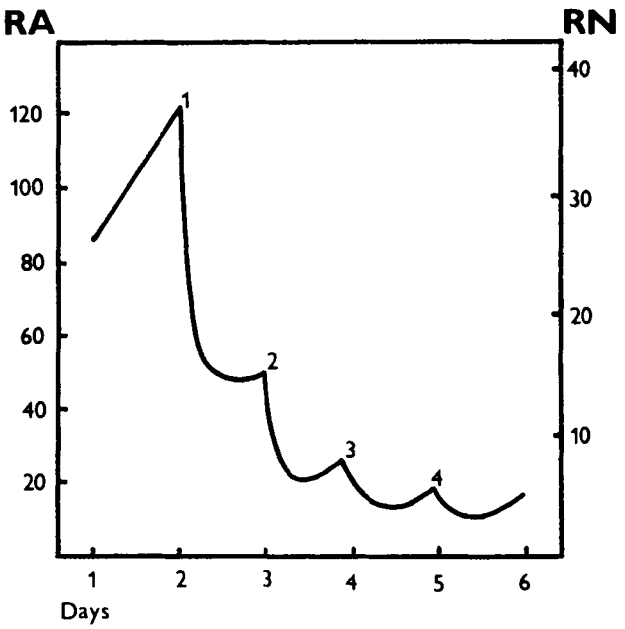

Fig.1: Tc-99 in Tc-99m preparations in dependence on generator age, repeated elutions.

Paper No.59

ON THE QUALITY OF ^{99m}Tc-ELUATES OBTAINED FROM FISSION MO-GENERATORS

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In a previous study (1) 8 different types of generators were studied. Since then 5 new types have emerged, all of which are based on fission-Mo-99.

The present study assesses the quality of eluates from these new types. 5 consecutive eluates from one 2oo mCi (nominal) generator of each type were assayed for nuclidic purity, Al^{+++} content, and radiochemical purity. In the latter case both the eluate and a 1:1 dilution (with isotonic saline) were assayed.

The results showed:

(a) none of the eluates contained α-emitters above the detection limit (5×10^{-8}% of the eluted Tc-99m activity)

(b) the amount of pure β-emitting impurities (including Tc-99) never exceeded 2×10^{-6}% of the eluted Tc-99m activity

(c) the amount of Mo-99 was in all cases but two below 10^{-3}% of the eluted Tc-99m activity

(d) only eluates from one generator type contained traceable γ-emitting impurities (other than Mo-99)

(e) only eluates from one generator type contained more than 0.5 ppm Al^{+++}

(f) all eluates and 1:1 dilutions of eluates had a radiochemical purity above 99%

The results indicate that the quality of eluates from fission-Mo-loaded ^{99m}Tc-generators are comparable to eluates obtained from "conventional" generators loaded with neutron-irradiated Mo.

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Paper No.60

CARRIER-FREE SELENIUM-73 CYCLOTRON PRODUCTION AND SELENO-RADIOPHARMACEUTICALS SYNTHESIS

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Short-lived selenium-73 has decay characteristics which are suitable for detection on a gamma camera as well as on a positron emission tomograph. Furthermore, the nuclear properties of this radionuclide suggest that selenium-73 will progressively replace selenium-75 in the future *in vivo* applications. The optimum physical and chemical conditions for the cyclotron production of high radionuclidic purity selenium-73 have been exhaustively determined.

The excitation functions for the production of ^{73}Se with a maximum energy of 37 MeV ^{3}He and 40 MeV ^{4}He using germanium of natural isotopic composition and ^{72}Ge isotopically enriched to 96.4% have been determined. The $^{72}Ge(^{3}He,2n)\ ^{73}Se$ nuclear reaction on GeO_2 with ^{3}He degraded from 37 MeV on a 151 mg/cm^2 target of GeO_2 is preferred. In such conditions, a thick target production rate of 933 $\mu C/\mu Ah$ can be obtained on isotopically GeO_2 enriched to 96.4% and of 403 $\mu C/\mu Ah$ on GeO_2 of natural isotopic abundance. With a low energy medical cyclotron, a sufficient yield of 218 $\mu C/\mu Ah$ can be reached on 71 mg/cm^2 of natural GeO_2 with incident alphas of 26 MeV. The extrapolation to 95% enrichment in ^{70}Ge indicates a production rate of approximately 1 $mC/\mu Ah$. Qualitative and quantitative data on ^{3}He and ^{4}He nuclear reactions resulting in radiocontaminants (^{75}Se, ^{71}As, ^{72}As and ^{76}As) are provided.

A method for rapidly separating the carrier-free selenium has been developed using a simplified sublimation method by means of a temperature-controlled quartz furnace heated in a HF coil. A reproducible radiochemical yield of 75% has been obtained.

Potential radiopharmaceuticals that have the capability of forming C-Se bonds can be labelled with chemically active intermediates such as NaHSe, Na_2Se_2, CH_2SeH, ... The synthesis of these intermediates from metallic radioselenium is described. In the aromatic series where the selenium atom is replacement for sulphur in the heterocyclic structure of benzoselenophene and derivatives, a fast radiochemical procedure starting from metallic selenium has been optimized to simplify the conventional time-consuming multistep synthesis.

In the aliphatic series, the introduction of the label into the product at the extremely last synthetic step has been successfully experimented for the preparation of the ^{73}Se-selenomethionine. The preparation time is now 4 hours after the end of bombardment and the over-all activity yield is better than 25% of the starting activity.

Paper No.61

POTASSIUM-38 PRODUCTION WITH A CYCLOTRON FOR MEDICAL USE

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Potassium-38 interests us as a myocardial imaging agent and as an *in vivo* indicator of rapid "new" potassium accumulations by other organs and tissues (1). Because of its 7.63-min half-life, and the resultant low radiation exposures, studies may be repeated frequently to follow physiological or pharmacological phenomena. Since K-38 is a 100% positron emitter, emission tomographic imaging may be exploited advantageously.

We have developed a convenient way to make K-38 by bombarding sodium chloride (NaCl) with 15-MeV He-4 ions (similar to the way K-38 was discovered in 1937 (2)) from our Cyclotron Corporation CS-15 cyclotron. The target chamber is diagrammed in the Figure. 100 mg of NaCl in water is evaporated on the tantalum plate target holder to form an ellipse of 11 mg/cm^2. This degrades the energy from 15 to 6 MeV, when irradiated at 30° to the beam axis. Bombardment of the NaCl occurs at the end of the evacuated external beam tube. After irradiation with a beam of He-4 ions defined by two 1.9-cm-diam collimators, a gate valve is closed, air is admitted to the target chamber, and the target is withdrawn by pulling on the water cooling tube. The bombarded NaCl is washed off and dissolved in 10 ml of water to form an isotonic saline solution containing "no-carrier-added" K-38. After sterilization by filtration it is ready for injection. No radioactive contaminants were detected, as expected from Q-values and threshold energies of possible nuclear reactions. The average yield and standard deviation for 21 production runs was 0.58 ± 0.17 mCi/μA for bombardments near saturation. A typical yield was 18 mCi of K-38 three minutes after bombardment for 20 minutes at 50 μA. "Thick-target" yields are about three times greater. Several targets are kept on hand to make repeated runs rapidly.

Our estimate for the total body radiation dose for this radionuclide is 12 mrad/mCi. Kidneys seem to be the critical organ receiving about 150 mrad/mCi. The radiation dose to the bone marrow and testes is estimated to be less than 10 mrad/mCi.

A pentobarbitalized rhesus monkey was scanned successively three times at about hourly intervals. Scans were started 8, 15, and 30 minutes after injection intravenously. The heart clearly was visualized well in all three images. Contrasts were good in all images, and did not change greatly, but was best in the first image. The acquisition of a positron tomographic imager or a positron camera would enhance greatly the usefulness of K-38 for high-resolution imaging of loci where accumulations of "new" potassium occur due to rapid turnover.

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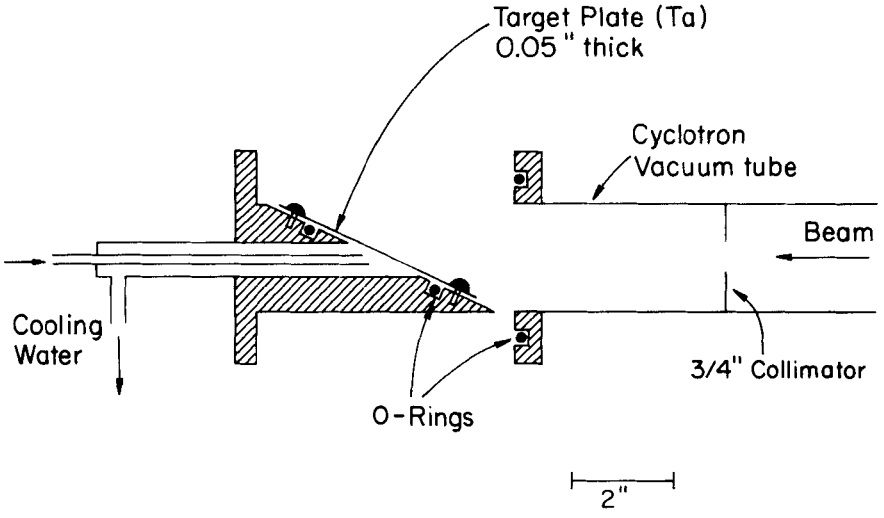
Table of Energetics of He-4 Reactions on NaCl

Target Nuclide	Nuclear Reaction	Product	Half-Life	Q Value (MeV)	E_{min} (MeV)
Cl-35	α,n	K-38	7.7 m	5.88	6.03
	α,d	Ar-37	34 d	8.77	9.24
	α,αn	Cl-34m	32 m	12.63	14.50
	α,2n	K-37	1.2 s	17.93	-
Cl-37	α,n	K-40	10^9y	3.88	3.99
	α,2n	K-39	stable	11.68	-
Na-23	α,n	Al-26	7×10^6y	2.96	3.07
	α,2n	Al-25	7.2 s	14.31	-
	α,αn	Na-22	2.6y	12.42	15.24

where E_{min} = Q value. $\frac{\text{Mass of Compound Nucleus}}{\text{Mass of Product}}$



Paper No.62

THE CYCLOTRON PRODUCTION OF ^{38}K, ^{51}Mn, ^{52m}Mn, AND ^{77}Kr FOR POSITRON EMISSION TOMOGRAPHY*

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Short-lived positron emitting radionuclides that can be used directly (without synthetic manipulation) at hospitals with a cyclotron and a positron emission tomograph are receiving increasing emphasis in nuclear medicine. The aims of our medical radionuclide development research program include the determination and optimization of new medical radionuclide production methods appropriate to cyclotrons, targetry and automated devices for radiochemistry, and delivery of radionuclides for direct or synthetic use. Results for four nuclides having demonstrated or potential application for myocardial or pulmonary studies in conjunction with a positron emission tomograph are discussed.

Potassium-38 ($t_{½}$ = 7.62 m) decays with a 100% abundance of 2.7 MeV positrons and 2.17 MeV photons. ^{38}K is ideal for myocardial perfusion studies in conjunction with positron emission transaxial tomography or focal plane tomography (1-2). The parameters for the routine cyclotron production of ^{38}K have been established using the $^{40}Ar(p,3n)^{38}K$ nuclear reaction. Argon was contained in a Ni target (37 ml) and irradiated with ∿8 μA of protons (E_p = 32→29.8 MeV) to 85% of saturation. Following the irradiation, the Ar (6.8 atm) was vented and 5 ml of water introduced into the target chamber. A single 5 sec wash resulted in ∿95% radiochemical yield of ^{38}K. The Al window of the target was punctured. The ^{38}Kr was withdrawn and purified of ^{38}Cl, produced by the $^{40}Ar(p,2pn)^{38}Cl$ reaction, by rapid passage through an ion exchange resin (Dowex 1-X8, 50-100 mesh), through a millipore filter and into a syringe for injection. The ^{38}K was obtained in a radionuclidic purity of >99.9% at an overall production yield of 4.4 ± 0.9 mCi/μAh. The time required from EOB for processing and delivery 1 km from the cyclotron was <4 min. The $^{38}Ar(p,n)^{38}K$ reaction on 30.8 mole percent ^{38}Ar is presently being considered as an alternate production route.

Manganese is an essential trace element which partitions into the mitochondria of heart, pancreas and liver (3). Mn may be a co-factor in cholesterol synthesis (4), and has been implicated as a respiratory co-factor in mitochondria (5). Chauncey et al (6) recently reported a significantly reduced uptake of ^{54}Mn by ischemic compared to normal canine myocardium. ^{51}Mn ($t_{½}$ = 46.2 m, β^+ = 2.2 MeV, 97%) and ^{52m}Mn ($t_{½}$ = 21.4 m, β^+ = 2.63 MeV, 97%) provide an opportunity to tailor the half life of the nuclide to the study. The $^{52}Cr(p,n)^{52m}Mn$ reaction with 12.3 MeV protons incident on a 258 mg cm^{-2} Cr_2O_3 target of 99.83% isotopic enrichment ^{52}Cr resulted in a production rate of 50 mCi/μAh. The radionuclidic impurity of ^{52g}Mn ($t_{½}$ = 5.63 d) form via the (p,n) reaction, and after complete decay of ^{52m}Mn was <0.013% of the ^{52m}Mn at EOB. Targetry was evaluated with a 10 μA fluence and E_p = 16→6 MeV. The $^{52}Cr(d,2n)^{52m}Mn$ reaction with incident deuterons of 18.0 MeV on 195 mg cm^{-2} Cr_2O_3 as 99.83% ^{52}Cr resulted in a production rate of 59 mCi/μAh. The total ^{52g}Mn impurity assayed after ^{52m}Mn decay was <0.007%. The $^{52}Cr(p,2n)^{51}Mn$ reaction on 195 to 226 mg cm^{-2} targets of isotopically enriched $^{52}Cr_2O_3$ and incident protons of 26 MeV resulted in a ^{51}Mn production rate of 35 ± 1.5 mCi/μAh. The yield of the $^{52}Cr(p,n)^{52m}Mn$ reaction is minimized, although the ^{52m}Mn impurity should not interfere. ^{51}Mn decays to ^{51}Cr ($t_{½}$ = 27.1 d). One mCi of ^{51}Mn yields ∿1 μCi of ^{51}Cr. The ^{51}Cr daughter does not preclude the use of ^{51}Mn clinically. In addition, the ^{51}Mn→^{51}Cr generator may be particularly

suitable for in vivo studies of the chemical effects of the nuclear transformation of radiomanganese decay while localized in mitochondria. The $^{50}Cr(d,n)^{51}Mn$ reaction was tested on isotopically enriched ^{50}Cr (96.15%), but the yield was low on a 104 mg cm^{-2} target of Cr_2O_3 irradiated with 10 MeV incident deuterons. A significant radionuclidic impurity of ^{52}Mn arises from the (d,xn) reactions on the isotopic impurities of ^{52}Cr (3.54%) and ^{53}Cr (0.24%). Work is in progress to develop a rapid radiochemical separation since Mn^{+2} in strong HCl is weakly absorbed on strong base anion exchange resin; whereas, Cr^{+3} is not absorbed.

The rare gases, particularly ^{81m}Kr and ^{133}Xe, are finding widespread use for the measurement of cerebral and myocardial blood flow, and for regional ventilation and perfusion studies. Krypton-77 ($t_{\frac{1}{2}}$ = 1.24 h) decays with the emission of 1.86 MeV positrons in 74% abundance, and 108 and 131 keV photons in 25% and 20% abundance, respectively. The nuclide may be appropriate for focal plane tomography of the lungs, provided the daughter (^{77}Br) does not significantly increase the absorbed radiation dose. ^{77}Kr may be useful for excitation labeling of ^{77}Br compounds. A production rate of 23 mCi/μAh was obtained via the $^{79}Br(p,3n)^{77}Kr$ reaction with E_p = 32→23 MeV and a KBr target. A 10 μA x 12 min irradiation produces ∿50 mCi of ^{77}Kr which was dynamically removed on-line with a dried stream of He (50 ml min^{-1}) from the salt target during the irradiation. The radiochemical yield was dependent on the beam current and the total irradiation dose. The ^{77}Kr was transported 25 m external to the cyclotron vault, and passed through an Ag furnace (350°C) to remove radiobromine impurities. The ^{77}Kr (free of ^{76}Kr impurity) was collected on a charcoal trap (77°K) which also serves to retain the ^{77}Br daughter when the trap is warmed to 130-200°K to vacuum distill the ^{77}Kr for use. A data acquisition system is useful to record the time, flowrate, and to monitor a sample of the ^{77}Kr in the He stream during the irradiation and collection process.

The use of ^{38}K, ^{51}Mn and ^{52m}Mn for positron emission tomography will be mentioned.

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Paper No.63

PRODUCTION OF ^{48}Cr FOR MEDICAL USE

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^{48}Cr ($T_{1/2}$ = 21.6 h) is a potentially useful radionuclide and may substitute the ^{51}Cr ($T_{1/2}$ = 27.7 d) in labelling of erythrocytes. Because of its shorter half-life and the high abundance of its γ-rays compared to ^{51}Cr, the activity of ^{48}Cr to be used and hence the radiation dose would be much lower than that of ^{51}Cr.

For production purposes, the excitation functions of the reactions $^{51}V(d,5n)^{48}Cr$, $^{nat}Ti(\alpha,xn)^{48}Cr$ and $^{nat}Ti(^{3}He,xn)^{48}Cr$ were measured. The ^{3}He induced reaction on natural titanium proved to be the best from the viewpoint of yield and purity. The maximum of the excitation function lies around 32 MeV, showing good production possibilities at the compact cyclotron CV 28. The yield of ^{48}Cr is about 35 μCi/μAh according to a degradation of energy of the ^{3}He particles from 36 to 0 MeV, and at EOB the radionuclidic impurity due to ^{51}Cr amounts to 0.5%.

The chemical separation procedure for ^{48}Cr consists of dissolution of the metallic titanium target material in hydrochloric acid, oxidation with hydrogen peroxide and extraction chromatography using tri-n-butylphosphate. The product is used for labelling erythrocytes. The chemical consequences of the EC-decay and the behaviour of the daughter product ^{48}V ($T_{1/2}$ = 15d) have also been studied.

Paper No.64

YIELD OF ^{28}Mg FROM Si, P, S, Cl, Ar and K BOMBARDED WITH 50-160 MeV PROTONS

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Magnesium is of fundamental biological importance as it is involved in numerous important biochemical reactions. Considering this, surprisingly little is known about the factors which regulate magnesium homeostasis in man. ^{28}Mg ($t_{1/2}$ = 21.1 h) is the only isotope of magnesium that can be used for metabolic studies. It is, however, difficult to produce carrier-free in high yields. Different production methods are reviewed by Probst *et al.* (1).

The aim of this work has been to investigate the possible use of spallation reactions with high energy protons for production of carrier-free ^{28}Mg in clinically useful amounts, and to establish a chemical separation method yielding a radiopharmaceutically acceptable preparation.

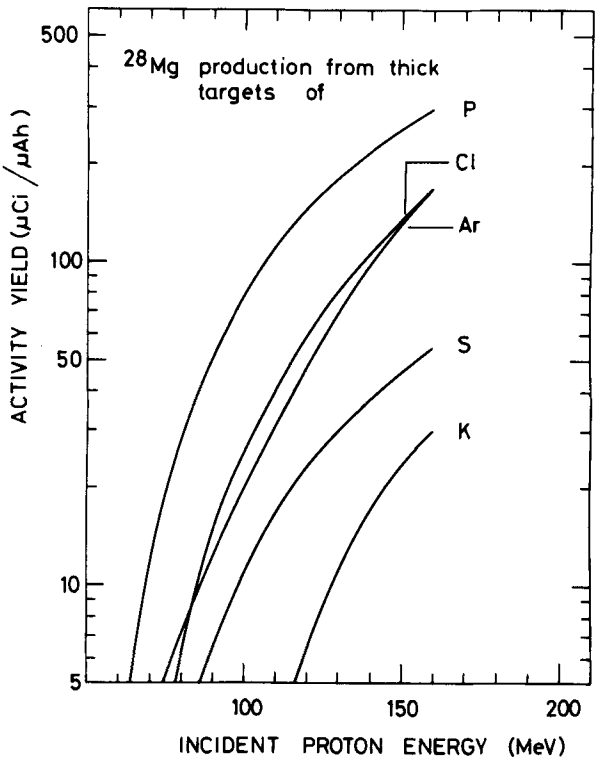
Targets of liquefied Ar were irradiated in the external proton beam of the syncrocyclotron at the Gustaf Werner Institute. Suitable forms or compounds of the other elements were irradiated in the internal proton beam. Simultaneously irradiated Al-foils were used to monitor the proton beam via the reaction $^{27}Al(p,3pn)^{24}Al$ (2,3).

Induced activities were chemically separated (except for Si and Ar) and measured with a 70 cc Ge(Li)-detector. Cross-sections for the production of ^{28}Mg and the main radionuclidic disturbances ^{7}Be, ^{22}Na and ^{24}Na were computed referring to the monitor-reaction (2,3). Some of our results are compared to the scarce literature cross-section data in the table below. Thick target yields were calculated and are given in the figure.

For routine production NaCl was used as target material and a carrier-free chemical separation method was worked out. In the internal, 1 μA beam, our optimum target configuration of pressed NaCl yielded around 15 μCi/μAh. A study of gastro-intestinal Mg-absorption in some 50 patients has been performed in collaboration with the Uppsala Academic Hospital (4).

Table comparing cross-section values obtained in this work to values found in the literature (proton energy = 130 MeV):

Reaction	This work	Literature	References
$^{30}Si(p,3p)^{28}Mg$	0.86 ± 0.10	2.05 ± 0.46	(5)
$^{31}P(p,4p)^{28}Mg$	0.190 ± 0.028	0.189 ± 0.022	(5)
$S(p,5pxn)^{28}Mg$	0.036 ± 0.005	0.00431 ± 0.00206	(5)
$Cl(p,6pxn)^{28}Mg$	0.115 ± 0.017	0.75 ± 0.2	(6)
$Ar(p,7pxn)^{28}Mg$	0.125 ± 0.019	-	-
$K(p,8pxn)^{28}Mg$	0.027 ± 0.004	-	-



Thick target yields of ^{28}Mg from P, S, Cl, Ar and K bombarded with protons.

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Paper No.65

ATTACHMENT OF BIFUNCTIONAL CHELATING AGENTS TO AMPHIPHILIC MOLECULES AND ANTIBIOTICS

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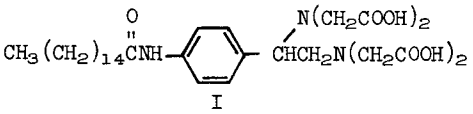
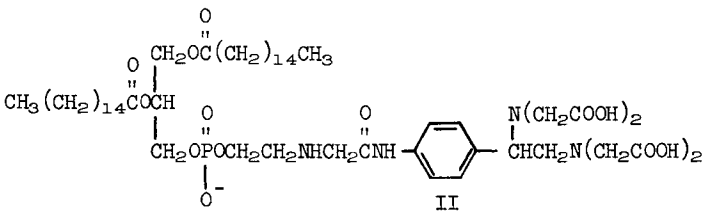
The coupling of para-substituted derivatives of 1-phenyl-ethylenediaminetetraacetic acid or related compounds to biological molecules such as fatty acids, phospholipids, or antibiotics can lead to products which possess both biological activity and powerful metal-chelating properties. Such compounds have important potential applications in nuclear medicine because they can rapidly form stable chelates with a variety of radioactive metal ions. With the aim of exploring new approaches to the radiolabeling of blood cells, we have prepared the palmitoyl derivative (I) by acylation of aminophenyl-EDTA, and the dipalmitoylphosphatidylethanolamine derivative (II) by alkylation of the phospholipid with 1-(p-bromoacetamidophenyl)-EDTA. Each compound has been radiolabeled using ^{111}indium citrate. Incubation of red blood cells in normal saline with the albumin complex of (I) leads to radiolabeling of the cells, presumably by intercalation of the hydrophobic palmitoyl chain into the cytoplasmic membrane. The label is not eluted from the cells by repeated washing with saline, but it is removed by washing with plasma. In contrast, the ^{111}indium chelate of (II) may be used to label cells *in plasma*, and the label remains well bound during repeated incubations with plasma. Investigation of the labeling of lymphocytes and platelets with (II) and *in vivo* experiments are in progress.



I



II

Because of its tumor-localizing properties, bleomycin is an attractive compound for radiopharmaceutical use. Since bleomycin forms suitably stable chelates only with the kinetically inert cobalt (III), the attachment of EDTA groups to the variable "terminal amine" moiety may provide products which retain the properties of bleomycin and are also able to form very stable chelates with a variety of metal ions.

Starting with bleomycin-demethyl A_2, we are exploring two routes to attachment of an EDTA group to the terminal amine moiety. Recent results indicate that an appropriate product is formed by S-alkylation with 1-(p-bromoacetamidophenyl)-EDTA followed by thermal demethylation to produce a thioether linkage.

Preparation of a chelating derivative of the antibiotic rifamycin has been achieved by N-alkylation of 3-aminoethylthiorifamycin with 1-(p-bromoacetamidophenyl)-EDTA. Studies of the in vivo behavior of this compound are underway.

Paper No.66

PREPARATION AND PROPERTIES OF ^{67}Ga-6-MERCAPTOPURINE COMPLEX AND ITS UPTAKE IN MORRIS HEPATOMA-3924A

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Much research has been devoted to finding elements and compounds that specifically concentrate in tumours.The synthesis of radiopharmaceuticals for tumour diagnosis has in recent years found much attention, and ^{57}Co-bleomycin and ^{67}Ga-citrate have proved so far to give the most satisfactory results, although the radiation risk in the use of the former and the low specificity of the latter have repeatedly been pointed out[2]. Diagnostic radiopharmaceuticals have been either (a) simple compounds of a radioelement of convenient half-life and γ-energy, e.g., ^{67}Ga citrate, or (b) labelled compounds, e.g., ^{57}Co-bleomycin, of which the nonradioactive component serves as a vehicle for the radioelement to the tumour. We have prepared and studied some properties in solution of ^{67}Ga-6-mercaptopurine, of which both ^{67}Ga and 6-mercaptopurine (6MP) have been shown to concentrate in neoplastic tissues[2,3]. The ^{67}Ga-6-mercaptopurine complex was prepared, according to the method of Kirschner and coworkers[4] for other metallo-6-mercaptopurines, by careful addition of a weakly alkaline solution of 6-mercaptopurine to an acidic solution of the ^{67}Ga salt. ^{67}Ga-6-mercaptopurine-citrate and ^{67}Ga-6-mercaptopurine-chloride were prepared and their properties studied chromatographically. The citrato complex seems to be more soluble than the chloro one as shown by its higher R_f value. The solutions of the two complexes are stable in dilute hydrochloric acid, solution of sodium citrate, and physiological saline. The scintigraphic examination of the two complexes injected to Morris hepatoma-3924A-bearing ACI rats showed that the two complexes have more tumour affinity than the initial ^{67}Ga salt. The tumour concentration of ^{67}Ga-compounds

varies in the order: ^{67}Ga-6-mercaptopurine-chloride > ^{67}Ga-6-mercaptopurine-citrate > ^{67}Ga citrate > ^{67}Ga-chloride.
Recently inoculated developing hepatomas concentrate the radiopharmaceuticals more than the older ones.

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Paper No. 67

INDIUM-^{111} DESFERRIOXAMINE COMPLEXES: PREPARATION AND STABILITY STUDIES

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Desferrioxamine (DF) is a class of sideramines, the naturally occurring iron containing growth factors found in many microorganisms (1). With ferric ions DF forms a 1:1 complex of high stability ($10^{30.6}$) (2) and is currently used as a therapeutic agent in patients with thalassemia (3). Because of its role in bacterial growth it is considered that DF labelled with a suitable gamma emitting radionuclide may potentially be useful for imaging infective lesions. The purpose of this study was to prepare DF complexes with ^{67}Ga and ^{111}In, to study their stability and evaluate their potential as imaging agents in animals bearing induced infective lesions.

Complexes were formed in 0.5 mM solution of DF in acetate buffers of different pH at room temperature. Chromatographic analysis (n-Butanol:n-propanol: H_2O::9:6:5, Rf M*-0.05, LM*-0.5 and L-0.9) (4) revealed that the hexadentate ligand chelates $^{67}Ga^{3+}$ ions quantitatively and had no influence of pH contrary to the slow and pH dependent ^{111}In complex formation. Results of further work indicated that an equimolar amount of Fe^{3+} ions completely displaced ^{111}In from its DF complex whereas ^{67}GaDF remained unaffected. Ga^{3+} ions did not dissociate either radioactive elements. This suggested the stability of ^{67}GaDF> FeDF> ^{111}InDf> CuDF.

Although EDTA and NTA dissociated both ^{111}In and ^{67}Ga from DF complexes in vitro, significantly reduced distribution of radioactivity was observed in a group of mice which i.v. received DF following the administration ^{67}Ga citrate, indicating the ability of DF to chelate ^{67}Ga even in the presence of transferrin. The radioactivity distribution of ^{111}InDF in rabbits on the other hand resembled the distribution of $^{111}In^{3+}$. The results indicate that ^{67}GaDF is a more suitable complex for in vivo studies than the ^{111}InDF complex. Work is in progress with ^{67}GaDF in rabbits with induced infective lesions.

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TABLE I
PREPARATION: INFLUENCE OF pH ON LABELLING
DF conc. 0.05 M, Incubation at R.T. Time 1 hr.

pH	^{111}In	^{67}Ga
2.9	35%	99%
4.3	48%	99%
5.7	49%	-
6.1	71%	99%
7.0	68%	-
7.5	82%	99%
8.5	76%	-

TABLE 2
PREPARATION: INFLUENCE OF TIME ON LABELLING
pH 6.1 Incubation Temperature 60°C

TIME	% ^{111}In
15 min.	85%
30 min.	90%
45 min.	87%
105 min.	88%

TABLE 3
STABILITY: INFLUENCE OF Fe^{3+}, Cu^{2+}, EDTA and NTA

	^{111}In DF	^{67}Ga DF
Cu^{2+}	100%	100%
Fe^{3+}	0.0%	100%
EDTA	0.0%	0.0%
NTA	0.0%	0.0%

IN VIVO: ALTERATION IN TISSUE DISTRIBUTION

Control: 5 mice received 17 to 20 μCi ^{67}Ga-citrate only

Test - 5 mice received 17 to 20 μCi ^{67}Ga-citrate, 2 hrs. later each mice received 20 mg DF

Animals were sacrified 20 hrs. later and concomitant radioactivity in the following tissues was determined. (% g ad. dose)

	Control Animals	DF animals
Blood	1.6 + .149	0.027 + 0.007
Urine	3.62 + 1.322	0.525 ± 0.174
Liver	4.64 ± 0.464	0.21 ± 0.033
Spleen	3.14 ± 0.03	0.1 + 0.058
Kidneys	5.09 ± 0.64	0.89 ± 0.12
Lungs	3.39 ± 1.65	0.05 ± 0.017
Muscle	0.76 ± 0.257	0.07 ± 0.039

Paper No.68

PARAMETERS AFFECTING THE PREPARATION AND CELL LABELLING OF LABELLED MACROCYCLES SUCH AS INDIUM-111 PORPHYRINS

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The use of macrocyclic chelating agents in the development of compounds useful for in vivo imaging procedures is a logical progression following the sometimes disappointing results obtained when trying to change the in vivo distribution of a radioactive metal by using linear multidentate or tris or ternary complexes of bidentate chelating agents. Their cyclic nature gives their metal complexes many advantages, not the least being high stability and fixed sterochemistry that make it easier to predict exactly what structure is present. The long biochemical interest in naturally occurring porphyrins and the availability of simple synthetic analogues makes this group of macrocycles attractive for determining structure-activity relationships.

m-Tetraphenylporphine (TPP) (I), the first readily available synthetic porphyrin has been used as the Cobalt-57, sulphonate complex (II) in an attempt to visualize tumours (1), but the results were not promising enough to sustain further effort. Later, the preparation of tetraphenylporphinato Indium (III) was reported and subsequently Indium-111 TPP was prepared and used in mice to determine the effect of ligands on the tissue distribution of Indium-111 (2). The tissue distribution of a number of other metalloporphyrins has also been determined.

The publications by McAfee & Thakur (3,4) stimulated an interest in cell labelling with Indium 8-hydroxyquinoline, but difficulties encountered due to the instability of this complex with respect to transferrin brought Indium-111 TPP back into favour as in the original study it was one of the few compounds that labelled cells to any extent in the presence of plasma.

The normal method of preparing metalloporphyrins is one of refluxing a large excess of the metal and the porphyrin in a dipolar aprotic solvent such as dimethylformamide (5). This method is not applicable to "carrier-free" chemistry and so a method was developed for the preparation of Indium-111 TPP which involved refluxing for 2 hours in a glacial acetic acid, sodium acetate mixture and which gave maximum yields before purification of ~35% (2). This was clearly unsatisfactory especially if radionuclides with shorter half lives than Indium-111 were to be used.

A study of the parameters of the incorporation of carrier free Indium-111 into TPP was performed and a reproducible yield of >95% incorporation in 30 minutes was obtained using ~100 µg TPP heated at 110° C in 1 ml glacial acetic acid. Analysis of the solution was performed by TLC using as a solvent Benzene:Methanol (99:1, v/v) and precoated silica gel plates. With this system the Rf values were TPP = 0.65, InTPP = 0.38, free In = 0.00. The study revealed a number of pertinent facts.

1. The incorporation of Indium-111 is very sensitive to the anions present but relatively insensitive to the cations. In terms of inhibition the order is $Cl > SO_4 > ClO_4$ — nitrate alters the porphyrin ring (Fig. 1). This order can be explained by the presence in solution of different indium species as found by Celeda, et al. (6). As one supplier of indium distributes the radionuclide in 0.45-0.9% NaCl, pH 2-3, this is a major problem.

2. Contaminating stable metal ions compete with the indium for the TPP.

Although indium is a labile metal others such as copper can compete effectively. Copper contamination of one batch of Indium-111 was great enough to be able to see the CuTPP by TLC (Rf = 0.74) and to observe the characteristic CuTPP electronic spectrum.

3. Attempts at chelating stable metal contaminants are partially successful and show that even in the presence of a 3-fold molar excess of 8-hydroxyquinoline ~100% Indium-111 TPP is obtained.

This is important because the tris 8-hydroxyquinoline Indium (III) complex has an exceptionally high stability (7) but ligand exchange still occurs in plasma. This requires cells to be washed free of plasma proteins if high labelling yields are to be achieved (3,4).

Indium-111 TPP is at present being investigated as an alternative to Indium-111 8-hydroxyquinoline for labelling cells. Initial studies with platelets in plasma, (which is needed to maintain the viability of the platelets), indicate rapid and complete labelling.

A drawback of this compound is its insolubility in aqueous media which necessitates the use of dipolar aprotic solvents such as dimethylsulphoxide for dissolution. Alternatives are the carboxyphenyl-(**III**) or 4-methyl pyridyl-(**IV**) derivatives which are water soluble. They are currently being investigated as to the best method of forming the carrier-free indium chelates and to subsequently determine their cell labelling efficiency as (**III**) binds to serum proteins but (**IV**) does not (8).

Finally the tertiary amine monocyclic ligands such as 1,4,8,11-tetrazacyclotetradecane (**V**) are under investigation as being perhaps more amenable to derivatisation than the porphyrins and thus potentially more useful in directing metals to specific tissues.

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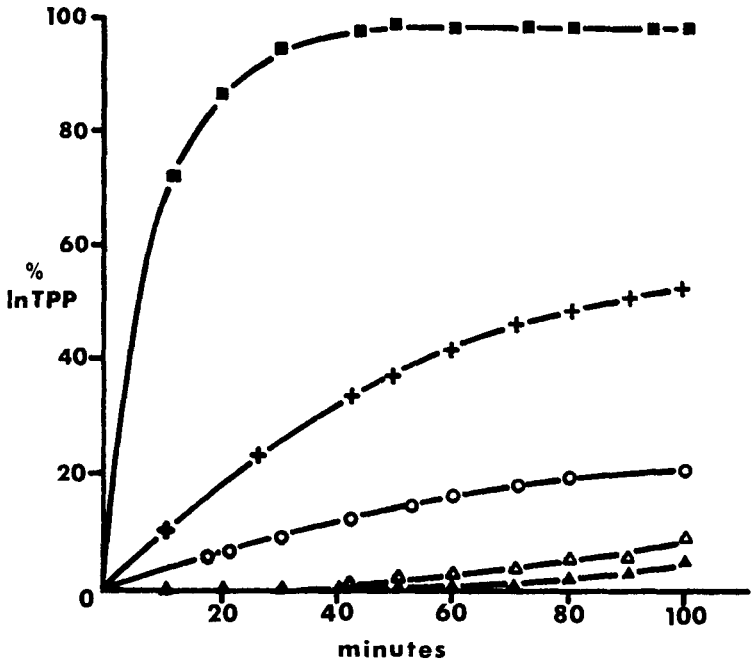
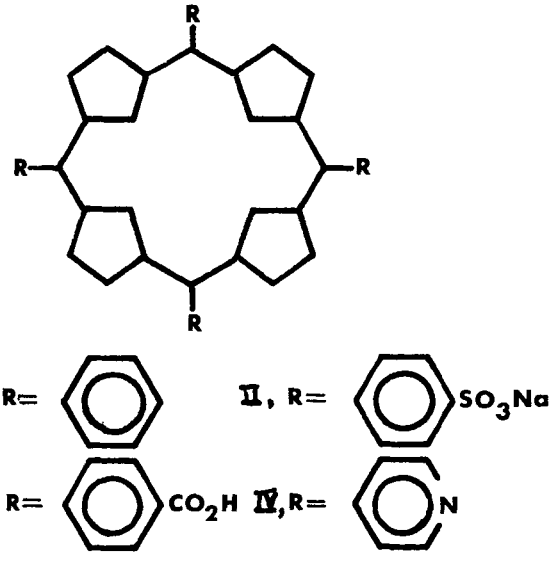
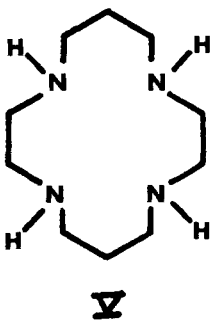


100 µl Indium-111 in 0.1 MHCl, 100 µl 0.15 M salt solution in H_2O evaporated to dryness and then heated at 110°C with 107 µg TPP in 1 ml Glacial Acetic Acid. ■ No salt solution, + $KClO_4$, ○ K_2SO_4, △ KCl, ▲ NaCl.





V

Paper No.A1

LONG-CHAINED ^{18}F-LABELLED FATTY ACIDS FOR THE STUDY OF MYOCARDIAL METABOLISM; ODD-EVEN EFFECTS.

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For the study of myocardial metabolism the kinetics of some ^{18}F-labelled fatty acids with different chain length and/or different position of the label have been investigated. The compounds, namely 2-^{18}F- and (9,10)-^{18}F-stearic acid, 16-^{18}F-hexadecanoic acid and 17-^{18}F-heptadecanoic acid were synthesized by nucleophilic F-for-Br exchange from the corresponding bromo-fatty acid ester systems in acetamide melt and hydrolysed to the free acids. The uptake data in the heart muscle in mice demonstrate some remarkable differences for the various compounds. For the 2-^{18}F-stearic acid only little heart activity is observed, but a slow increase of liver activity takes place within 30 minutes after injection. Similar to ^{11}C-, ^{34m}Cl-, ^{77}Br- and ^{123}I-halofatty acids (1), the compounds with the ^{18}F-label in the middle or at the end of the chain lead to a fast increase of heart activity rising to a maximum within about one minute. The clearance data indicate the existence of a fast and a slow elimination process.

In contrast to the corresponding iodinated compounds (1), 16-^{18}F-hexadecanoic and 17-^{18}F-heptadecanoic acid exhibit different half-lives of elimination (16-^{18}F-hexadecanoic acid: T_{fast} = 2.2 min, T_{slow} = 35-52 min; 17-^{18}F-heptadecanoic acid: T_{fast} = 6 min, T_{slow} = 18 min). These results can be attributed to a mechanism known as the odd and even rule, i.e. alternation of toxicity between ω-fluorinated odd and even numbered fatty acids. The difference of biochemical behaviour between the two compounds was further confirmed by the analysis of the heart muscle at maximum accumulation. While the concentrations of free fatty acids and glycerides were similar for both acids, the most striking contrast was the high amount of free ^{18}F-fluoride in the case of 17-^{18}F-heptadecanoic acid. Free fluoride, however, could not be detected among the metabolites of 16-^{18}F-hexadecanoic acid, a fact which is strongly supported by a concomitant increase of bone activity within 60 minutes after injection for the odd-numbered fatty acid.

The results can be interpreted in terms of a very fast fatty acid catabolism via ß-oxidation, ending with a C_2 or C_3 units respectively. Further degradation, i.e. dehalogenation, takes place only in the case of 17-^{18}F-heptadecanoic acid, while ^{18}F-fluoroacetic acid, resulting from the catabolism of 16-^{18}F-hexadecanoic acid, obviously undergoes further reactions in the citric acid cycle.

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Paper No.A2

[^{18}F]-L and DL-FLUOROARYL AMINO ACIDS. PRESENT STATUS.

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Although these compounds have been available since 1970 (1—4) they have not yet given successful results as pancreas imaging agents in man. On the other hand they have not been definitely rejected. The materials previously prepared could have contained labelled impurities and were originally made at relatively low specific radioactivities. Also the comments of ref (5) concerning the instability of derivitives of DOPA should be noted. The recent visualisation of the pancreas in man using [^{11}C]-valine(6) and the opportunities opened up by the β^+-camera encourage further work with the ^{18}F-amino acids. We consider that as many as possible of the amino acids should be available for the user.

A re-evaluation of the preparation and use of these compounds has been undertaken. The key intermediates were the protected formamido, III(a-d) or acetamido, III(e-j), malonic esters(6). The protected ^{18}F-labelled compounds (III) were obtained by thermal decomposition of the corresponding diazonium fluoroborates (II). Because of chemical and physical differences between the various compounds (III) a variety of methods of decomposition and subsequent de-protection(7) were needed.

^{18}F-labelling of the diazonium fluoroborates (I) :

$$\underset{\text{I(a-j)}}{RN_2^+\ BF_4^-} \xrightarrow[\text{neon}]{^{18}F} \underset{\text{II(a-j)}}{RN_2^+\ B^{18}F_4^-} \xrightarrow[140\text{-}200^\circ]{\text{dec.}} \underset{\text{III(a-j)}\sim 35\%}{R^{18}F} + N_2 + B^{18}F_3 + \underset{\sim 65\%}{\text{Imps/Tar.}}$$

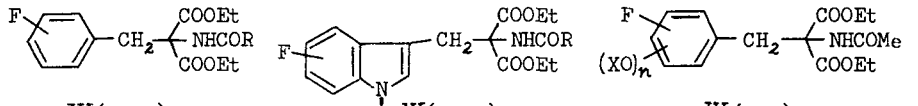
The aryl diazonium fluoroborates (I) were labelled by heterogeneous exchange between ^{18}F/neon(80 psi) and the organic solid (13mg) dispersed in a filter element, a 3.5cm diameter GFD disc (Fig 1) in the recirculatory target system(7,8). The effect of additives in the target gas was investigated. With pure neon, or neon containing oxygen (15%) or nitrogen (15%), about 42% of the fluorine-18 produced was trapped on the filter disc. When neon containing hydrogen (15%) was used, virtually all the fluorine-18 produced remained on the glass walls(8) of the target vessel. These results suggest that the species $NO^{18}F$ or $O_2{}^{18}F$ may be the carriers of fluorine-18 between the target vessel and the filter element, but this idea can be criticised.

Decomposition of the ^{18}F-diazonium fluoroborates (II) :
A variety of methods were investigated for this stage. Speed and ease of purification and of handling of the product (III) were desired. It was not possible to use a single method for all cases (a-j). Decomposition by heating alone ("dry") had the advantage that the crude product could be taken up in a volatile solvent for subsequent purification by preparative TLC, LC or GLC. The previously described inert solvent (tetralin) decomposition(9)suffered from several disadvantages and so was not used.

When the fluoro-ester was sufficiently volatile, III(a—d,i,j) it was feasible to purify (III) by vacuum sublimation or GLC. Vacuum sublimation did not prove to be useful because the sublimate still contained many impurities. On the other hand GLC using a 5ft × ¼in column of 3% OV1 or QF1 at 200°C gave a pure product (Fig 2) that was readily trapped at the chromatograph outlet. It was even possible to inject the ^{18}F-diazonium fluoro

-borate in acetone directly into the injector of the chromatograph (glass column essential).

FLUORO-ESTERS III(a–j).



III(a–d) III(e–h) III(i,j)

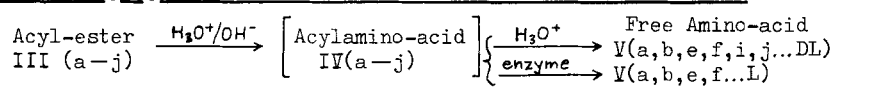
(a)p-F (b)m-F, R = H. (c)p-F (d)m-F, R = Me. Protected forms of fluorophenylalanines V(a p-F, b m-F).

(e)5-F (f)6-F, R = H. (g)5-F (h)6-F, R = Me. X = Ac. Protected forms of fluorotryptophans V(e 5-F, f 6-F).

(i)3-F, X = 4-Me (n = 1) (j)5-F, X = 3,4-Me_2 (n = 2) Protected forms of fluoro-tyrosine V(i), fluoro-DOPA V(j).

Routine Procedure : The dry decomposition was always used. The GFD-disc with ^{18}F-diazonium fluoroborate was placed between two stainless steel discs (5 X 1cm) preheated to 150°C (170°C for indoles) and left for 7min. The crude products III(e–h) were extracted into chloroform and purified by preparative TLC on a 5 X10cm "analytical" silica gel plate eluted with chloroform, ethyl acetate (5 : 1-vols), R_F~0.7 (Fig 3). The crude products III(a–d,i,j) were purified by preparative GLC as described above, t_R 8–10 min (Fig 2).

Hydrolysis of [^{18}F]-Fluoro-esters (III) including enzyme resolution.



The acetyl esters III(c,d,g,h) gave the N-acetyl-DL-amino acids (IV) by mild hydrolysis (Method 1. In g,h X→H also). These intermediates were stereo-selectively converted to the free L-amino acids V(a,b,e,f) using an amino acylase ex Aspergillus Orizae, Kindly supplied by Dr I. Chibata (Method 2) (9,10). Cleavage of the acetyl esters III(c,d,i,j) with 47% HBr gave the DL-amino acids V(a,b,i,j)*(Method 3). Compounds III(a,b,e,f) containing the labile N-formyl group are reported to give the DL-amino acids directly by Method 1 (2) but analysis of the products on the Biogel column indicated that this hydrolysis was incomplete, even negligable in 30min. [*(i,j X→H).

Method 1 did not work for compounds III(i,j) because of the great stability of the O-methyl group(s). A more labile protecting group is required. The O-isopropyl ethers would be suitable but the synthetic problems are great.

Routine Procedure : Method 1. The fluoro-ester III(a,b,e,f) was refluxed with 0.3N-NaOH (400µl) for 20min, and after the addition of 2N-HCl (75µl) refluxed a further 15min. The final solution was adjusted to pH 7.

Method 2. The enzyme (3mg, as supplied) was made up in water (200µl) with sodium acetate (7mg) and activated by the addition of Co^{+2}(50µl of 0.0001M $CoCl_2$). The enzyme solution was incubated with the acyl amino acid solution for 20min. The L-amino acid was isolated as below.

Method 3. The fluoro-ester III(c,d,i,j) was refluxed with 47%-HBr (200µl) for 45min. The resulting solution was diluted 5-fold with water and passed through a 7 X 1cm column of Amberlite IR4B(OH^-).

Purification. The final solution (0.5–1.0ml) was passed through a 30 X 1 (10 X 1 for tryptophan) cm column of Biogel P2 (200–400mesh) eluted with de-ionised water at 0.3ml/min (amino acid t_R~30min, Fig 4).

FIGURES

Fig 1 : ^{18}F-exchange vessel used in recirculatory neon target system. (Ref 8 - complete system).

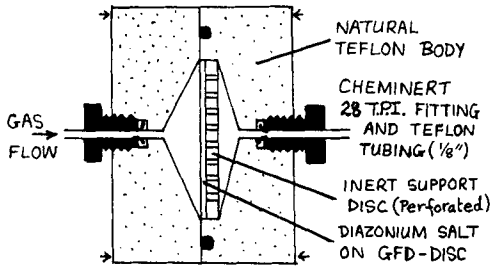


Fig 2 : Purification of fluoro-ester III(j) by preparative GLC. (See text).

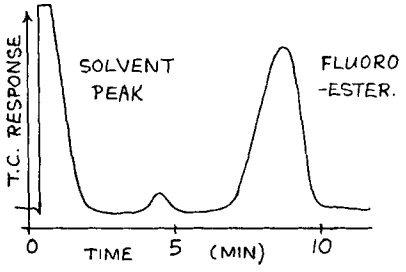


Fig 3 : Purification of fluoro-ester III(g) by preparative TLC. (See text).

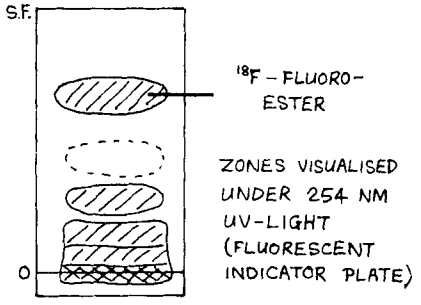
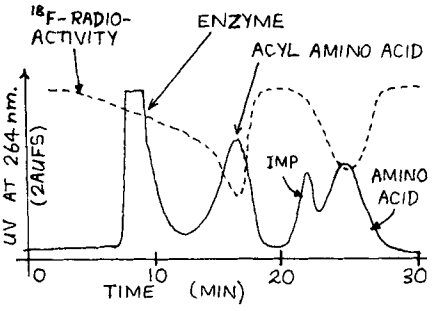


Fig 4 : Purification of fluoro-amino acid V (c) by preparative LC (after enzyme resolution —conditions in text)



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Paper No.A3

^{18}F-LABELLED L-AMINO ACIDS AS PANCREATIC SCANNING AGENTS

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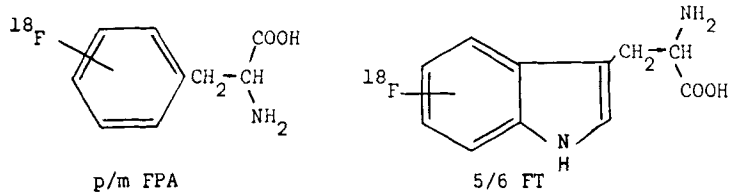
The use of ^{75}Se-L-selenomethionine (SM) for diagnostic scanning of the pancreas was initially demonstrated by Blau and Manske (1) in 1961. Since then ^{75}Se-selenomethionine has remained the only radiopharmaceutical routinely in clinical use for pancreatic scanning despite the drawback of long physical and biological half-lives, low incorporation into the pancreas and a relatively high uptake by the liver which can overlap the pancreas (reviewed in 2). The trend in the design of new radiopharmaceuticals for pancreatic scanning has been to label DL or L-amino acids with either ^{11}C, ^{13}N or ^{18}F which enables imaging with a positron camera.

Previous reports of ^{18}F-labelled amino acids have been for the DL mixtures (3,4) eg p-fluorophenylalanine (p-FPA) and 5 and 6 - fluorotryptophan (5FT,6FT), but clinical studies with ^{18}F-labelled DL-p-FPA and DL-5FT have proved disappointing (5,6). The structure of these compounds is shown in Figure 1. The L isomers of ^{18}F-pFPA, ^{18}F-mFPA and ^{18}F-5FT have been synthesised from the DL mixture by treating the DL-acylamino acid with L-amino acylase and then separating the L amino acid on a polyamide gel column (7,8).



p/m FPA 5/6 FT

Figure 1.

A distribution study of the L isomer of ^{18}F-mFPA and ^{18}F-5FT has been made in male Wistar rats with respect to time and compared with the distribution of ^{75}Se-L-SM. The rats (weight range 200-250g) were injected with either 7μCi of the ^{18}F-labelled amino acid or 2μCi of ^{75}Se-SM via the lateral tail vein while under light ether anaesthesia. Both the ^{18}F-labelled amino acids show a considerably higher pancreas to liver ratio (per g of tissue) than SM over a period of 30-120 minutes following injection (Table 1). The reason for this increase is apparent from Table 2. Although the pancreatic uptake is increased slightly for the ^{18}F-labelled amino acids, there is a sharp fall in the liver uptake from 17% for SM to 7-8% injected dose for mFPA and 5FT.

TABLE 1. Pancreas to liver ratio (per g of tissue) in rats of 3 L-amino acids.

L-Amino Acid (No. of animals)	Min. after injection 30	60	90	120
^{18}F-mFPA (3)	8.3	7.6	6.2	4.9
^{18}F-5FT (4)	6.4	4.5	6.3	6.4
^{75}Se-SM (5)	3.1	2.7	3.1	3.3

TABLE 2. Tissue uptake and tissue concentration of 3 L-amino acids 30 minutes after iv injection.

L-Amino Acid	Pancreas % Inj. dose	% Inj. dose/g	Liver % Inj. dose	% Inj. dose/g
^{18}F-mFPA	8.8	7.4	7.2	0.90
^{18}F-5FT	7.2	5.9	8.3	0.92
^{75}Se-SM	6.7	5.2	17.2	1.94

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Paper No. A4

INCORPORATION OF FLUORINE-18 IN PERHALO COMPOUNDS USING THE $^{20}Ne(d,\alpha)^{18}F$ REACTION

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The recoil chemistry of fluorine-18 and simple organic substrates has been extensively studied, mainly at low radiation dose using the $^{19}F(\gamma,n)^{18}F$ and $^{19}F(n,2n)^{18}F$ reactions (1). A variety of gaseous inorganic fluorinating agents have been labelled with high levels of radioactivity using the $^{20}Ne(d,\alpha)^{18}F$ reaction , where due to the high radiation doses encountered the effects of radiation chemical reactions would be expected to predominate (2-5). Sulphur hexafluoride and several halomethanes (I-V) have now been labelled in high activities by the bombardment of 0.3-2% mixtures of an appropriate substrate in neon with 14MeV deuterons. This work was undertaken in order to make some of the compounds available for pharmacodynamic studies. In addition sulphur hexafluoride-^{18}F is of interest for the study of regional pulmonary diffusion.

The target was a cylindrical aluminium vessel 45 cm long by 6 cm diameter with a 4 x 2 cm beam entry window of 0.050 mm stainless steel. A cylindrical copper liner electroplated internally with silver to a thickness of 0.1 mm was introduced via the detachable backplate. After evacuation to a pressure of 10^{-3} mm Hg the target was filled with C.P. grade neon and the substrate (0.5 - 4 m moles) to a pressure of 3.5 Kgf.cm^{-2}. Following irradiation the gaseous products were analysed by radio-gas chromatography on a 1.55 m x 6.25 mm column of 80-100 mesh Porapak-Q using a carrier gas flow rate of 20 ml min^{-1}. The gaseous contents of the target were vented to a trap at -196° at a flow rate of 50-100 ml min^{-1} and the extracted radioactivity measured.

The results obtained for sulphur hexafluoride and compounds in the series $CCl_{4-n}F_n$ (n = 1 to 4) as substrates are given in Table 1. The radiochemical yields were calculated using previously reported data for the $^{20}Ne(d,\alpha)^{18}F$ reaction (6,7). With sulphur hexafluoride labelling of the substrate was the predominant reaction and the two major gaseous impurities were sulphuryl fluoride-^{18}F and thionyl fluoride-^{18}F (identified by glc - mass spectrometry) arising from the presence of traces of oxygen in C.P. neon. A cryogenic purification system for sulphur hexafluoride-^{18}F has been developed.

Similarly, labelling of the substrate in high activity was also observed in the case of tetrafluoromethane (V). In this case traces of ^{18}F-labelled hexafluoro ethane and octafluoropropane were detected (by glc-mass spectrometry) together with other unidentified impurities. With tetrachloromethane (I) and the chlorofluoromethanes (II, III, IV) ^{18}F for Cl and ^{18}F for F replacement reactions were observed, products other than the major one being other chlorofluoromethanes with minor amounts of unidentified material. Mass peaks on analytical glc were observed for all the identified labelled products. The silver plated target liner was essential in order to obtain the products in the yields reported here, indicating that chemical processes at the liner surface are important. Bombardments with these substrates result in chemical deposits on the

surface of the liner and it is likely that this unidentified material plays a part in the reactions. Neither the liner nor the target were chemically cleaned between bombardments.

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TABLE 1.

Substrate	m.moles	Beam Current (μA)	Int.Beam current (μA hrs)	Major gaseous ^{18}F labelled product (%)+		Activity EOB (mCi)	Radiochemical yield % *
SF_6	2.04	10	2	SF_6	(96)	23.8	35
	0.61	10	2		(92)	25.7	37
	0.61	20	10		(85)	71.4	22
CCl_4 (I)	4.15	10	2	CCl_3F	(99)	18.7	27
	4.15	20	10		(95)	72.6	22
CCl_3F (II)	2.23	10	2	CCl_2F_2	(67)	10.1	15
	2.23	10	8		(58)	27.7	11
CCl_2F_2 (III)	2.23	10	3	CCl_2F_2	(72)	20.3	20
$CClF_3$ (IV)	2.23	10	2	$CClF_3$	(48)	11.7	17
CF_4 (V)	0.67	10	2	CF_4	(89)	23.6	34
	0.67	10	6		(77)	48.9	26

\+ Fraction of total extracted gaseous activity
* Based on total ^{18}F produced.

Paper No.A5

ISOTOPE EXCHANGE WITH ^{18}F ON SUPERCONJUGATE SYSTEM

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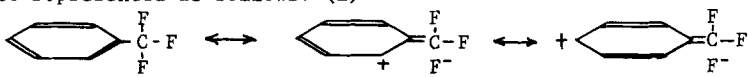
An isotope exchange reaction is an effective method for a preperation of radioactive halogeno organic compounds. But this reaction dose not occur on organic fluoro compounds under the usual conditions because of the high chemical bond energy of C-F (107-121 kcal). If the isotope exchange reaction could be applied to the preparation of ^{18}F-labelled organic compounds, a synthesis procedure should become more easy and a number of ^{18}F-positron emitting radiophamaceuticals should be developed. In this work, the isotope exchange reaction between CF_3-aromatic compounds and ^{18}F -18-crown-6 potasium fluoride has been investigated and attempted to apply to the ^{18}F-labelling of some biological active fluoro compounds.

CF_3-aromatic compounds show the properties of superconjugation or no-bond resonance represented as follows: (1)



On other hand, 18-crown-6 is a good solvent for KF to make "naked"fluoride which is a strong nucleophilic reagent. (2)

Benzotrifluoride selected as model compound of this reaction was dissolved in benzene and heated with 18-crown-6 and quartz sand coated with $K^{18}F$ in a sealed glass tube at 100°C for 2 hours. After cooling, the reaction mixture was placed on a short column of silic acid. The 12.7% of exchanged ^{18}F-benzotrifluoride was washed off the column with benzene and the chemical and radiochemical purity were checked by gas chromatography. This isotope exchange yield was the highest value of several experiments in heterogeneous condition. Unreproducible results in benzene solution may depend on a presence of trace amounts of water and heterogeneous condition.

The reaction rate, solvent effect and substituent effect were also examined under the homogeneous condition. The longer reaction time gave higher exchange yield but the loss of ^{18}F onto glass wall were also increased (table-I). Benzene was good solvent for this reaction but acetonitrile and benzene-methanol did not give any ^{18}F-organic compound (table-II). And the reactivity of various kinds of substituted benzotrifluoride (I), 2-trifluoromethylbezothiazole (II), 2-(trifluoromethyl)-phenothiazine (III) and 5-trifluoromethyluracil (IV) is summarized in table III.

These results suggest that the isotope exchange yield may be a good indicator of superconjugativity of CF_3-compounds and this labelling method may be usefull for a complicated compound such as 2-trifluoromethybenzothiazole.

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Table I. Isotope exchange yield for various reaction time

Reaction time	Exchange Yield	^{18}F on glass wall
1 hour	1.2%	47%
2 hours	1.5%	54%
3 Hours	2.5%	62%

Benzotrifluoride: 100μmol $K^{18}F$: 0.36μmol 18-crown-6: 500μmol/ml

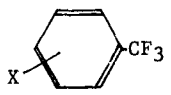
Table II. Isotope exchange yield in various solvents

Solvent	Exchange Yield	
Benzene	2.2%	Benzotrifluoride: 200μmol
Benzene + MeOH	0.1%	$K^{18}F$: 0.1μmol
Acetonitrile	0.01%	18-crown-6: 100μmol/ml
Diethylene glycol diethyl ether	1.0%	Heated at 100°C for 2hours in glass tube

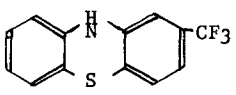
Table III. Isotope exchange yields of various kind of CF_3-compounds

Compound	μmol	$K^{18}F$ μmol	18-crown-6	Solvent	Exchange Yield
(I)	42	0.069	20μmol/ml	Benzene	4.0%
2-NH_2-(I)	41	0.065	20	Benzene	3.4%
3-NH_2-(I)	41	0.063	20	Benzene	12.9%
3-OH-(I)	41	0.067	20	Benzene	4.9%
4-OH-(I)	40	0.062	20	Benzene	0.9%
2-CN-(I)	38	0.065	20	Benzene	0
4-CN-(I)	40	0.064	20	Benzene	0.5%
(III)	40	0.066	20	Benzene	7.2%
(II)	42	0.063	20	Benzene	14.9%
(IV)	3.4mg	0.1	100	D.G.D.E.	0.68%

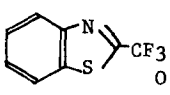
Heat at 100°C for 30 min in plastic tube made from poly-fluorocarbon



(I)



(III)



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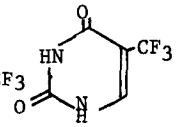

(IV)

Paper No.A6

5-^{18}F-5-FLUORO-2'-DEOXYURIDINE: A POTENTIAL DIAGNOSTIC AGENT FOR TUMOR METABOLISM

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The importance of developing a non-invasive technique to determine the metabolic status of a tissue mass has been demonstrated (1). The potential of radioiodinated nucleosides and the problems encountered with their use for tumor imaging and metabolic studies has been well documented (2,3). The use of fluorinated pyrimidines may circumvent some of the difficulties observed with the iodinated nucleosides due to their different metabolic pathways (4). The widely used cancer chemotherapeutic agent, 5-Fluorouracil, has been synthesized using ^{18}F and investigated for differential tissue distribution (5,6). Comparison of the short term distribution of 5-Fluoro-2'-deoxyuridine-6-^{3}H and 5-Fluorouracil-6-^{3}H in Lewis Lung Carcinoma and Ehrlich Ascites Carcinoma models had indicated that the deoxyribonucleoside of 5-Fluorouracil exhibits tumor to blood ratios and blood clearance values that are more compatible with nuclear medicine techniques than the free base (7).

The solution of 2'-deoxyuridine in glacial acetic was allowed to react with the F_2-^{18}F at 25°C until no substrate was observed on APLC analysis (Reverse Phase eluted with 2.5% Methanol/H_2O). The solvent was removed *in vacuo* at 50°C and the product treated with 5% NH_4OH in methanol. The crude product was subsequently purified by preparative HPLC using a Merck prepacked silica gel 60 column eluted with ethanol: chloroform (1:4 v/v). The elution volume containing the 5-Fluoro-2'-deoxyuridine-5-^{18}F was reduced to dryness and the product taken up in physiological saline and sterilized by millipore filtration.

Tissue Distribution work is currently under investigation.

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Paper No.A7

TELLURO AMINO ACIDS-SYNTHESIS, CHARACTERIZATION AND PROPERTIES OF A NEW AND POTENTIALLY USEFUL CLASS OF COMPOUNDS.

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The Te-123m nuclide emits 159 keV photons suggesting that agents labeled with this nuclide would be attractive candidates for tissue imaging. Amino acids labeled with Te-123m are of particular interest since some of these compounds would be isosteric with the sulfur analogs and might behave similarly in vivo. Such agents could possibly be useful for pancreatic imaging and for other biomedical applications. The goal of this investigation was to develop a general chemical method for the preparation of telluro amino acids. Attempts by other workers to prepare such compounds by microbiological methods have been unsuccessful (1). Since telluro amino acids were unknown prior to our studies we attempted the synthesis of a representative member of this class of compounds by several routes. Two general approaches were considered which involved either the introduction of an (organo telluro) 'reagent' into a 'substrate' that contained the protected -CH(NH_2)COOH moiety or introduction of the 'reagent' into a 'substrate' that could subsequently be converted to the α-amino acid after the coupling step.

The feasibility of preparing telluro amino acids by several of the methods reported for the synthesis of seleno amino acids (2) was investigated. A pivotal step in many of these methods involves introduction of the (benzyl seleno) group as the 'reagent' species by reaction with various 'substrates.' This strategy involves the mild removal of the benzyl group with Na/NH_3 (du Vigneaud) followed by CH_3I methylation. The applicability of preparing telluro amino acids by these methods was studied. Benzyl tellurol (Ph-CH_2-Te-H) can be prepared from dibenzyl ditelluride but is very unstable. Attempts to introduce this moiety into a variety of amino acid substrates were unsuccessful. In fact, the facile decomposition of dibenzyl ditelluride in oxygen-free solvents was studied in detail by nuclear magnetic resonance spectroscopy. These results precluded the preparation of (benzyl telluro) intermediates which could serve as substrates for subsequent du Vigneaud reductive-methylation. Our extensive studies have thus clearly indicated that (benzyl tellurol) cannot be used for the synthesis of such compounds. Our early studies demonstrated the greater stability of compounds containing the (phenyl telluro) moiety (Ph-Te-R) compared to similar compounds containing (alkyl telluro) groups (R-Te-R'). For this reason model syntheses in which the (phenyl telluro) moiety was the 'reagent' were investigated.

One potential route that was conceived involved the coupling of (phenyl tellurol) (I) with (halo alkyl) substituted acetals. The preparation of β-(phenyl telluro) alanine (V) by this method (route 1) appeared particularly attractive since the crucial substrate, β-bromo-acetaldehyde dimethyl acetal (II), was available commercially. The strategy involved coupling of (I) and (II) to form β-(phenyl telluro)-acetaldehyde dimethyl acetal (III) with subsequent removal of the O-alkyl groups to yield the free aldehyde (IV) which could be subjected to a Strecker-type synthesis to form the telluro amino acid (V). The substituted acetal (III) was prepared in high

yield (> 80%) and fully characterized. Unfortunately (III) was decomposed by acid treatment and the free aldehyde (IV) could not be obtained and this route was abandoned. Since the intermediate species such as (III) are formed in high yield it is conceivable that route 1 should be further investigated with a γ- or δ-halogenated acetal that would not be subject to facile β-elimination.

An alternate strategy involved the coupling of the 'reagent' to a 'substrate' containing the protected -CH(NH_2)COOH moiety such as reaction of (I) with a 5-(halo alkyl) hydantoin (VI) (route 2). Selenomethionine was prepared sometime ago by such a route via a (benzyl seleno) hydantoin intermediate (3). We have found that modifications of this method can be used successfully to prepare telluro amino acids. Using the modified method the (telluro) substituted hydantoins yield the free (telluro) amino acids upon basic hydrolysis. We have prepared DL-α-amino-γ-(phenyl telluro) butyric acid (VII) as a model compound and DL-α-amino-γ-(methyl telluro) butyric acid (VIII, "telluromethionine") has also been prepared. More recently a variety of branched and α-alkylated compounds have been prepared. This "telluro hydantoin" method is a general route and the Te-123m labeled amino acids can also be prepared.

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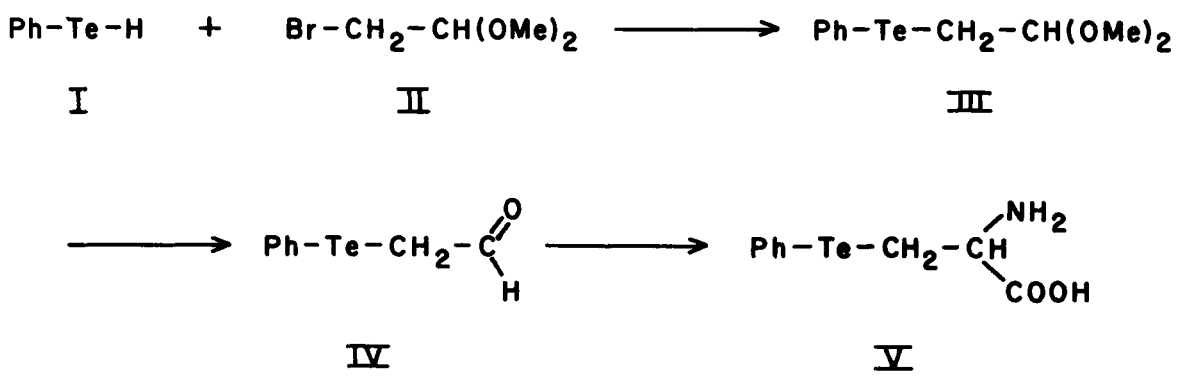
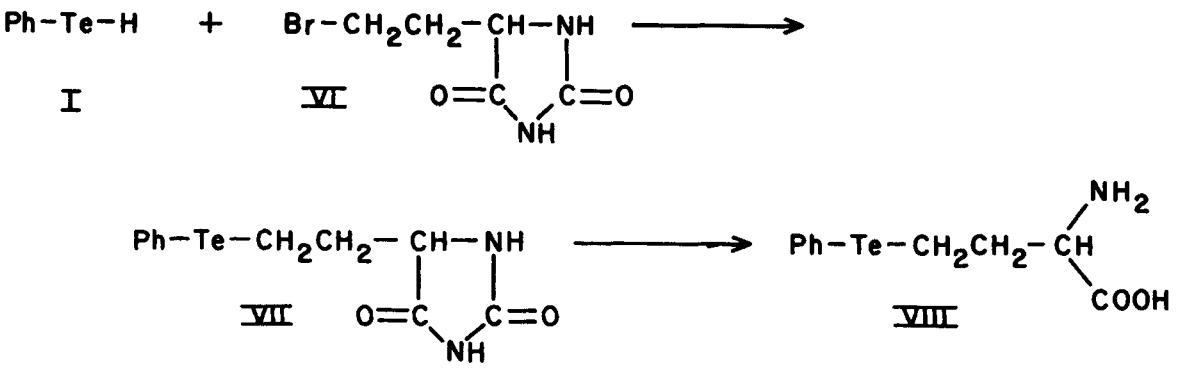
*Research sponsored by the Department of Energy under contract with Union Carbide Corporation.

ROUTE 1



ROUTE 2



Paper No.A8

16-METHYL-TELLURO-Te-123m-9-HEXADECENOIC ACID: A POTENTIAL MYOCARDIAL AGENT

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The demand for noninvasive methods for myocardial perfusion imaging has led to the development of various analogs of fatty acids containing I-123, C-11, Cl-34m and Br-77 (1-5). Fatty acids have a high affinity (6) for myocardial cells through active transport whereby they are rapidly utilized as a source of energy via beta oxidation. Virtually all of the fatty acid radiopharmaceuticals possess major disadvantages (7) such as halogen exchange which create problems in the use of these agents in myocardial studies. Thus there is a need for fatty acid analogs containing a stable radionuclide which possess suitable characteristics for imaging.

The incorporation of tellurium-123m, a radionuclide with a long half life and a single γ-photon of 159 KeV, into 9-hexadecenoic acid as a methyl-telluro group has been achieved via a simple reaction from Te-123m metal utilizing sodium borohydride in aqueous ethanol:

$$CH_3\text{-Te-123m-}CH_2\text{-}(CH_2)_5\text{-CH=CH-}(CH_2)_7\text{-COOH}$$

The Te-123m containing fatty acid is synthesized by adding 16-bromo-9-hexadecenoic acid to an aqueous ethanolic solution of disodium ditelluride (Na_2Te_2-123mTe) which is generated from Te-123m by the action $NaBH_4$ under reflux conditions in a nitrogen environment. The nucleophilic disodium ditelluride displaces the bromine at the 16-position to form a dialkyl ditelluride (R-TeTe-R). The ditelluride is then reduced with sodium borohydride to form the 16-telluro derivative of 9-hexadecenoic acid which is stabilized as 16-methyl-telluro-123m-9-hexadecenoic acid by treatment with sodium ethoxide and methyl iodide.

$$\text{Te} + NaBH_4 \xrightarrow[NaOEt]{EtOH/H_2O} \text{NaTeTeNa} \xrightarrow{\text{R-Br}} \text{R-TeTe-R}$$

$$\text{R-TeTe-R} + NaBH_4 \longrightarrow \text{R-TeH} \xrightarrow{NaOEt} \text{R-Te-Na} \xrightarrow{CH_3I} \text{R-Te-}CH_3$$

$$(\text{R= } -CH_2\text{-}(CH_2)_5\text{-CH=CH-}(CH_2)_7\text{-COOH})$$

Synthesis of non-radioactive and Te-123m containing 16-methyl-telluro-9-hexadecenoic acid was accomplished within 3 hours. The non-radioactive fatty acid has been characterized by NMR, IR, mass spectral and elemental analysis and the Te-123m analog was found to have identical chromatographic properties in two solvent systems. The Te-123m labeled compound is being evaluated as an agent for myocardial perfusion in experimental animals.

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Paper No.A2

[^{18}F]-L and DL-FLUOROARYL AMINO ACIDS. PRESENT STATUS.

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Although these compounds have been available since 1970 (1—4) they have not yet given successful results as pancreas imaging agents in man. On the other hand they have not been definitely rejected. The materials previously prepared could have contained labelled impurities and were originally made at relatively low specific radioactivities. Also the comments of ref (5) concerning the instability of derivitives of DOPA should be noted. The recent visualisation of the pancreas in man using [^{11}C]-valine(6) and the opportunities opened up by the β^+-camera encourage further work with the ^{18}F-amino acids. We consider that as many as possible of the amino acids should be available for the user.

A re-evaluation of the preparation and use of these compounds has been undertaken. The key intermediates were the protected formamido, III(a-d) or acetamido, III(e-j), malonic esters(6). The protected ^{18}F-labelled compounds (III) were obtained by thermal decomposition of the corresponding diazonium fluoroborates (II). Because of chemical and physical differences between the various compounds (III) a variety of methods of decomposition and subsequent de-protection(7) were needed.

^{18}F-labelling of the diazonium fluoroborates (I) :

$$\underset{\text{I(a-j)}}{RN_2^+\ BF_4^-} \xrightarrow[\text{neon}]{^{18}F} \underset{\text{II(a-j)}}{RN_2^+\ B^{18}F_4^-} \xrightarrow[140\text{-}200^\circ]{\text{dec.}} \underset{\text{III(a-j)}\sim 35\%}{R^{18}F} + N_2 + B^{18}F_3 + \underset{\sim 65\%}{\text{Imps/Tar.}}$$

The aryl diazonium fluoroborates (I) were labelled by heterogeneous exchange between ^{18}F/neon(80 psi) and the organic solid (13mg) dispersed in a filter element, a 3.5cm diameter GFD disc (Fig 1) in the recirculatory target system(7,8). The effect of additives in the target gas was investigated. With pure neon, or neon containing oxygen (15%) or nitrogen (15%), about 42% of the fluorine-18 produced was trapped on the filter disc. When neon containing hydrogen (15%) was used, virtually all the fluorine-18 produced remained on the glass walls(8) of the target vessel. These results suggest that the species $NO^{18}F$ or $O_2{}^{18}F$ may be the carriers of fluorine-18 between the target vessel and the filter element, but this idea can be criticised.

Decomposition of the ^{18}F-diazonium fluoroborates (II) :
A variety of methods were investigated for this stage. Speed and ease of purification and of handling of the product (III) were desired. It was not possible to use a single method for all cases (a-j). Decomposition by heating alone ("dry") had the advantage that the crude product could be taken up in a volatile solvent for subsequent purification by preparative TLC, LC or GLC. The previously described inert solvent (tetralin) decomposition(9)suffered from several disadvantages and so was not used.

When the fluoro-ester was sufficiently volatile, III(a—d,i,j) it was feasible to purify (III) by vacuum sublimation or GLC. Vacuum sublimation did not prove to be useful because the sublimate still contained many impurities. On the other hand GLC using a 5ft × ¼in column of 3% OV1 or QF1 at 200°C gave a pure product (Fig 2) that was readily trapped at the chromatograph outlet. It was even possible to inject the ^{18}F-diazonium fluoro

to the target organs, and as binding sites in the target organs become saturated an increasing excess of the agent is spilled over into non-target tissues (8).

With the selenium-75 that can currently be produced in UK and other reactors it should be possible to chemically synthesise L-seleno-methionine [^{75}Se] at specific activities approaching 200-300mCi/mg. However, this would mean developing a production process that would involve handling materials on an extremely small chemical scale. Rather than embarking on this work, it occurred to us that the effects of administering constant activity dosages of selenium-75 at increasingly high specific activities might be simulated by administering readily available L- selenomethionine [^{75}Se] at constant specific activity (10mCi/mg) but at increasingly lower chemical dose (and therefore radioactivity) levels, and measuring any changes in the resulting biodistribution of activity. It was found that as little as 0.5 nCi of selenium-75 could be counted in samples of rat tissue with reasonable accuracy (coefficient of variation 4%). Using this approach the biodistribution of selenium-75 in rats has been measured, following injection of quantities of L-selenomethionine [^{75}Se] that were equivalent to injections of 250µCi in human patients at "human equivalent specific activities" covering the range 4.0 to 400 mCi/mg selenomethionine. Throughout that range no significant differences could be observed in the percentage of the injected activity that was taken up by the pancreas, or in the pancreas to liver concentration ratio, although there was some increase in the proportion of activity taken up by the kidneys as the chemical dose level was decreased.

The chemical dose levels used by Kirschner, if extrapolated to human patients, corresponded to levels much higher than those normally injected, and this probably accounted for the increases in liver to pancreas uptake ratios he observed. The present study indicates that, based on work in rats, no further improvement in pancreas uptake or pancreas to liver ratios could be achieved by increasing specific activities of L-selenomethinine [^{75}Se] beyond those that are currently available. It is suggested that the approach used above could be used to check the effects on specific organ uptake of changing chemical dose levels of other radiopharmaceuticals, including potential new radiopharmaceuticals, without resorting to the synthesis of the products over a wide range of specific activities.

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Paper No.A10

RAPID RADIOHALOGENATION OF SMALL MOLECULES

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The reactions of ^{80m}Br and ^{125}I with tyrosine, uracil, cytosine, uridine and histidine have been studied using the Chloramine-T method and using immobilised enzyme catalysts. Following reaction the radiolabelled product from each reaction was separated using reverse phase high pressure liquid chromatography and distilled water as the eluant.

Radiochemical yields were obtained as functions of many parameters of the reactions, including reaction time, reactant concentration and catalyst concentration. Optimum radiochemical yields were as follows:

Reactant	(Chloramine-T) Label		(Enzymatic)	
	^{125}I	^{80m}Br	^{125}I	^{80m}Br
Tyrosine	86%	65	85	83
Uracil	95	83	83	89
Cytosine	85	76	77	68
Uridine	75	-	92	-
Histidine	86	-	90	-

Paper No.A11

SYNTHESIS OF RADIOBROMINATED ANDROGENS

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The research to be described was conducted with the objective of obtaining a radiobrominated androgen with sufficient stability, specific activity and androgen receptor binding affinity to permit imaging of the prostate. The approach involved three stages: screening of potential steroids by *in vitro* competition assay, designing a synthetic technique for the efficient synthesis of ^{82}Br, ^{80m}Br, or ^{77}Br-labelled target compounds and evaluation of the biological stability and selectivity of radiolabelled products.

Initial screening showed that 2α-bromo-17β-hydroxy-5α-androstan-3-one (1) and 2-bromo-17β-hydroxy-5α-androst-1-en-3-one (2) possessed high binding affinity for the androgen receptor. The biological instability of α-halo ketones has prevented the successful use of the former compound (3). The latter steroid (II, reaction scheme) has been previously synthesized by way of an intermediate 1,2-epoxide (4), 2,2-dibromide (5), and 1,2-dibromide (6) but all methods possess limitations which render radiobromination impractical.

The need for a fast, selective, and high yield reaction led to the development of a reaction sequence which employed silver tetrafluoroborate as co-reactant in order to catalyze and direct bromine addition by coordination of the silver cation with the π-system in the A-ring (7). 17β-Hydroxy-5α-androst-1-en-3-one (I) and $AgBF_4$ were dissolved in methylene chloride and a solution of bromine in CH_2Cl_2 was added dropwise. Dehydrohalogenation occurs upon addition of the base 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) and the reaction was quenched with water. The reaction sequence took less than 1 1/2 hr and there was no side-product formation. Curiously, the same procedure, when applied to 17β-hydroxyandrosta-4,6-dien-3-one(III) led to the formation of the product of addition rather than addition-elimination.

Both products (II and IV) were then radiolabelled with bromine-82. Quite high radiochemical yields were obtained using this method. While compound (IV) proved biologically unstable, compound (II) was sufficiently stable to allow the isolation and identification of the steroid-receptor complex. Incorporation of carrier-free bromine-77 now appears feasible and is in progress.

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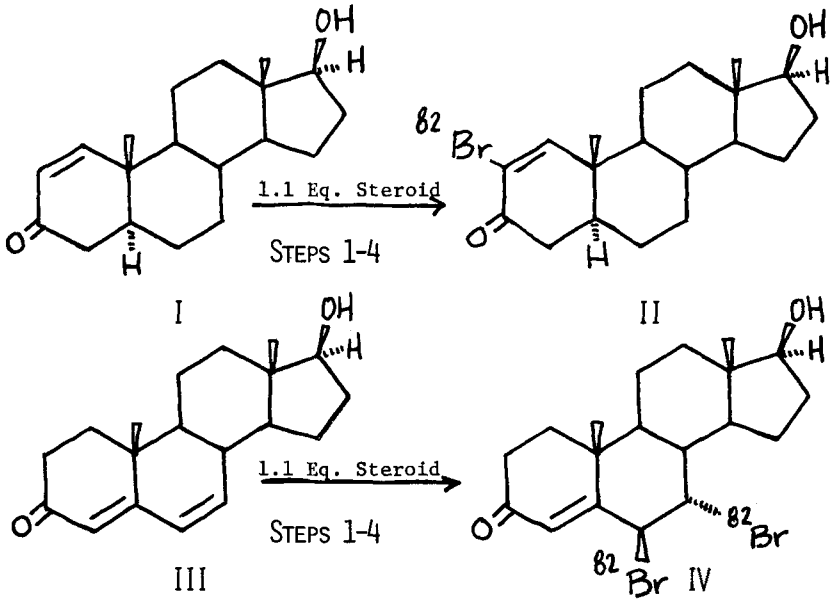

Steps 1-4 involve: 1. Addition of a solution of 0.9 eq. $AgBF_4$ in CH_2Cl_2.

2. Addition of a solution of 1.0 eq. of $^{82}Br_2$ in CH_2Cl_2 and stirring for 15 minutes.

3. Addition of a solution of 2.0 eq. of DBN in CH_2Cl_2 and stirring for 30 minutes.

4. Addition of water, separate phases and concentrate.

Compound Number	Radiochemical Yield (%)*	Androgen Receptor Binding Affinity (%DHT)+
II	95	101
IV	100	100

* Percent yield calculated on the basis of the number of moles of $AgBF_4$ used with correction for decay.

\+ Determined by the steroid's ability to compete with [^{3}H]-5α-DHT for binding sites in the rat ventral prostate mince.

Paper No.A12

2^1-[^{82}Br]-BROMO-2^1-DEOXYURIDINE FOR TUMOR UPTAKE STUDIES

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A general and efficient synthesis of 2^1-bromo nucleosides has been developed to allow incorporation of radiobromine into the 2^1-position. Reaction of uridine (I) with diphenylcarbonate using the procedure of Hampton and Nichol (1) affords 2,2^1-anhydrouridine (II) in 52 percent yield. Treatment of II with bromide anion in the presence of one equivalent of *p*-toluenesulfonic acid in dimethylformamide at 100° for 4 hr gave 2^1-bromo-2^1-deoxyuridine (III). The yields of III using the ammonium, sodium and lithium bromides, as determined by quantitative preparative HPLC, were 62, 80 and 70 percent respectively. The reaction of II with ammonium bromide did not proceed in the absence of *p*-toluenesulfonic acid; the latter is likely necessary to protonate the 2,2^1-anhydro ether linkage of II to give the protonated intermediate IV which subsequently reacts with bromide anion due to the increased electrophilicity of the 2^1-position.

Although the time scale and reaction conditions are suitable for direct radiochemical synthesis with ^{82}Br anion, ^{82}Br-labelled III was prepared routinely by irradiation of III at a thermal neutron flux of 1×10^{12}n $cm^{-2}s^{-1}$ in the University of Alberta Slowpoke reactor. Specific activities were routinely in the order of 0.6-0.7 mCi mM^{-1}, after decay of ^{80}Br. Radiolytic decomposition was negligible during the 2 hr irradiation period.

High pressure liquid chromatography of the reaction mixture or the irradiated products containing III, was effected on an E. Merck Silica Gel 60 prepacked column (size B) with chloroform:methanol (1:1 v/v) as solvent, with UV detection at 254 nm. At 5 ml min^{-1}, III and II had elution times of 18 and 32 min respectively.

Preliminary distribution studies of [^{82}Br]-III in BDF_1 mice with subcutaneous solid Lewis Lung carcinomas showed little tissue uptake. Accumulation in the kidney appeared to be associated with urinary excretion. Blood levels persisted for longer periods aft i.v. injection than observed with other halopyrimidine nucleosides (2,3).

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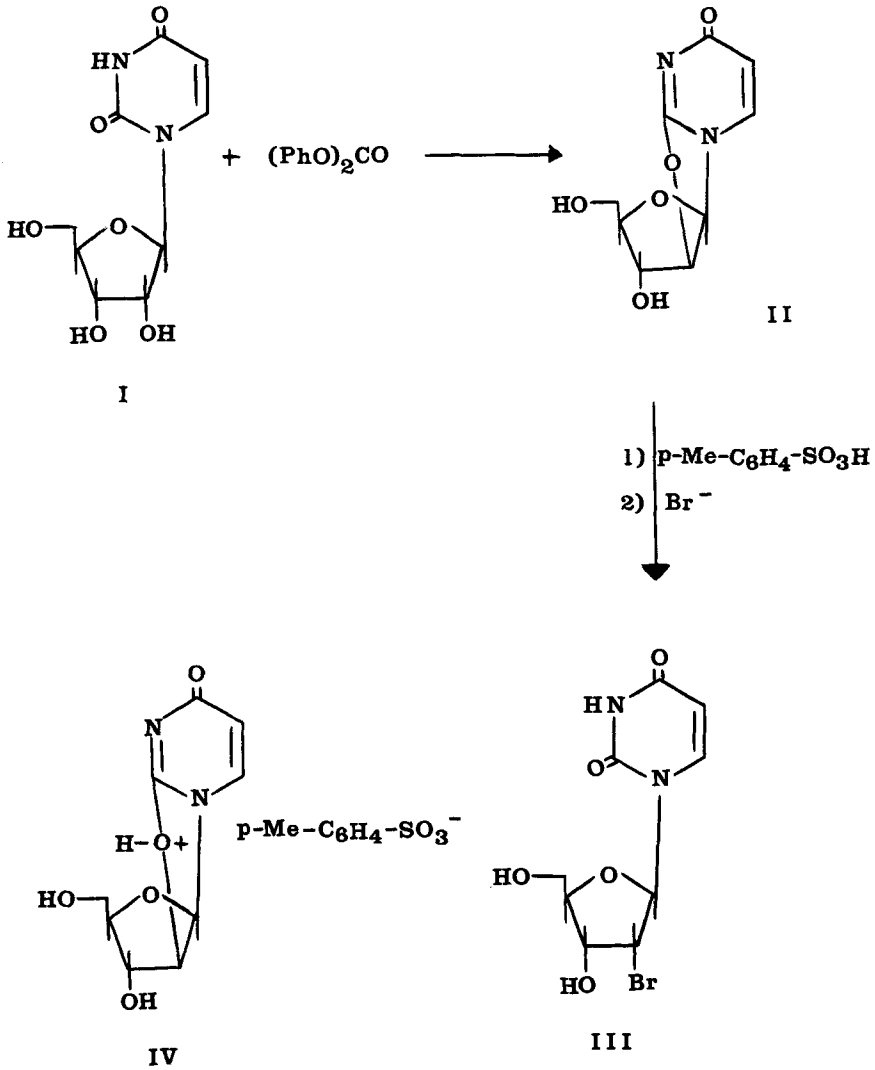
HN
O
N
HO
O
HO
OH
+ (PhO)2CO
N
O
HO
OH
II
I
1) p-Me-C6H4-SO3H
2) Br−
H-O+
p-Me-C6H4-SO3−
IV
III
Br

Paper No.A13

STRUCTURAL RELATIONSHIP BETWEEN RADIOACTIVE 6β-IODOMETHYL-19-NORCHOLEST-5(10)-EN-3β-OL AND ITS ANALOGUE FOR ADRENAL ACCUMULATION

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It is known that iodine-131 labelled 6β-iodomethyl-19-norcholest-5(10)-en-3β-ol (NCL-6-I-131) is accumulated strikingly into adrenal gland and makes superior adrenal image as compared to iodine-131 labelled 19-iodocholest-5-en-3β-ol (CL-19-I-131)(1-3). Further, it has been shown that 6β-methyl-19-norcholest-5(10)-en-3β-ol shows higher accumulation in adrenal gland than that of cholesterol, demonstrating that 6β-methyl analogue has the basic structural feature having high affinity for adrenal (4). On the other hand, it has been shown that the substitution of bromine for iodine in NCL-6-I results in decrease of the adrenal accumulation (5). To obtain further information regarding the structure and adrenal localization of cholesterol analogue is of potential value in designing radiopharmaceuticals for specific diagnostic purposes. The synthesis and tissue distribution studies of the corresponding 3α-ol epimers to CL-19-I and NCL-6-I were carried out in order to prove the stereochemical requirement of the hydroxy group at C-3 for adrenal accumulation.

The synthetic sequence utilized in the preparation of 19-iodocholest-5-en-3α-ol (I) and 6β-iodomethyl-19-norcholest-5(10)-en-3α-ol (II) is outlined in the Scheme. Iodine-131 labelled (I) and (II) were obtained by isotope exchange with sodium iodide-I-131 in acetone at 15° and in refluxing acetone, respectively. (II)-I-131 is considerably stable and maintains its radiochemical purity of >95% even after 8 days at 15°, whereas (I)-I-131 is unstable and about 50% of the activity was lost in 3 days at 15°.

The Table compares the concentration of radioactivity into adrenal, liver and thyroid following administration of (I)-I-131 and (II)-I-131 to rats with that of the reported CL-19-I-131 and NCL-6-I-131. For (I)-I-131, most of the radioactivity was found in the thyroid even after 1 day with little radioactivity appearing in other tissues, indicating significant in-vivo deiodination consistent with the data of its thermal stability. The rat adrenal accumulates (II)-I-131 about ten times more than (I)-I-131. However, (II)-I-131 shows much lower adrenal accumulation as compared to the corresponding 3β-ol analogue. This fact suggests that the β-configuration of the hydroxy group at C-3 in norcholesterol series is one of the most important factors required for adrenal gland specificity.

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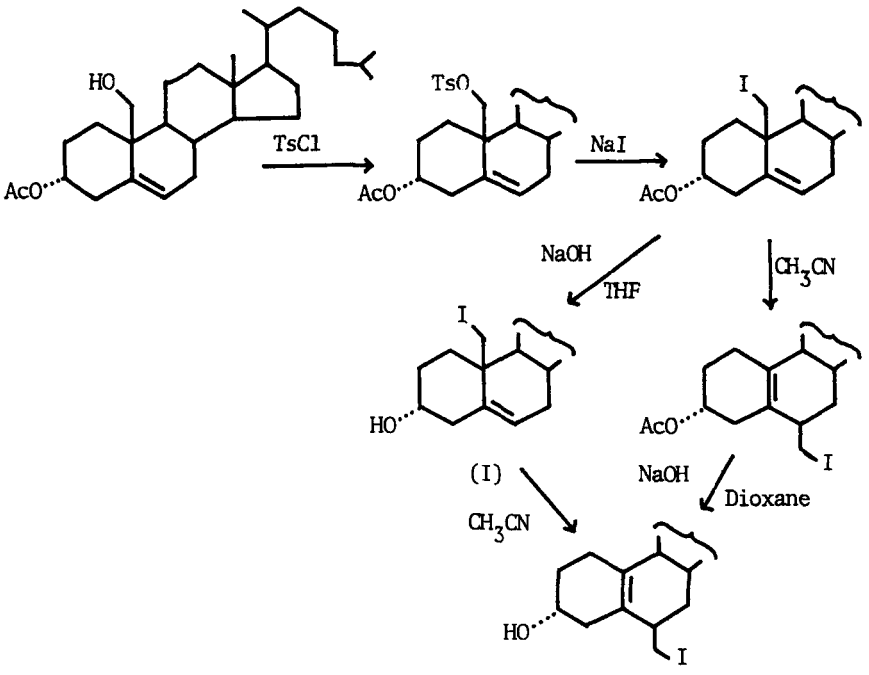

(II)

Table of Tissue Accumulation of Iodine-131 Labelled Steroids[a)]

Compd	Adrenal			Thyroid			Liver		
	days			days			days		
	1	3	7	1	3	7	1	3	7
(I)	1.95	2.78	2.82	1306	1170	1720	1.27	0.23	0.18
CL-19-I[b)]	17.3	20.6	18.2	377	359	294	0.56	0.15	0.07
(II)	13.6	21.1	21.9	199	265	125	1.2	0.3	0.2
NCL-6-I[b)]	141	163	208	90	149	80	1.65	0.55	0.19

a) Percent administered dose per gram of tissues.
b) Ref. (1).

Paper No.A14

SYNTHESIS OF RADIOIODINATED ω-(p-AMINOPHENYL)-SUBSTITUTED FATTY ACIDS

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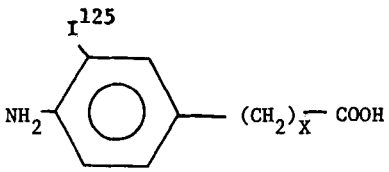
The rapid myocardial turnover of ω-radiolabeled fatty acids makes multiple projection imaging of regional myocardial blood flow difficult (1). In 1904 Knoops' classical experiments with ω-phenyl labeled fatty acids revealed the whole-body metabolism of these derivatives to be similar to the parent fatty acids. However neither the rate of metabolism nor the myocardial extraction of the phenyl analogs has been determined. These observations as well as the synthetic ease and stability of radioiodinated anilines has prompted the synthesis of two I-125 labeled ω-(p-aminophenyl)-substituted fatty acids.

Reaction of 11-bromoundecanoic and 16-bromohexadecanoic acids with a four-fold excess of diphenyl copper lithium in ethyl ether at ambient temperature gave the 11-phenyl-and 16-phenyl-substituted fatty acids in 30-50% yields. Nitration of the compounds gave inseparable mixtures of the ortho- and para-nitro isomers. However the methyl esters could be readily purified by HPLC on Porasil using $CHCl_3/CH_3OH$ (95/5) as eluant.

Hydrolysis followed by catalytic reduction of the pure ω-p-nitrophenyl fatty acids gave the respective ω-p-anilino derivatives. Use of carrier-free NaI-125 with chloramine-T as oxidant gave exclusive mono-radio-iodination (compounds I and II) when an excess of the aniline compounds was employed. Yields ranged from 40-75% with the shorter-chain fatty acid giving consistently higher yields. The myocardial uptake and retention of these radiochemicals are presently undergoing evaluation in dogs.



I: X = 10

II: X = 15

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Paper No.A15

RADIODIDE EXCHANGE STUDIES WITH 5-IODONICOTINIC ACID (NIACIN) ANALOGS

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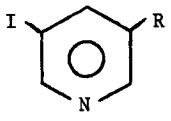
Nicotinic acid (niacin) and its derivatives such as nicotinamide, ethyl nicotinate and 3-hydroxymethyl pyridine (Ronicol) are well known for their ability to reduce the blood free-fatty acid and cholesterol levels (1). Nicotinic acid itself is known to bind to myocardial tissue (1) and nicotine shows high uptake in the brain (2). This host of biologically important pyridines has prompted a study of methods of labeling the pyridine nucleus with radioiodine. Basic information on the variables affecting nucleophilic radiodide exchange in aromatic systems is generally lacking. We report here our initial findings on the radioiodide exchange labeling of 3-substituted-5-iodopyridines.

Compounds I-IV were synthesized from the respective 5-amino compounds by reaction of the diazonium salts with potassium iodide. Exchange labeling experiments were run in propylene glycol at 135°C using approximately 2 mg of the idodpyridine and 2 mCi of carrier-free NaI-125. The electron withdrawing substituents in compounds III and IV might be expected to increase



	R
I	H
II	CH_2OH
III	COOH
IV	$COOC_2H_5$

the exchange rate via their inductive effect. However during the first hour I and II exchange labeled at over twice the rate of III and IV. The exchange rate in all cases was increased by adjusting the pH to 4-5 with glacial acetic acid. Protonation of the pyridine nitrogen seemingly stabilizes the negative charge build up during the transition state. Even greater enhancement of the exchange rate was achieved by using the respective pyridine-N-oxides. The N-oxides can be readily reduced to the pyridine compounds without loss of the iodine label by reduction with Fe/acetic acid at 50°C.

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Paper No.A16

SYNTHESIS OF RADIOLABELED INHIBITORS OF PHENYLETHANOLAMINE N-METHYLTRANSFERASE

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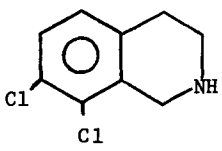
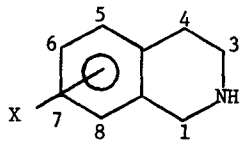
Phenylethanolamine N-methyltransferase (PNMT), the enzyme that catalyzes the final step in epinephrine biosynthesis, is essentially unique to the adrenal medulla. Thus an inhibitor of PNMT, labeled with a suitable gamma-emitting isotope, could potentially image the adrenal medulla and its epinephrine producing tumors. Although many in vitro inhibitors of PNMT have been developed over the years, only recently has an inhibitor (SKF 64139) been found effective in vivo (1). The presence of the lipophilic chlorine atoms in the 7 and 8 positions of SKF 64139 suggested an approxi-



SKF 64139



	X
I	6-Iodo
II	7-Iodo
III	8-Iodo

mate bioisosteric substitution of a single iodine atom for the two chlorine atoms.

Iodination of 1,2,3,4-tetrahydroisoquinoline with $I_2/AgSO_4/H_2SO_4$ followed by preparatory TLC separation and repeated recrystallization of the HCl salts gave 6- and 7-iodotetrahydroisoquinolines (I and II) and minor amounts of the 8-isomer (III). Purity of the compounds was determined by GLC analysis of the trifluoroacetamide derivatives on 5% DEGS/Supelcoport. Structural proof rests on the proton NMR spectra and confirmatory syntheses of I and II from the respective 6- and 7-aminotetrahydroisoquinolines. Compounds I and II were I-125 labeled in 50-60% radiochemical yield by diazotization of the respective 6- and 7-aminotetrahydroisoquinolines in the presence of carrier-free NaI-125 and copper bronze (Gatterman reaction). In the absence of copper bronze, yields were <5%. Compound III was exchange labeled with NaI-125 in propylene glycol at 140°C (10% yield in 15 hr).

The inhibitory constants (Ki) of I-III have been determined and correlate directly with the uptakes of the I-125 labeled compounds in the adrenal medulla.

(1) Pendleton R.G., Kaiser C. and Gessner G., J. Pharmacol. Exp. Ther. 197, 623 (1976).

Paper No.A17

Radio-iodination of aromatic compounds with ^{123}I and ^{131}I by exchange

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In 1976 Elias (1) extended the possibilities of aromatic exchangelabelling by introducing molten acetamide as a solvent for aromatic iodination.

As we use this procedure to label iothalamate with ^{123}I for clinical use, we decided to study the scope and mechanism of this reaction. It turned out that only in a limited amount of cases exchange was actually possible: Only when the aromatic was substituted with very strongly electronwithdrawing groups (e.g. 2-iodo nitrobenzene, 2-iodo benzoic acid (OIBz), 2-iodo hippuric acid (OIH) and iothalamate).

After addition of a small amount of ICI also with other aromatic iodocompounds (iodo benzene, 2-iodo toluene, 2-iodo phenol and 2-iodo aniline) exchange was possible (a).

Thus it is clear that the reactionmechanism of the first group (without ICI) is an example of nucleophilic aromatic substitution. This is a relatively rare phenomenon with a mild reagent like I^-.

We then wanted to further elucidate the mechanism of this nucleophilic substitution, especially of hippuran (OIH) and its side product 2-iodo benzoic acid (OIBz).
A remarkable observation was that at low temperatures the exchange reaction gave a larger percentage of O^xIBz than at higher temperatures (e.g. at 140° in acetamide < 5% and at 85° 20% was found) whereas the percentage of inactive OIBz was much lower.
We therefore started some semi-kinetic experiments. In these we used formamide as the solvent, because the reaction was too fast in molten acetamide, even at 85°. To account for the observed results thermodynamic data are clearly necessary.
As was already reported on the First symposium by Elias (2) second order kinetics seem to apply.

Table of radiochemical yield of the exchange reaction of $^xI^-$ with OIH and OIBz in formamide at 59°.

	Amount of formamide (mg)	Amount of OIH (A)/ OIBz (B) (mg)	Starting activity (^{131}I μCi)	Time (min.)	Yields $^xI^-$ (unreacted)	O^xIH (%)	O^xIBz	$^xI_2$
A	27.1	5.0	196	30	22	56	20	2
	24.5	5.0	96	30	61	28	9	2
	27.9	10.0	94.2	30	26	58	14	2
	28.1	5.0	94.5	10	84	11	3	2
B	28.9	4.1	210	30	2		96	2
	25.4	4.1	196	15	33		65	2

(1) Elias H. and Lotterhos H.F., Chem. Ber., 109 (1976) 1580
(2) Elias H., Lotterhos H.F., Sinn H. and Maier-Borst W., 1st Int. Symp. Radiopharm. Chem. (1976) nr. 56

(a) All experiments were performed at 178° during 6 minutes in evacuated ampoules unless otherwise stated.

Paper No.A18

RADIOIODINATION CATALYZED BY INSOLUBLE POLYMERIC REAGENT

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One of the most important methods for labelling of organic compounds with radioactive iodine is the halogen exchange carried out in polar organic solvents or in molten organic systems (1). There is a pressing need for rapid, efficient and mild radioiodination procedures, particularly those adaptable to facilitate the utilization of iodine-123 in nuclear medicine. In recent years the application of insoluble polymers in the fluorine-18 labelling procedures has been reported (2-4). We report that the insoluble polymeric phosphonates (I) have efficient catalytic activity for isotope exchange reactions between alkyl iodides and carrier-free sodium iodide-I-131 in two-phase and tri-phase systems.

The catalysts (I) were prepared by the reaction of the corresponding trialkyl phosphite with chloromethylated polystyrene (1.34 m mol of Cl/g of polymer, 100-200 mesh) cross-linked with 2% divinylbenzene. The polystyrene beads (I) were suspended in a heterogeneous mixture of various alkyl iodides in benzene and aqueous carrier-free sodium iodide-I-131.

The Table shows the results of radioiodinations in a tri-phase system with catalysts (I). The catalytic activity for the polymeric phosphonates (I) for alkyl iodides examined was in the following order: Ic > Ib > Ia, indicating that structures of alkyl chain on the phosphonate groups bound to the polymer have strong influence in determining the catalyst's activity. With 1-iodohexadecane, the catalysts (I) exhibit almost equally poor efficiencies. The presence of carrier decreased considerably the rate of iodine exchange. The monomeric analog, diethyl benzylphosphonate in place of Ib shows no catalytic activity, suggesting the presence of cooperative effect of phosphonate groups on catalysis. The insoluble reagents (I) also proved to be efficient catalysts in a two-phase system (solid-organic phase) for the run with 1-iodohexane.

Radioiodination catalyzed by insoluble polymeric reagents we developed has a certain formal similarity to the phase-transfer catalyst (5) and, as an immobilized reagent, facilitate rapid work up after the reaction. Also it may be adapted to the labelling with other radiohalogens.

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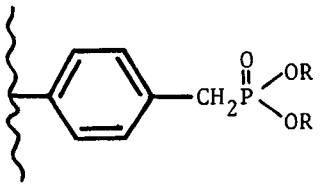

I a, R=CH_3
b. R=CH_2CH_3
c, R=$CH(CH_3)_2$

Table of Iodine Labelling in a Tri-Phase System Catalyzed by Insoluble Polymeric Reagent

Alkyl Iodide	Catalyst	Mol.equiv. of catalyst	Time/h	Labelling yield (%)
1-Iodohexane	*		2	4
	Ia	0.042	2	45
	Ib	0.037	2	75
	Ic	0.038	2	89
1-Iodooctane	*		2	11
	Ia	0.034	2	40
	Ib	0.03	2	79
	Ic	0.03	2	88
1-Iododecane	*		2	4
	Ia	0.032	2	21
	Ib	0.029	2	59
	Ic	0.029	2	75
1-Iodohexadecane	*		2	4
	Ia	0.019	2	2
	Ib	0.017	2	29
	Ic	0.017	2	47

Reaction of 3.0 - 6.5 X 10^{-4} m mol of alkyl iodide in 1 ml of benzene with carrier-free sodium iodide-I-131 (ca. 25 μCi) dissolved in 1 ml of water at 40°. For this system the polystyrene beads reside at the interface of the organic and aqueous phases.
* Reaction carried out in the absence of catalyst.

Paper No.A19

PREPARATION OF 2α-$^{125}I_1$-DIHYDROTESTOSTERONE BY EXCHANGE LABELLING AND DIRECT RADIOIODINATION

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Despite several attempts in the visualization of the prostate (1-3) the development of reliable organotropic radiopharmaceuticals for this target gland is still in its infancy. Radioiodinated diethylstilbestrol diphosphate (1,2) and estradiol-17-phosphates (3,4) were used for scanning the prostate (1,3), for distribution studies of these radiopharmaca in the multicompartment system (1,3), or for the investigation of the binding capacity of the radiotracers for cytoplasmic receptors (4). Procedures applied to the preparation of these synthetic hormones were the ^{131}ICl method (1), utilization of the positive radioiodine generated on a platinum anode (2), and cuprous ion catalyzed exchange labelling (4,5). Naturally occuring androgens or their metabolites seem to be promising carrier molecules for radionuclides to the prostate (6). Therefore, we have continued the interest in the investigation of potentially useful imaging agents for the prostate by synthesizing 2α-$^{125}I_1$-dihydrotestosterone (I). This radiotracer was prepared during our study utilizing both exchange labelling procedure (5) and direct radioiodination (7). Unlabelled analog of the compound (I) was obtained in the reaction involving dihydrotestosterone, iodine, cupric chloride, and HCl in chloroform-ethanol 1:1 at 65° during 30 minutes. The sole product of the reaction examined was purified on a preparative silica TLC plate using benzene-ethyl acetate 1:1 as a solvent system (R_F value 0.68). IR, UV and mass spectral data taken on (I) when compared with those on numerous 2-halo-3-keto steroids (8) made the structural assignement of the product possible.

We believe that the positive iodine species are generated in the process between iodine and cupric ion which form a double redox couple. The resulting electrophile attacks the Δ^2-enol of dihydrotestosterone (8). The α-halogenation of the enol is a consequence of both steric hindrance to the β-face attack and a "half-chair" conformation of ring A during iodination (8). Apparently, this process strictly follows the stereoelectronic

control (9).

The same process was performed during 15 min in the presence of $Na^{125}I$ instead of iodine. The reaction products were examined radiochromatographically on the crude mixture. Only one peak (R_F 0.68) was observed and corresponded to the radioactivity yield greater than 95%. This carrier free product was purified using the usual work-up procedure (10). Unlabelled (I) was added to the radioiodinated product and on the developed chromatogram both compounds were located at the same spot (R_F 0.68).

The exchange labelling of 2α-$^{125}I_1$-dihydrotestosterone with $Na^{125}I$ was carried out in chloroform-ethanol 1:1 and in the presence of CuCl at ambient temperature during 4 hours. Approximately 40% of the total radioactivity was associated with (I) as proven actigraphically. We found that radiopharmacon (I) is stable both at -20° in ethanol and at ambient temperature in the crude reaction mixture during 25 days as yet.

It seems that the reported method might be a suitable procedure for labelling the bioactive substance with short-lived radioiodine as an alternative to other routes employed in radipharmacy.

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Paper No.A20

RAPID SYNTHESIS OF 2-DEOXY-D [1-^{14}C]-GLUCOSE SUITABLE FOR LABELLING WITH ^{11}C.

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2-deoxy-D [1-^{14}C]-glucose was used for the measurement of the local brain metabolism in animals (1). To extend this method to an "in vivo" study in man, labelling with a gamma emittor was necessary. 2-deoxy- D [2-^{18}F] has already been synthetized by Ido et al (2). To avoid modifications of structure of the sugar, we have attempted to develop a method suitable for a labelling with ^{11}C (half life 20 min). Because of the short time available for a such synthesis, a route with the smallest number of radioactive steps was studied. ^{14}C was used as a tracer to improve chemical yields and purification procedures.

The chosen method (scheme I) was an adaptation of that of Bayly and Turner (3). 2,3-4,5-di-O-isopropylidene-D-arabitol (II) was prepared from D-arabitol (I) (4). The esterification of the primary alcohol group at C-1 with methane sulfonic anhydride (5) gave 2,3-4,5-di-O-isopropylidene-1-O methane sulfonyl-D-arabitol (III) which was converted into 1-deoxy-2,3-4,5-di-O-isopropylidene-1-iodo-D-arabitol (IV) by heating with sodium iodide (3) in anhydrous methylethylketone. The labelled carbon was introduced by action of sodium [^{14}C] cyanide (3) on compound (IV) : 2-deoxy-3,4-5,6-di-O-isopropylidene-D[^{14}C] glucononitrile(V)was obtained (2 min, 100°C in anhydrous DMF ; yield based on sodium cyanide was 75%).

From the compound (V), two sequences of reactions were possible : - a complete reduction of the nitrile in an amine followed by an oxidation in a -CHO group - or an hemireduction of the nitrile directly in a CHO group ; this second route is preferable because it avoids one step. Catalyst, time, temperature and pressure influenceson the reaction yield were studied. Compound (V) was then converted into 2-deoxy-D [1-^{14}C]glucose (VI) by hemihydrogenation using palladium black as a catalyst (6) (10 min, 95°C) in dilute hydrochloric acid which removed protecting groups. The yield in compound VI based on compound V was 45% ; other products were present : the corresponding amine (1-amino-1,2-dideoxy-D glucitol), a small amount of glucononitrile (V) and an unidentified impurity.

The different stages of the synthesis were checked by chromatographic methods and the identity of each obtained product was confirmed by ^{13}C NMR.

The overall yield of the synthesis was 30% (based on sodium cyanide) and after an HPLC purification on a Partisil PXS 10/25 PAC WHATMAN column with an elution system acetonitrile 80 - water 20 (elution time 5 min) the radiochemical purity was 98 % . The overall time of this synthesis with two active steps and an HPLC purification was within one hour, time suitable for labelling with ^{11}C.

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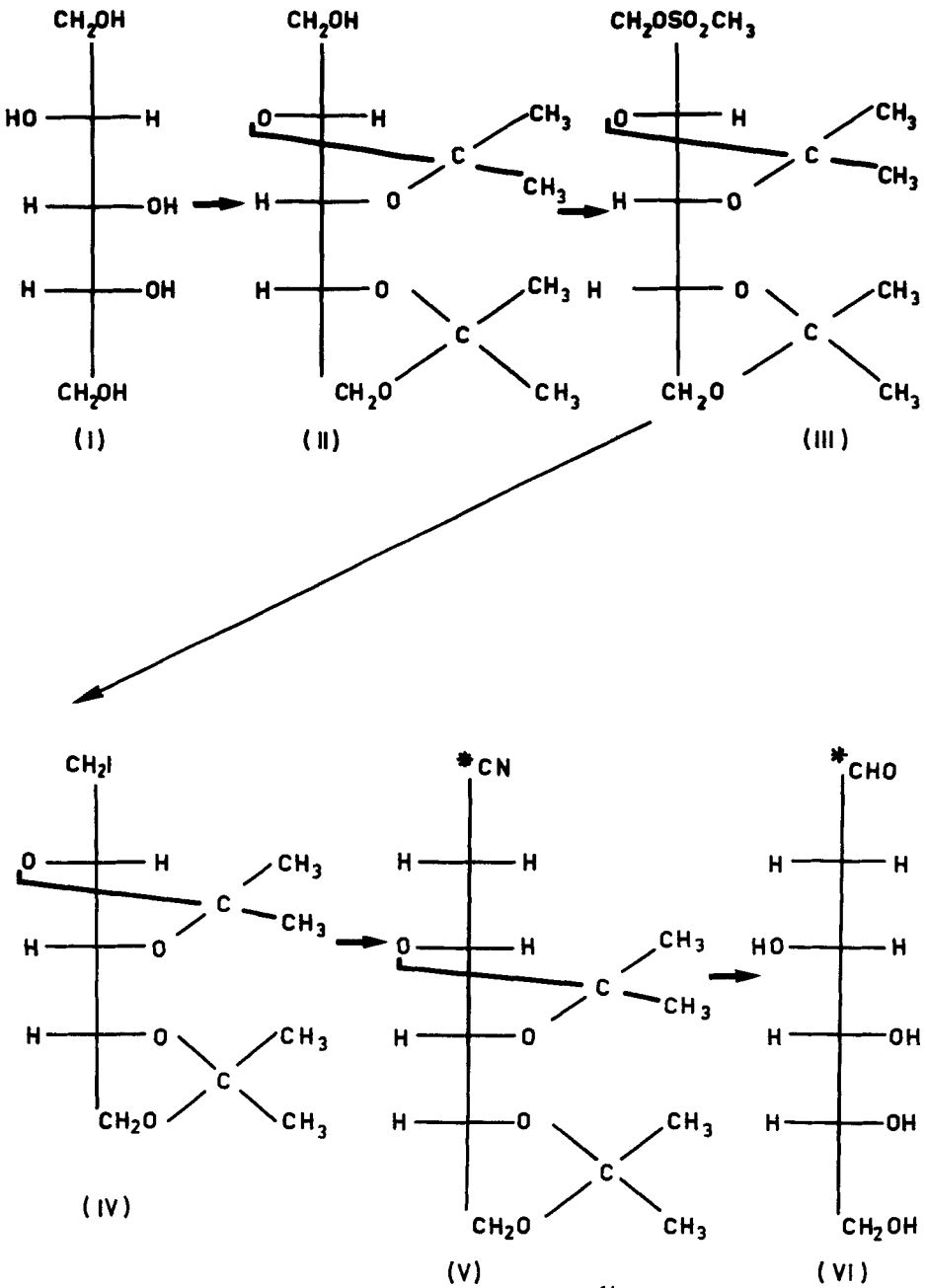

SYNTHESIS OF-2-DEOXY-D[1-^{14}C] GLUCOSE

Paper No.A21

SYNTHESIS OF ^{11}C-HIPPURIC ACID FROM ^{11}C-BENZOIC ACID AND GLYCINE USING RAT-LIVER MITOCHONDRIA

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The classical agent for quantitation of renal tubular secretion is p-amino-hippuric acid. **In Nuclear Medicine studies, this has been replaced** by ^{131}I-o-iodohippuric acid ("hippuran"). We decided to try to synthesize the parent compound, hippuric acid (benzoyl-glycine) labelled with carbon-11, to compare its usefulness with that of "hippuran". The method chosen was to carbonate phenylmagnesium bromide with $^{11}CO_2$ to make ^{11}C-benzoic acid, and then to attach glycine to this biosynthetically using rat liver mitochondria. Optimal conditions for mitochondrial synthesis of hippuric acid from benzoic acid, and details of analysis of the two compounds, have been previously described (1).

It is logical to use biosynthetic methods for making carbon-11 compounds, because of the stereo-specificity and rapidity of enzymatic reactions, and because high specific activities can be obtained. Previous use of biosyntheses have included production of ^{13}N-amino acids from $^{13}NH_3$ with isolated enzymes (e.g. (2)) and ^{11}C-glucose from $^{11}CO_2$ with bean leaves (3). **The rapid recent development of high pressure liquid chromatography** has greatly increased the feasibility of using biological systems as synthetic tools.

A typical rat liver cell contains about a thousand mitochondria which are easily isolated from liver homogenates by differential centrifugation. Techniques for preparing and handling these organelles are given in ref. (4). Mitochondria are often termed the "power houses of the cell" and indeed their main function is production of adenosine triphosphate, the high energy compound which is used to drive otherwise endergonic biosynthetic reactions. However, liver mitochondria also contain other enzymes, among them those responsible for detoxification of benzoic acid by its conversion to hippuric acid.

Method. Carbon-11 CO_2 was produced by bombardment of N_2 with 11 MeV protons from the U.W. Tandem Van de Graaff accelerator. A 2 μA beam in 40 minutes gave 100 mCi of activity which was collected in a metal trap cooled in liquid nitrogen; a fore pump maintained a vacuum of 28 inches of mercury in the trap during irradiation. **The trap which contained 2 μmol** of carrier CO_2 was then connected by vacuum hose to a 50 ml glass carbonation vessel (5) containing 6 ml of frozen ether and 1 mmol of PhMgBr. After the carbon dioxide had been distilled *in vacuo* onto the ether, both stopcocks on the flask were closed and the contents were continuously shaken in an ice-bath for 10 minutes. The mixture was added to 5 ml of 2N-H_2SO_4 in a separating funnel and after a vigorous shaking the ether layer was removed and rotary evaporated to dryness. **The residue was** dissolved in 5 ml of 240 mM-KCl containing 20 mM-Hepes (hydroxyethyl-piperazine ethylsulphonate) buffer and adjusted to pH 7.6. The solution was filtered through glass wool and then added to an incubation mixture so that the final composition was in a volume of 10 ml: 120 mM-KCl, 10 mM-Hepes, 2.5 mM-potassium phosphate, 10 mM-glutamate (to support oxidative

generation of adenosine triphosphate), 5 mM-glycine and 100 mg of mitochondrial protein. The suspension was stirred for 10 minutes at 30°. Reaction was stopped by 10 ml of 2N-HCl and precipitated protein removed by spinning for 5 minutes at full speed in a desk-top centrifuge. The supernatant was extracted twice with ether, which was evaporated as before; the residue was dissolved in 0.9% NaCl and adjusted to neutral pH with 0.1N-NaOH.

Results. Yields in our first two experiments were 0.41 and 3.4 mCi in 71 and 67 minutes respectively. Subsequent paper chromatography (SG81 paper; ether:formic acid; 95/5 v/v) showed a hippuric acid/benzoic acid ratio of 5:1. In each case about 100 µCi of material was administered I.V. to a rabbit. As expected the radioactivity was rapidly excreted through the kidneys, and the renograms were comparable to those made with "hippuran" in rabbits.

Liver mitochondria contain other enzyme systems which could make potential radiopharmaceuticals. For example CO_2 is "fixed" at pyruvate carboxylase producing oxaloacetate, an intermediate in the central Krebs cycle; incubation conditions and specific inhibitors can be chosen to direct the flow of carbon to any of several compounds including the amino acids aspartate, glutamate and glutamine. Entry into the Krebs cycle could also be made by ^{11}C-acetate or ^{11}C-propionate produced from the appropriate Grignard reagents.

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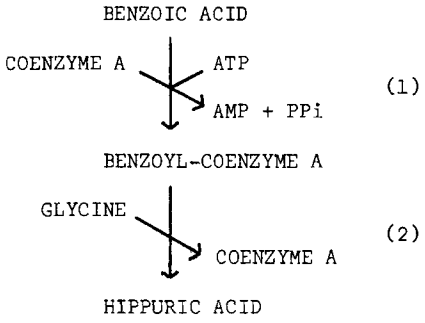
FIGURE 1. MITOCHONDRIAL SYNTHESIS OF HIPPURIC ACID.



Enzyme (1) is benzoyl-CoA synthetase; (2) is glycine acyltransferase. Coenzyme A used in the first reaction is returned in the second. Adenosine monophosphate (AMP) produced by benzoyl-CoA synthetase is rephosphorylated by respiratory energy. A more detailed explanation of the reactions is given in reference (1).

Paper No.A22

REDUCTION REQUIREMENTS OF TECHNETIUM-99M PERTECHNETATE FOR THE FORMATION OF TECHNETIUM RADIOPHARMACEUTICALS

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This study was undertaken to establish the chemical requirements for the reduction of technetium-99m pertechnetate in order that various technetium-99m radiopharmaceuticals might be formed. The radiopharmaceuticals were by the electrolytic generation of the reducing stannous ion in a solution containing the chelating agent and the pertechnetate at the appropriate pH. By controlling the electric charge passed it is possible to accurately control the quantity of reducing ion liberated into the solution. In all these studies 100 mCi of technetium-99m with a total chemical content (technetium-99m + technetium-99) of 4×10^{15} atoms of technetium[1]. This quantity was chosen as it is sufficiently large that it is possible to generate chemically equivalent quantities of stannous ion yet it is still within the normal range encountered in clinical practice since it corresponds to 100 mCi obtained from the Monday elution of a standard commercial Tc-99m generator. The studies involve the effect on the labelling efficiency of the independent variation of the quantity of stannous ion, the pH and the quantity of complexing agent in five different technetium-99m radiopharmaceuticals.

1) Pyrophosphate. The effect of pH on the labelling efficiency of pyrophosphate was studied with the pyrophosphate content maintained at 6×10^{19} molecules and the stannous ion content at 5.6×10^{15} ions in a total volume of 20 mls. The results, shown in figure 1, indicate that the optimum pH is between 2 and 3. The effect of variation of the quantity of pyrophosphate was studied with the pH maintained at 2.5 and the stannous ion content at 2.8×10^{15} ions. The results, shown in figure 2, indicate a semilogarithmic dependence on the pyrophosphate content below 1.8×10^{20} molecules above which the labelling efficiency remains constant. The dependence of the labelling efficiency on the stannous ion content was studied using the optimum pH of 2.5 and a pyrophosphate content of 2.4×10^{20} molecules. The results, shown in figure 3, show that the labelling drops off rapidly below 5.6×10^{15} stannous ions. If the two extremes of this curve are interpolated they intersect at a value of 4×10^{15} stannous ions suggesting that the minimum ratio of stannous ion to pertechnetate required for labelling is 1:1, ie it is technetium in the plus five oxidation state which is involved in this complex.

2) Human Serum Albumin. The effect of pH on the labelling efficiency was investigated with an albumin content of 10^{17} molecules and a stannous ion content of 5.6×10^{15} ions in a total volume of 20 ml. The results, figure 1, show that the optimum pH for labelling is below 0.8. Therefore, the effect of the albumin content was studied at pH 0.75 with a stannous ion content of 2.8×10^{15} ions. The results, figure 2, show a semilogarithmic dependence below 8×10^{16} molecules of albumin while above that point essentially complete labelling is achieved with this quantity of stannous ion. The dependence of the labelling efficiency on the stannous ion content was determined at pH 0.75 and with an albumin content of 10^{17} molecules. The results, figure 3, show that the labelling efficiency does not start to drop sharply until the stannous ion content is below 2×10^{15} suggesting that the oxidation state of technetium in technetium human serum albumin is plus six.

3) D.T.P.A. The effect of pH on the labelling efficiency was investigated with a D.T.P.A. content of 6×10^{19} molecules and a stannous ion content of 5.6×10^{15} ions in a total of 20 mls. The results, figure 1, shows that the labelling is optimum below pH 3. The effect of the D.T.P.A. content was studied at pH 3.0 with a stannous ion content of 5.6×10^{15}. The results, figure 2, show a semilogarithmic dependence below 3×10^{19} molecules of D.T.P.A. above which essentially complete labelling occurs. The effect of the stannous ion content on the labelling efficiency was studied at pH 3.0 with a D.T.P.A. content of 6×10^{19} molecules. The results, figure 3, shows that less than 10^{15} stannous ions are necessary for the labelling of D.T.P.A. with 4×10^{15} technetium atoms, suggesting that the stannous ion acts in some sort of catalytic role and that the oxidation state of technetium in the technetium D.T.P.A. complex is plus seven.

4) Gluconate and Glucoheptonate. Studies of the effect of pH on the labelling efficiency of these two complexing agents showed that labelling was favoured by more alkaline pH's, figure 1. Under such conditions the hydrolysis of the stannous ion introduces considerable interference and optimum pH's could not be determined as they appeared to be over pH 7.5; however, the reproducibility at these higher pH's was very poor. The effect of the gluconate or glucoheptonate content on the labelling efficiency was investigated at pH 6. The semilogarithmic dependence observed, figure 2, is somewhat reduced probably due to the non optimum pH used as similarly decreased dependence on complexing agent content was observed for the other agents studied when non optimum pH's were used. The effect of the stannous ion content was also evaluated at pH 6, figure 4, and shows that a considerable excess of stannous ion is necessary to obtain high labelling efficiencies. Presumably this excess is necessary to overcome the effects of hydrolysis of the stannous ion.

In summary then, it has been shown that large excess quantities of stannous ion are not necessary for the labelling of technetium-99m pertechnetate to complexing agents provided the labelling is carried out at the optimum pH and with an adequate excess of complexing agent. Furthermore, it has been shown that the oxidation state of technetium in technetium pyrophosphate is probably plus five, in technetium human serum albumin probably plus six and in technetium D.T.P.A. probably plus seven.

It is important to note that these preparations were analysed by TLC immediately following preparation with no attempts being made to evaluate the in vitro stability which would be expected to be low[2].

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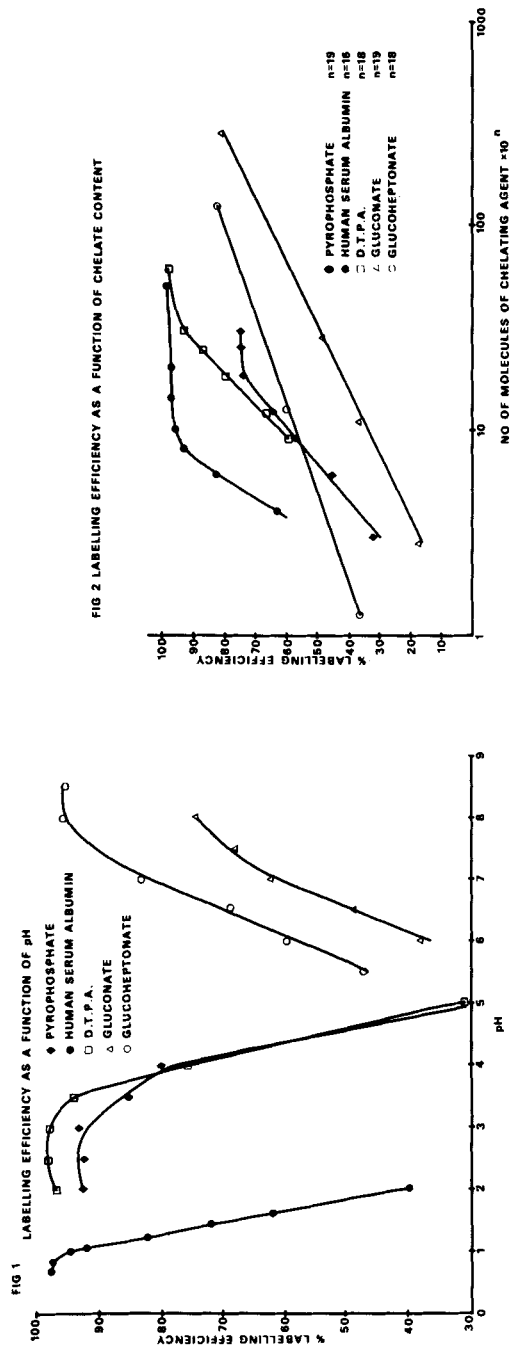
FIG 1 LABELLING EFFICIENCY AS A FUNCTION OF pH
◆ PYROPHOSPHATE
● HUMAN SERUM ALBUMIN
□ D.T.P.A.
△ GLUCONATE
○ GLUCOHEPTONATE
% LABELLING EFFICIENCY
pH

FIG 2 LABELLING EFFICIENCY AS A FUNCTION OF CHELATE CONTENT
● PYROPHOSPHATE n=19
● HUMAN SERUM ALBUMIN n=16
□ D.T.P.A. n=18
△ GLUCONATE n=19
○ GLUCOHEPTONATE n=18
% LABELLING EFFICIENCY
NO OF MOLECULES OF CHELATING AGENT =10^n

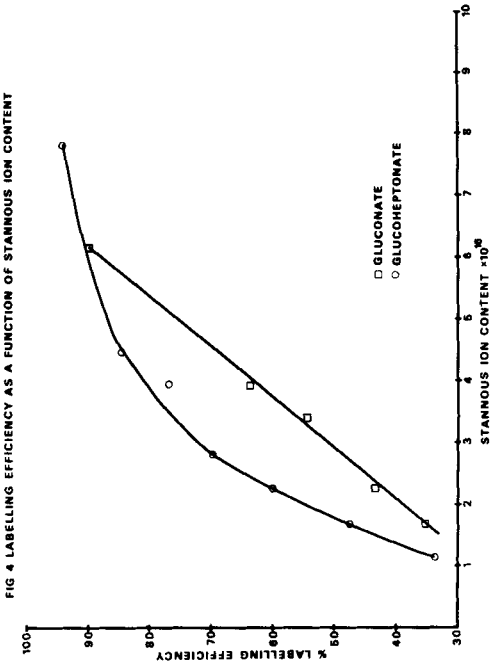
FIG 4 LABELLING EFFICIENCY AS A FUNCTION OF STANNOUS ION CONTENT
□ GLUCONATE
○ GLUCOHEPTONATE
% LABELLING EFFICIENCY
STANNOUS ION CONTENT =10^10

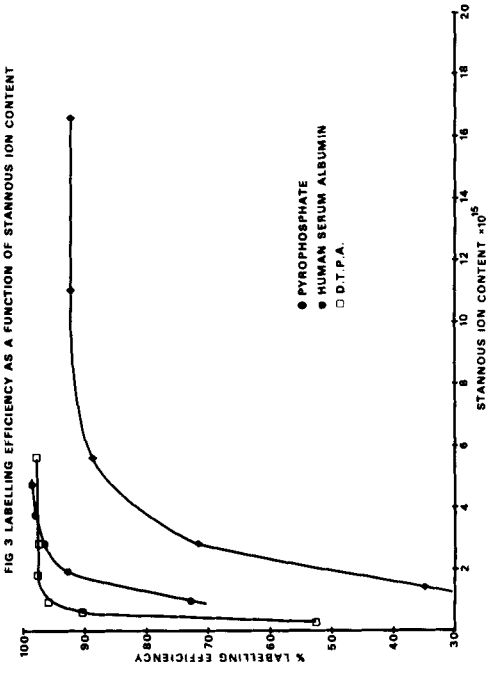
FIG 3 LABELLING EFFICIENCY AS A FUNCTION OF STANNOUS ION CONTENT
● PYROPHOSPHATE
● HUMAN SERUM ALBUMIN
□ D.T.P.A.
% LABELLING EFFICIENCY
STANNOUS ION CONTENT =10^15

Paper No.A23

PHTHALEIN AND FLUORESCEIN DERIVATIVES WITH AN EFFECTIVE 99m-TECHNETIUM LABELED STATE FOR A HEPATOBILIARY TRANSPORT: 99m-Tc-PC, A NEW HEPATOBILIARY RADIOPHARMACEUTICAL.

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In an attempt to develop a new hepatobiliary agent labeled with 99m-Tc, compounds structurally related with phthalein and fluorescein dyes such as Rose Bengal and sulphobromophthalein (BSP) were surveyed. The chemical state of Tc in the molecule of the labeled agent played an important role. A low hydrolyzed state of 99m-Tc was inferred to be important in the bile excretion mechanism (1,2).

Evaluated compounds include Calcein, Methylxylenol Blue (MXB), Phenolphthalein Complexone (PPC), Phthalein Complexone (PC) and Thymolphthalein Complexone (TPC) (see Fig. 1), and they were labeled with the low hydrolyzed 99m-Tc. The labeling efficiency with 99m-Tc was quantitative in MXB, PPC and PC while approximately 90 % in TPC and Calcein.

Their biological behavior was analyzed and compared with one another. The amount of radioactivity recovered from the bile of rats during the first 1 hr decreased in the order 99m-Tc-PC > 99m-Tc-TPC > 99m-Tc-PPC > 99m-Tc-Calcein (Fig. 2). This order shows that the biliary excretion behavior of 99m-Tc chelates is remarkably affected by slight changes in their structures, and their relationship with the biological behavior is discussed.

Among the tested compounds, 99m-Tc-PC displayed the most effective excretion (61 % at 1 hr in rats), similar to data reported with 99m-Tc-HIDA and higher than 131-I-Rose Bengal and 99m-Tc-PG (pyridoxalglutamate). Its distribution studies in mice showed that the complex rapidly excreted through the biliary tract into the intestine. Scintigraphic studies in rabbits compared well with results obtained in mice and rats. Excellent images of the gallbladder were obtained in 40 min in rabbits.

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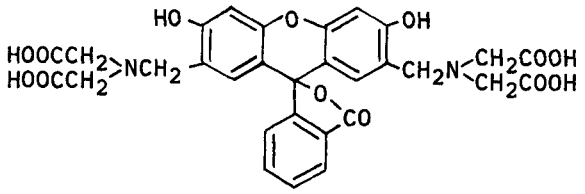
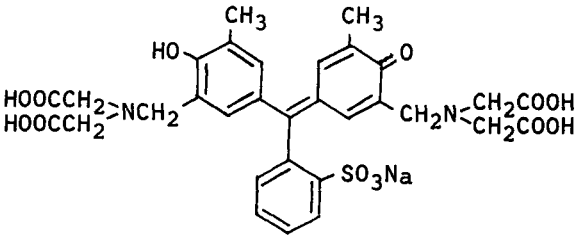
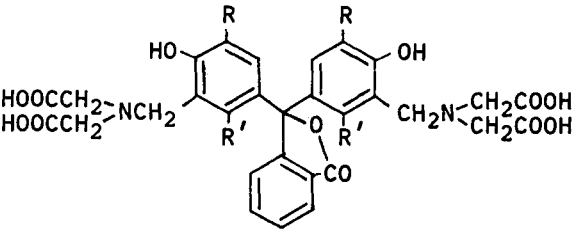
Fig. 1.



CALCEIN

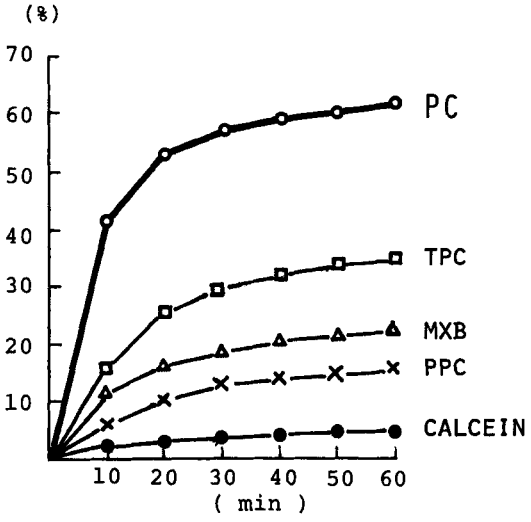


MXB



PC: R= CH_3
R'= H
PPC: R=R'= H
TPC: R= $CH(CH_3)_2$
R'= CH_3

Fig. 2.
Bile Excretion of ^{99m}Tc compounds in rats.



Paper No. A24

ROLE OF REDUCING AGENT ON Tc-99m-GLUTATHIONE BIODISTRIBUTION: RELATIONSHIP TO CHEMICAL PROPERTIES

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Recent reports have described a dependency of organ localization of Tc-99m radiopharmaceuticals on reducing agent. The renal agent dimercaptosuccinate (DMSA) prepared with stannous ion, electrochemically and with sodium borohydride showed both chromatographic and biological distribution differences (1). Tc-99m-Ethylthiomethylphosphonate prepared with stannous chloride ($SnCl_2$) and formamidine sulfinic acid (FSA) resulted in increased renal retention with stannous ion as the reducing agent (2,3). Differences were also noted with Tc-99m-glutathione (γ-glutamylcysteinylglycine, GSH). Preparation with $SnCl_2$ resulted in values of 12.2% in the kidneys and a kidneys to liver ratio of 9.8 in mice at 2 hr. When prepared with the cysteinyl sulfhydryl group as reducing agent, 1.72% was found in the kidneys and a kidneys to liver ratio of 0.9 resulted at the same time period. A study of the properties of Tc-99m-GSH was therefore initiated in order to ascertain the chemical basis for the biodistribution differences.

Several studies have considered whether or not tin is retained in technetium complexes. Results have in some cases indicated the retention of tin (4,5) and in others not (6). Dual label studies of Tc-GSH were performed with Sn-113 and Tc-99 in order to answer this question. Complexed forms of GSH prepared with Sn-113-$SnCl_2$, Tc-99m and sufficient Tc-99 to react with one half of the stannous ion present were separated by paper electrophoresis. No Sn-113 activity overlapped with that of technetium as determined by Tc-99m activity indicating that tin was not retained in the complex.

Preparations of Tc-99-GSH complexes via both modes of reduction did have different spectral and electrophoretic properties, however. Without added $SnCl_2$ a green complex with an absorption maximum at 665 nm (ε~3700) resulted; with stannous ion a brown complex with absorptions at 390 (ε~4750) and 560 nm (ε~1630) resulted. The radioactivity in the electrophoresis of the complexes was centered at 4.0 cm for the stannous preparation and 5.8 cm for the sulfhydryl preparation when reference bromcresol green was centered at 3.0 cm. The brown complex ($SnCl_2$) was converted to green by heating or by added reducing agents sodium borohydride or dithionite. Reduction with sodium borohydride alone gave initially a brown complex which subsequently turned green.

These results suggest that different oxidation levels result from stannous and sulfhydryl group reduction and may be the reason for the biodistribution differences. The action of the other reducing agents on the stannous preparation suggests also that a lower oxidation level results from sulfhydryl reduction. This conclusion is consistent with the higher net negative charge on the sulfhydryl preparation as indicated by the electrophoresis results.

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Paper No.B1

INCREASING THE TUMOUR SPECIFICITY OF ^{67}Ga-RADIOPHARMACEUTICALS.

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^{67}Ga-citrate is so far the most widely employed tumour localizing radiopharmaceutical[1]. Numerous factors which determine its tissue distribution[2] and its extensive uptake in the liver, spleen and bowel are, however, serious drawbacks[3]. Irreproducibility of the scintigrams by using ^{67}Ga-citrate from different suppliers have also been reported[4]. The reason for these undesirable observation has been investigated in the present work. Several samples of ^{67}Ga-citrate and ^{67}Ga-chloride from different suppliers was examined chromatographically and electrophoretically, and their uptake observed by scintigraphy after injection into healthy and Morris hepatoma-3924A-bearing ACI rats.
An excess of chloride, citrate, malate and tartrate converted ^{67}Ga into a single anionic species which was taken up in the liver but not in the tumour. The presence of both chloride and citrate anions in the ^{67}Ga-citrate and ^{67}Ga-chloride solutions respectively was found to favour the tumour uptake of the radiopharmaceutical. At an optimal citrate-chloride ratio of the two anions in the injected solution, ^{67}Ga citrate was found to concentrate selectively in the tumour with comparatively neglegible uptake in the liver or other organs of the rat. The reason of this behaviour of ^{67}Ga will be discussed.

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Paper No.B3

CONSEQUENCES OF INDIUM-111 DECAY IN VIVO : CALCULATED ABSORBED RADIATION DOSE TO CELLS LABELLED BY INDIUM-111 OXINE.

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^{111}In-oxine has been accepted in many quarters as a useful label for cellular blood components, but we still "need to be sure that the observed shifts of radioactivity accurately represent natural phenomena and that the movements of the cells are not modified by the labelling procedure (1)". Amongst the factors which might influence the behaviour of labelled cells is possible radiation damage caused by the Auger electrons emitted as part of the decay process of the ^{111}In atoms within them. The absorbed radiation dose to such cells may be calculated, provided the intracellular distribution of the ^{111}In is known.

The location of ^{111}In atoms in labelled human neutrophils has been studied by Thakur et al (2) who concluded that most of the ^{111}In binds to various intracellular components. They could not be more specific, however, so for present purposes we may assume uniform distribution within these cells (diam. $\sim$ 10μm). By contrast, it has recently been shown (3), by combined autoradiography and electron microscopy, that in labelled platelets (diam. $\sim$ 2μm) the ^{111}In is highly concentrated in specific organelles (diam. $\sim$ 0.2μm).

The gamma rays, conversion electrons and x-rays emitted in the decay of an ^{111}In atom all have ranges which are long relative to cell diameters, and may therefore be neglected. The radiation absorbed within a labelled cell is assumed to be due to the Auger electrons emitted. (Table 1.)

Calculations show that the radiation dose to a neutophil resulting from the decay of a single ^{111}In atom is 0.135 rad. In a typical experiment in which 10^8 cells are uniformly labelled with 100μCi of ^{111}In, the integrated radiation dose per cell is 1.75 krad (17.5 Gy), and the initial dose rate is 18 rad/hour.

In the case of labelled platelets, the radiation dose resulting from the decay of each ^{111}In atom is 14.5 rad (0.145 Gy).

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TABLE 1

Radiation	Mean No. per disinteg. n_i *	Mean energy per particle $\bar{E}_i$ (MeV) *	$n_i\bar{E}_i$ (MeV)	Range ** µm
KLL Auger Elect	.1103	.0192	0.0021	∿8
KLX " "	.0441	.0223	0.0009	∿10
KXY " "	.0067	.0254	0.0002	∿12.55
LMM " "	.9867	.0024	0.0024	0.230
MXY " "	2.2886	.0006	0.0014	0.025
		Total	0.0070 MeV/disinteg.	

* from Dillman and von der Lager, MIRD Supplement No.10 (Soc. Nuc. Med. New York) 1975

** from Lea, D.E., "Actions of radiation on living cells" (Cambridge Univ. Press) 1967

Paper No.B4

THE BINDING OF ^{113m}In TO DTPA AND A COMPARISON BETWEEN GFR ESTIMATED WITH ^{113m}In - DTPA AND ^{125}I - IOTHALAMATE

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Diethyl triamino penta acetic acid (DTPA) is excreted almost completely by glomerular filtration, and labelled with technetium it has been used for determination of glomerular filtration rate (GFR) (1).

As has been proposed by Best et al. (2), a label with ^{113m} Indium might be equally suitable provided ^{113m}In was entirely bound to DTPA; free indium ions could otherwise be bound to serum proteins (3) and introduce error in the calculations.

A study of the binding of ^{113m}In to DTPA versus binding to serum proteins has been carried out. In addition the ^{113m}In - DTPA clearance was compared to the clearance of ^{125}I - iothalamate, a well known agent for determination of GFR, using constant infusion technique.

^{113m}In was obtained from an Indium generator (Amersham) eluted with 5 ml 0.04 M hydrochloric acid. ^{113m}In - DTPA was prepared by adding this eluate (1-3 mCi) into a vial containing 1.6 mg DTPA (Amersham). After mixing, the solution was left for 10 min to equilibrate. Another aliquot of the ^{113m}In eluate was buffered with tromethamin to a pH of 7.4. The separation of ^{113m}In bound to DTPA from ^{113m} In bound to serum proteins, was performed with an ACA 44 ultrogel column on the basis of their difference in molecular size.

In vitro studies: Free indium eluate and ^{113m}In - DTPA were mixed separately with serum from 2 fasting subjects and applied to the ultrogel column. Fig 1 shows the separation patterns.

In vivo studies: Similarly indium eluate and ^{113m}In - DTPA were injected separately intravenously to 3 fasting subjects on two consequtive days. Serum samples taken 10 min after the intravenous injection, was applied to the ultrogel column. The separation pattern was as in the in vitro studies (fig 1).

The separated fractions were calculated in per cent of the radioactivity applied to the column after correction for decay.

The radioactivity within the ^{113m}In - DTPA peak (fig 1) represented 100.6, 107.1 and 99.6 per cent of the amount of radioactivity applied to the column in the in vivo studies, and 102.0 and 101.4 per cent in the in vitro studies.

The glomerular filtration rate was determined in fifteen subjects aged 21-76 years. ^{113m}In - DTPA and ^{125}I - iothalamate were dissolved in 0.9 per cent sodium chloride for constant intravenous infusion. Blood samples were taken with 10 min intervals. Constant levels of radioactivity in plasma was obtained in 13 of 15 subjects. The values for GFR obtained with

^{113m}In - DTPA were nearly equal to those obtained with ^{125}I - iothalamate (r = 0.996) (Fig 2).

The represented data show that ^{113m}In is firmly bound to DTPA, both in the in vivo and in the in vitro studies. Furthermore, the clearance determined with ^{125}I - iothalamate and ^{113m}In - DTPA gave close to identical results.

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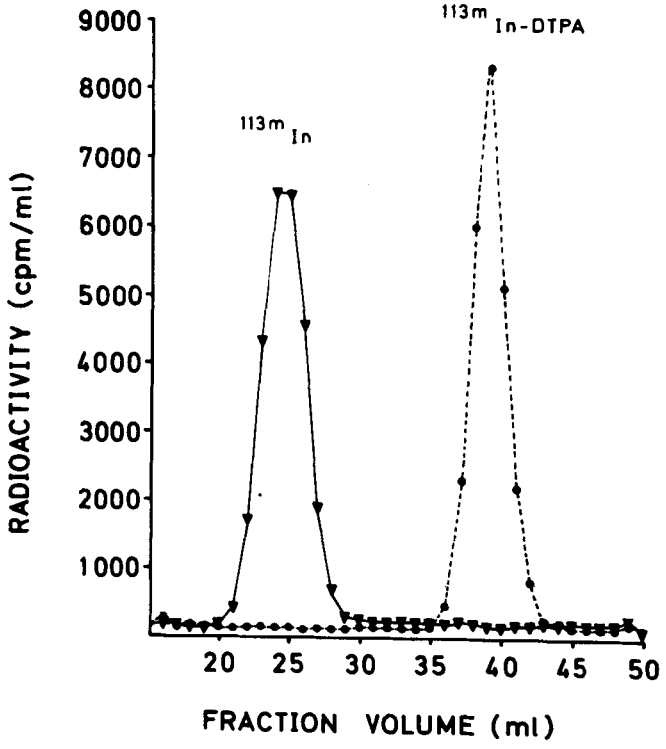

Fig 1. Representative elution diagrams for ^{113m}In when bound to serum proteins and DTPA.

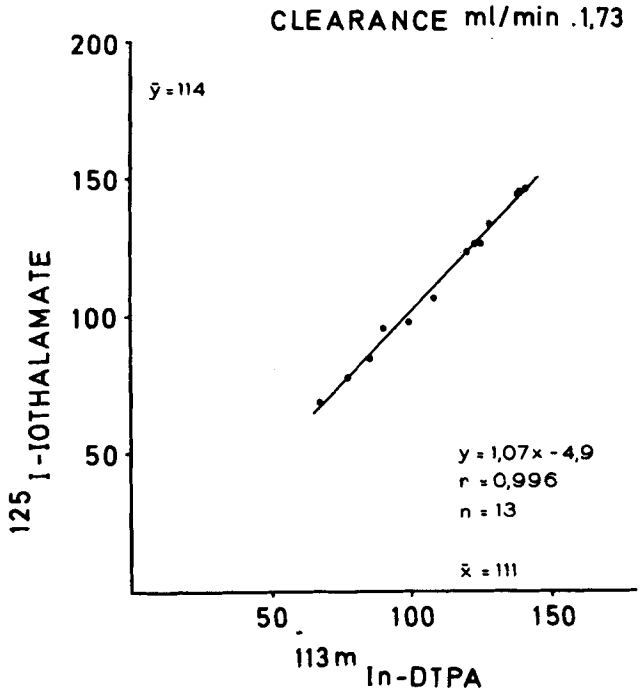

Fig 2. The clearance of ^{113m}In - DTPA compared to the clearance of ^{125}I - iothalamate.

Paper No.B5

INVESTIGATION OF ^{203}Pb-OXINE AS A POTENTIAL LABEL FOR PLATELETS.

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The high affinity of serum transferrin for ^{111}In requires that plasma be removed prior to cell labeling with ^{111}In-oxine. This constitutes an additional step in the labeling procedure and introduces the possibility of damaging the platelets. Since Pb(II) was not expected to bind strongly to serum proteins, ^{203}Pb-oxine was investigated to determine whether cell labeling can be achieved in plasma. ^{203}Pb (279 keV γ, $t_{\frac{1}{2}}$ 52 hrs.) offers the additional advantage of a lower radiation dose than ^{111}In.

^{203}Pb-oxine was prepared according to the procedure of Thakur, et. al. (1). The chloroform extraction of oxine, $Pb(ClO_4)_2$ and Pb-oxine, as a function of the pH of the aqueous phase, is shown in Figure 1. At pH > 10, the extraction of the complex is excellent with little extraction of uncomplexed oxine. Below pH 8, however, less than 40% of ^{203}Pb activity is extracted indicating that a neutral, lipophilic species is not formed at physiological pH.

Base titration of aqueous solutions containing varying ratios of Pb^{2+} to oxine (Bjerrum Method) suggest that the species which predominates at pH 7 is $Pb(Ox)^+$, where Ox is the anionic form of oxine. An insoluble complex, $Pb(Ox)_2$ [or possibly Pb(Ox)(OH)] forms above pH 8.

In several experiments using microcurie amounts of ^{203}Pb, the chloroform extract containing $^{203}Pb(Ox)_2$ was evaporated to dryness and redissolved in 50μl of ethanol and 200μl of saline. This solution was added to a suspension of rabbit platelets in 4 ml unbuffered saline or human platelets in Tyrode's solution. Up to 90% of ^{203}Pb activity was taken up by the platelets within 20 minutes. If the $^{203}Pb(Ox)_2$ solution is added to platelet suspensions in citrate buffered saline or unbuffered plasma, less than 15% labeling is obtained, presumably due to rapid formation of $^{203}Pb(Ox)^+$ at pH 7.5 or lower.

No significant binding of $^{203}Pb(ClO_4)_2$ or $^{203}Pb(Ox)_2$ to serum proteins was found by electrophoresis, TCA precipitation, nor dialysis against physiological saline.

These studies indicate that ^{203}Pb-oxine offers no advantages for cell labeling. Current work involves the evaluation of more stable lipophilic complexes of ^{203}Pb.

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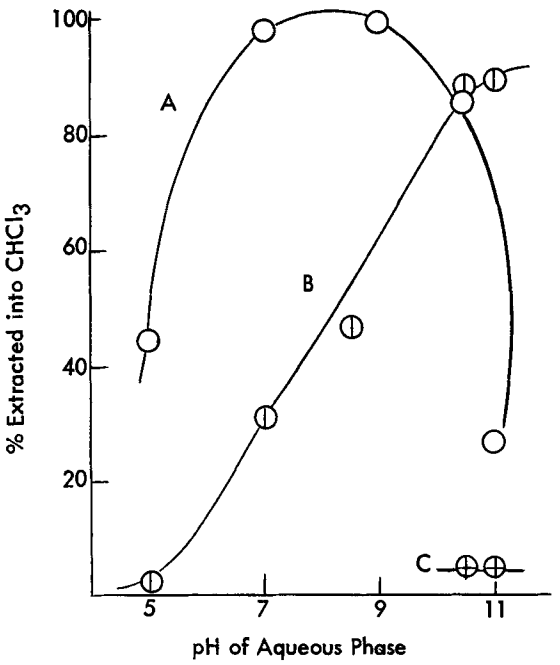
FIGURE I



Chloroform Extraction from aqueous solutions containing:

A. 6.9×10^{-3} M oxine

B. 5×10^{-5} M $Pb(ClO_4)_2$ with 1μCi of ^{203}Pb and 5×10^{-4} M oxine

C. 5×10^{-5} M $Pb(ClO_4)_2$ with 1μCi of ^{203}Pb

Paper No.B6

PREPARATION, ISOLATION AND IDENTIFICATION OF BLEOMYCIN-^{57}Co CHELATE WITH SPECIAL REFERENCE TO ITS CHELATE STRUCTURE

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To obtain information on bleomycin-Co 57 chelate, an attempt was carried out 1) to prepare cobalt chelate with a sufficient amount of stable Co, 2) to isolate chelate compounds with a high speed liquid chromatography, 3) to study the relative stability of these compounds in respect to pH and time amd, 4) to study the conformation of Co chelated bleomycin, a NMR study was performed to find the changes of signals occuring in chelation. Commercial bleomycin mixture contains A2(67%), B2(25%) and A1(8%). The chelated bleomycin with stable cobalt yielded six components separated by TLC, giving rise to double spots to each of A2, B2 and A1, namely, A2-I, A2-II, B2-I, B2-II, A1-I and A2-II respectively. A separation by high speed liquid chromatography with micro-Bondapack C-18 eluted with 0.05M ammonium acetate and acetonitrile gave better separation than TLC. A determination of mole ratio of cobalt vs bleomycin was carried out after mixing of each component with an excess cobalt at pH 6-6.5 and after separation with high speed liquid chromatography. Atomic absorption and UV absorption revealed one mole cobalt bound with one mole bleomycin in A2 and B2 chelate. However, A1 yielded unstable chelate. These ratios are not changed with concentration of bleomycin in the presence of excess cobalt. However, those of freshly prepared chelates changed with a progress of time, giving rise to the increase of the first component in A2 as well as B2, i. e., A2-I and B2-I, with the corresponding decrease of the second peak. Because the width of the first peak increased, it was thought that the second was not converted to the first. It may be converted to different components, or it was decomposed. The NMR study of bleomycin A2-Co chelate revealed a low field shift of the signals of methyl protons in pyrimidine ring and two protons in imidazole ring. Other patterns of signals remained identical. Further study on chelate conformation by NMR and optical circular dichroism is in progress.

Paper No.B7

QUALITY CONTROL OF ^{99m}Tc-PLASMIN PREPARATIONS FOR SCINTIGRAPHIC DETECTION OF DEEP VENOUS THROMBOSIS

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At the 1st International Symposium on Radiopharmaceutical Chemistry we reported on the methods for labelling of plasmin with ^{99m}Tc (1). Since then we have used ^{99m}Tc-plasmin in several patient series for comparison with both phlebography and the ^{125}I-fibrinogen test for diagnosis of deep-vein thrombosis. In a study of 106 consecutive patients 97 % of the thrombi were detected with ^{125}I-fibrinogen within 2 days and 94 % of the thrombi with ^{99m}Tc-plasmin within 5 minutes (2). Only a few foot or calf thrombi of small size 8-10 mm were missed.
The ^{99m}Tc-plasmin used in these patient series was prepared from a kit manufactured by NOVO Industry A/S Denmark according to our previous prescription (3). The preparation of ^{99m}Tc-plasmin took place by adjusting the pH-value to about 2.1 by adding 0.2-0.3 ml 0.1 M HCl to the kit. Then ^{99m}Tc-pertechnetate was added to a final volume of 3.5 ml. The vial was inversed vigorously for about one minute and equilibrated in 45 minutes at room temperature before the preparation was tested and ready for use.

We used the method of Gel chromatography column scanning (GCS) to analyze the labelling yield and the radiochemical purity of each preparation before administration to patients. Columns of various dimensions were tested in order to find the one which is best suited for clinical routine work.
The research and development of ^{99m}Tc-plasmin has been performed by using columns of 15 mm inner diameter and 300 ml length filled with Sephadex G-25 Fine (Pharmacia Fine Chemical Uppsala Sweden). A 0.1 ml aliquot of ^{99m}Tc-plasmin preparation was applied to the top of the gel column which was developed with 10.0 ml 0.9 % NaCl/HCl solution (pH 2.1) for about 15 minutes. Different ^{99m}Tc-species are thus fractionated at various zones of the column which are recorded by scanning with a slit collimated NaI(Tl) detector.

For the routine control of each preparation for the patient study we first used shorter columns (5.5 cm) of the same diameter.
The resolution was not as good as for the longer columns but enough to distinguish ^{99m}Tc-plasmin from ^{99m}Tc-pertechnetate and reduced hydrolyzed ^{99m}Tc. The time of development was only about 5 minutes.
Next step was to use columns of only 9 mm inner diameter and 125 mm gel bed. The development of these columns are even faster and we believe that this type fulfils the need for quality control in routine radiopharmaceutical work. They are also possible to use for quality control of all other kinds of radiopharmaceuticals labelled with ^{99m}Tc or other shortlived gamma emitting radionuclides.

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Paper No.B8

QUALITY CONTROL OF ^{99m}Tc-LABELLED COLLOIDS FOR SCINTIGRAPHIC STUDIES OF THE LYMPHATIC SYSTEM

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By using ^{99m}Tc-labelled colloids it is possible to study the dynamics of the lymphatic system and to obtain information about the spread of cancer to the lymph nodes (1).

The uptake in the lymph nodes depends on the size of the particles. Rapid enhanced and reproducible uptake is recorded for the preparation with small particle size (in the order of 10 nm). Colloid preparations with larger particle size, however, are accumulated to a much lower extent and with varying results (2). There is thus need for a rapid, simple and accurate quality control method which gives information about the distribution of ^{99m}Tc-activity on particles of various size and the presence of ^{99m}Tc-pertechnetate and other labelled species.

We have studied the possibilities of using gel chromatography column scanning (GCS) which was previously developed for quality control of ^{99m}Tc radiopharmaceuticals. The use of Sephadex^{R} (Pharmacia Fine Chemicals Uppsala Sweden) as column filling has, however, achieved limited success for quality control of ^{99m}Tc-labelled colloids. The present investigations were therefore carried out with Sepharose as column filling. Sepharose^{R} (Pharmacia Fine Chemicals Uppsala Sweden) is supplied as a dense suspension of swollen agarose beads of 40-150 µm size.

The sample to be analyzed was applied at the tope of a column filled with Sepharose 4B and developed with 10.0 ml 0.9% NaCl. Thereafter the column which still retained all the radioactivity was scanned with a slit collimated NaI(Tl)-crystal detector.

The scanning profiles as shown in the figure give information on the size distribution of the labelled colloid and the presence of the ^{99m}Tc-labelled species in the preparation.

This method has been used for comparative quality control studies of some ^{99m}Tc-colloid kits commercially available and of some colloids prepared by ourselves at various conditions.

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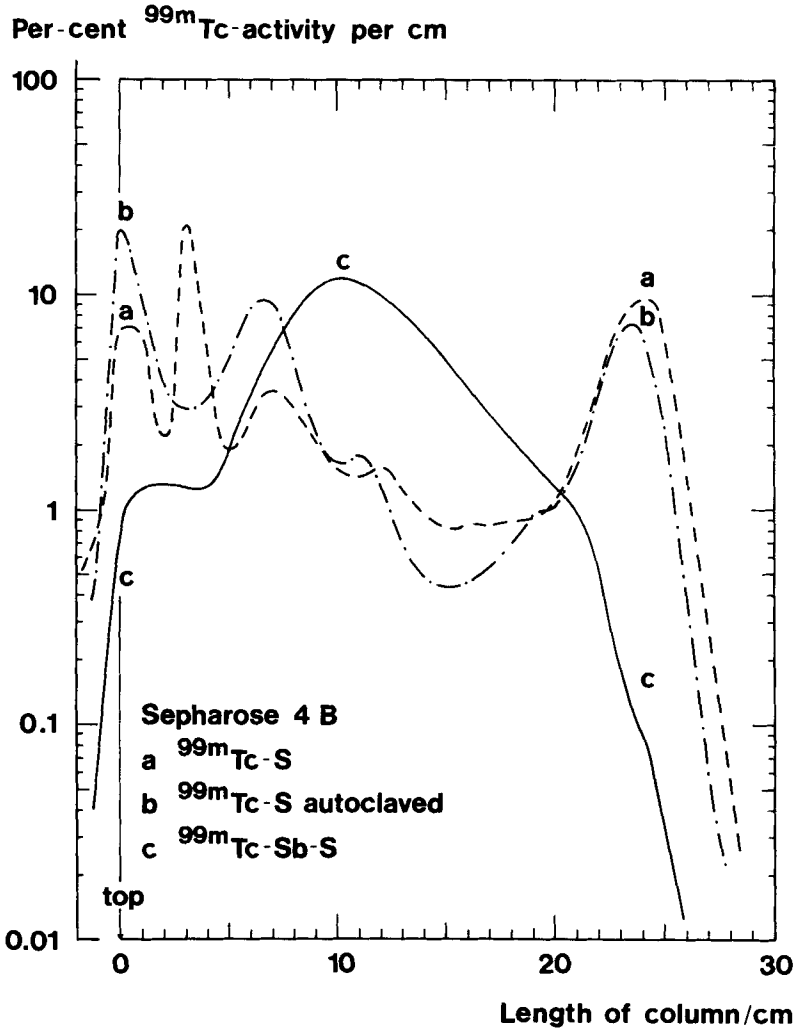

Figure: GCS-scanning profiles on Sepharose 4B of

a) ^{99m}Tc-sulphur colloid of large particle size before autoclaving

b) same as a) after autoclaving

c) ^{99m}Tc Sb-S colloid

Paper No.B9

DEVELOPMENT OF ELECTROCHEMICAL LABELLING PROCEDURES USING MEK EXTRACTED Tc-99m

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Technetium 99m labelled compounds have provided a wide spectrum of useful radiopharmaceuticals for the scintigraphic evaluation of almost all important organs. The most widely used method for the preparation of these labelled compounds involve the reduction of pertechnetate with stannous chloride. However, this method suffers from several disadvantages such as atmospheric oxidation of stannous tin, necessity for multiple pH adjustments, irreversible hydrolysis of tin etc.

Although freeze drying the kit components has overcome these drawbacks, kits using electrochemical methods offer an attractive and simple alternative. The main advantage in this case is the in-situ generation of stannous ions avoiding aerial oxidation and the consequent need for smaller amounts of reducing agent enhances the radiochemical yield and purity of the final product. The authors have carried out detailed studies on the electrolytic reduction of Tc-99m-pertechnetate using tin electrodes and standardised optimum conditions to prepare Tc-99m labelled gluconate and ethane hydroxy diphosphonic acid (EHDP), which are used for kidney and skeletal scanning respectively. These studies are described in the first part of this paper.

For the preparation of human serum albumin microspheres which are used for lung scanning, a rapid and convenient procedure based on spheridization of the albumin using a spinning disc, followed by direct oven heating for denaturation and sterilisation has been developed. Different methods have been studied to label these albumin microspheres with technetium 99m including electrochemical methods and these studies are described in the second part of this paper.

Paper No.B10

PREPARATION OF TRI-*n*-BUTYLTIN TRITIDE AND ITS USE IN THE SYNTHESIS OF 9α-^{3}H STEROIDS

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Steroids labeled with tritium at C-9 have not, to our knowledge, been prepared. Yet such compounds would be quite desirable since C-9 is, metabolically, a highly stable position.

An obvious route to 9α-^{3}H steroids would be by reductive dehalogenation of an analogous 9α-bromo compound using a tritiated reducing agent. 9α-Bromosteroids bearing an 11β-hydroxyl or halogen function are readily available from 11β-hydroxysteroids by well described methods (1, 2).

The use of *n*-Bu_3SnH as a selective reducing agent toward halogen is well known and has been reviewed by Kuivila (3, 4), yet there have been no attempts either to reduce 9-bromosteroids with this reagent or to prepare its tritium labeled analog. We felt that the desired transformation, i.e. 9α-bromosteroid to 9α-^{3}H steroid, could be effected using *n*-Bu_3SnH for two reasons. First, although certain vicinal dihalides undergo elimination reactions, bromohydrins are reduced to their corresponding alcohols (5). Furthermore, as in the case of $Cr^{II}(OAc)_2$/BuSH [the only reagent found to reduce 9-bromosteroids (6)], reductions involving *n*-Bu_3SnH are believed to proceed by a free radical mechanism.

Two methods were used to prepare *n*-$Bu_3Sn^{3}H$ (III). In method (a) *n*-Bu_3SnLi (7) was quenched with freshly prepared $^{3}H_2O$. In method (b) *n*-Bu_3SnCl was reduced with $NaB^{3}H_4$. In each case (III) was reacted *in situ* with either IV or VI. The 9α-tritiated products, V and VII respectively, were isolated by extraction and purified by TLC. Yields using methods (a) and (b) were 0.75% and 60% respectively. Chromatographic mobility and UV spectra of the labeled products were identical to those of cold standards prepared using *n*-Bu_3SnH. NMR and mass spectra of these standards were consistent with their proposed structures. Experimental details, advantages of each method used to generate III, and the use of *n*-Bu_3SnH in the preparation of 11β-fluorosteroids will be discussed.

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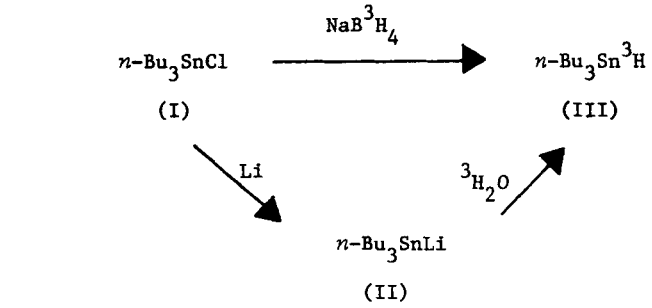
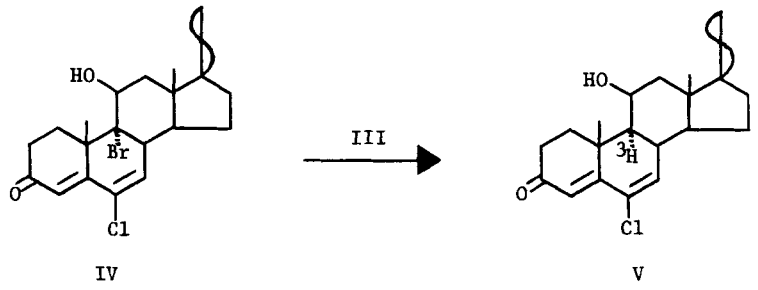
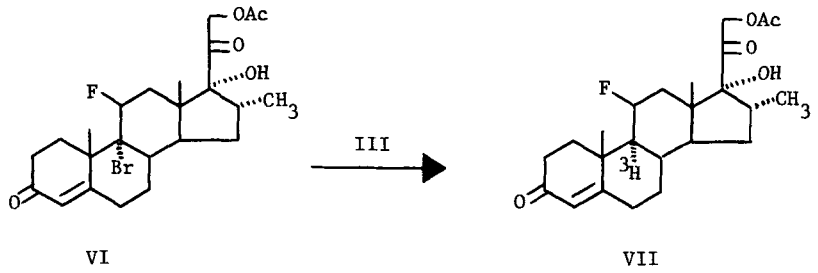

NaB^{3}H$_4$
n-Bu$_3$SnCl
(I)
n-Bu$_3$Sn^{3}H
(III)
Li
^{3}H$_2$O
n-Bu$_3$SnLi
(II)


HO
Br
O
Cl
III
HO
^{3}H
O
Cl

IV V


OAc
O
OH
F
CH$_3$
Br
O
III
OAc
O
OH
F
CH$_3$
^{3}H
O

VI VII

Paper No.B11

The Application of HPLC to Labelling with Short-Lived Nuclides.

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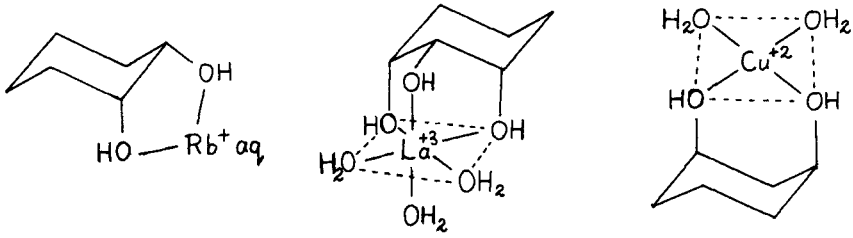
Many chemical reactions can be applied to the preparation of compounds labelled with the short lived β^+/γ-nuclides ^{13}N ($t_{1/2}$ 10min), ^{11}C ($t_{1/2}$ 20min) and ^{18}F ($t_{1/2}$ 110min)(1) but it is usually necessary to separate the desired labelled product from other products and/or starting materials. Enzymatic reactions or biosyntheses can be very rapid but the separation problem remains.

An elegant solution is the use of liquid chromatography (HPLC). It is necessary to use a stationary phase with high selectivity in order to differentiate between the components of the mixture that may be structurally very close(2). It is necessary to use a column with high efficiency in order to reduce the time for the separation(2). It is desirable to use water or a non-toxic buffer as the eluant so that the collected fraction containing the labelled product is immediately available for use *in vivo*. Finally it is desirable to have a large column capacity because the volume of solution applied to the column is typically 0.5—1.0ml, which volume is rather high compared with the usual HPLC column dimensions (~50 X 0.3cm).

^{11}C-labelled sugars and polyols have been prepared by photosynthesis (Table 1)(3,4) but the ^{11}C-products are mixtures of two or three labelled products with very similar structures. In fact the only significant difference between the various products $R(OH)_n$ is the stereochemical relationship of the hydroxyl groups. It was found that metal cations, $M^{+x}aq$, immobilised on a cation exchange resin (Aminex A5) formed a selective stationary phase for the separation of sugars and polyols with water as the eluant (5,6). The selectivity varied with the choice of $M^{+x}aq$. Cations such as Na^+—Rb^+ interacted only with adjacent pairs of *ax-eq* or *cis-cis* hydroxyl groups, a weak interaction (k′ low). Cations such as Ca^{+2}— Ba^{+2}, La^{+3} interacted with an *ax-eq-ax* or *cis-cis* sequence of three hydroxyls, and Cu^{+2} interacted with an *ax-...-ax* sequence of two α,γ-hydroxyls, both relatively strong effects, leading to high k′-values for polyols with such stereochemistry on these stationary phases.

Fig : 2- and 3-centre coordination of $R(OH)_n$ with metal cations.



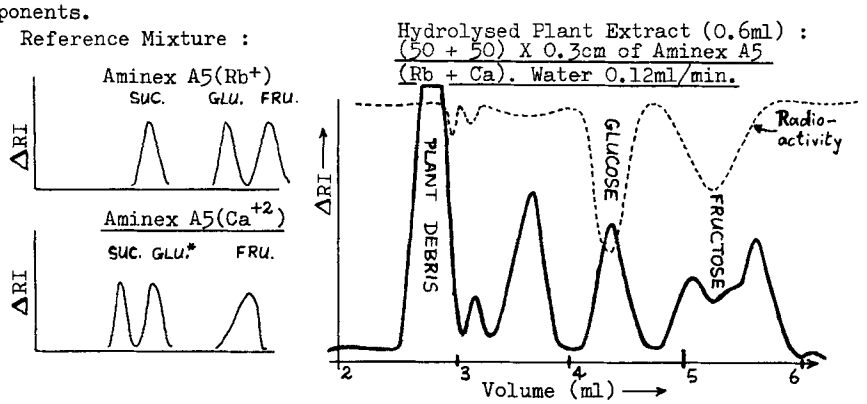
The ^{11}C-storage glycoside is extracted from the plant material(3,4,6) and hydrolysed at acid pH to liberate the two component C_6/C_3-polyols. Unfortunately in the limited time available it is not possible to guarantee complete hydrolysis and so it is necessary to devise an efficient LC

Table 1 : ^{11}C-labelled products produced by biosynthesis from $^{11}CO_2$.

Species	^{11}C-components to be separated	
	Storage Glycoside (I)	Component Polyols (II,III)
Fucus evesiculosus	Mannitol (free)	—
Girgartina stellata	Floridoside	Galactose, Glycerol
Polysiphonia lanosa	Mannoglyceric acid	Mannose, Glyceric acid
Land plants e.g. leguminosae	a glyceryl-glycoside Sucrose	Glucose, Fructose

separation of a 3-component mixture. Although it was possible to separate any 2-component test mixture by the correct choice of M^{+x}, it was not possible to separate the 3-component mixture of glycoside (1st peak, unwanted) and its component polyols (2nd, 3rd peaks, both required). The problem was solved by the combination of two columns in series as detailed below

(1) Separation of Sucrose (I), Glucose (II) and Fructose (III).
Aminex A5(Rb^+) gave a good separation of sucrose from the other two polyols. Aminex A5(Ca^{+2}) gave a good separation of fructose from the other two. A combination of the two columns optimised the separation of the three components.

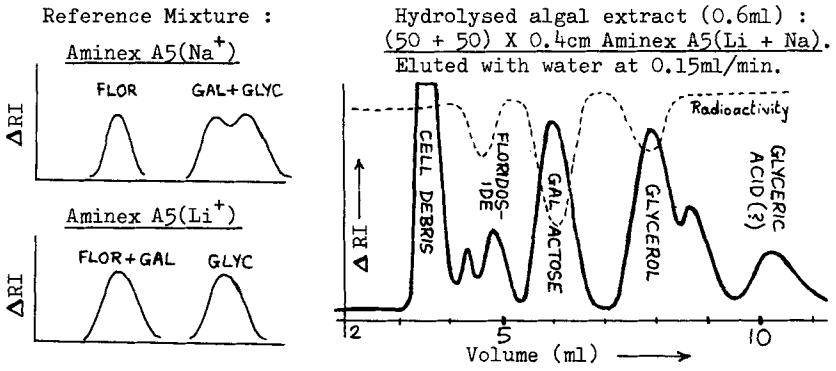


(2) Separation of Floridoside (I), Galactose (II) and Glycerol (III).
Aminex A5(Na^+) gave a good separation of floridoside from galactose, but galactose and glycerol were unresolved. Aminex A5(Li^+ or La^{+3}) gave a good separation of galactose and glycerol but floridoside and galactose were unresolved. A combination of two columns was mandatory and either of the above two combinations allowed the three polyols to be efficiently separated.

(3) Separation of Mannoglyceric acid (I), Mannose (II) and Glyceric acid (III).
Aminex A5(Na^+) (I,II) separated, (II,III) unresolved. Aminex A5(Li^+ or La^{+3}) (I,II) unresolved, (II,III) well separated. Therefore the systems of Example 2 above were used.

*The glucose peak is split into a doublet (due to the α/β-anomeric forms). The effect is not noticable except with low-volume injections (<200µl).

Separation of Floridoside, Galactose, Glycerol.



(4) Purification of Mannitol :
On Aminex A5(Ca^{+2}) mannitol had a relatively high k′-value (~ 2) and was well separated from other non-labelled impurities.

DISCUSSION :
I have demonstrated that HPLC has a unique role to play in the synthesis of short-lived labelled compounds. It is not possible to separate ^{11}C-glucose and fructose by classical LC or, in fact, any other method with sufficient rapidity. HPLC is essential and the systems described fulfil all the stated requirements to an acceptable degree.

It is quite possible to separate sucrose (if present), glucose, fructose on a single column, e.g. on Aminex A5(Na^{+}) so that the complexities described here are not obligatory. On the other hand, Floridoside (always present), galactose, glycerol cannot all be separated on any single column. In this case the dual-column system is mandatory, and stationary phase selectivity is more important than the column efficiency.

^{11}C-sugars probably will not be useful as radiopharmaceuticals because they are difficult to make and are extensively metabolised *in vivo*. ^{18}F-sugars are showing promise and the HPLC systems described might have some preparative or analytical application. Also of present or future interest are Y-labelled biogenic amines and antagonists. The analysis of such compounds and their extensive metabolites could be facilitated by the use of highly-selective stationary phases, especially Cu^{+2}-form cation exchange resin(7).

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Paper No.B12

AN APPROACH TO THE REMOTE AUTOMATED PRODUCTION AND SIMULTANEOUS LOADING OF MULTIPLE $^{81}Rb \rightarrow ^{81m}Kr$ GENERATORS FOR SHIPMENT TO REGIONAL HOSPITALS*

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One facet of our research effort is to determine optimum conditions (excitation functions, targetry, radiochemical parameters, etc. for cyclotrons having different beam and particle characteristics) and to develop automated methods for the production and on-line use of short-lived radionuclides. The $^{81}Rb \rightarrow ^{81m}Kr$ generator (1-5) is rapidly gaining acceptance as a result of the earlier efforts which concentrated on the concept and the definition of operational characteristics and clinical utility of the generator. Beam and technician time restrictions coupled with requests for early AM delivery of generators at 1 to 4 institutions necessitated the adoption and improvement (where possible) in the presently described (1-5) generator technology and loading of the generators without operator intervention.

A target of natural krypton gas (9.5 atm) was irradiated with 32-MeV protons to encompass both the $^{82}Kr(p,2n)^{81}Rb$ and $^{83}Kr(p,3n)^{81}Rb$ reactions. The ^{81}Rb thick target production yield was 10.9 ± 0.8 mCi/μAh. The relative EOB radionuclidic composition of ^{81}Rb, ^{82m}Rb, ^{79}Rb, ^{86}Rb, ^{84}Rb, and ^{83}Rb on the generator is 1.0, 1.2, 0.2, 0.06, 0.008, and 0.007, respectively. During the first three hours post-irradiation attention must be directed to discarding the ^{79}Kr ($t_{½}$ = 34.9 h) resulting from the decay of ^{79}Rb ($t_{½}$ = 23 m). The other Rb isotopes interfere only in the context of requiring additional shielding.

The Inconnel-600 target was remotely positioned into the beam or retracted into Pb shielding. At EOB a network of remotely operated solenoids vents the Kr and facilitates a single pass of 0.75 liter of H_2O through the target to effect the dissolution and collection of ∿95% of the Rb activity in an external reservoir 5 m from the target. The aqueous solution containing the ^{81}Rb was purged through zirconium phosphate or Dowex-50W-X4 columns at a rate of ∿30 ml min^{-1} with an overall radiochemical extraction yield of ∿90%. The bromine isotopes produced via (p,2pn) reactions are not retained on the generator. The generators (1 to 4) are simultaneously and uniformly loaded in parallel. The loading process, including a 25 ml H_2O rinse, a 10 sec purge with N_2, and manual removal of the generators from the system for shipment requires <10 min. The generators are typically loaded with >25 mCi of ^{81}Rb.

Preliminary experiments with low beam currents indicate that bromoform can be used as a target material in a dynamic target with the $^{79}Br(\alpha,2n)^{81}Rb$ reaction. The ^{81}Rb was extracted by $ZrPO_4$ with >90% efficiency independent of flowrate (4-35 ml min^{-1}). Thereby the target system may permit the generator to be placed in the flowing system throughout the irradiation and be fully loaded at EOB. The extraction of ^{81}Rb from $CHBr_3$ with Dowex-50W was typically ∿60%. This system may be useful at cyclotrons not having high energy protons available for ^{81}Rb production via the Kr(p,xn) reaction.

The elution of ^{81m}Kr from the generator was examined under a variety of elution conditons. In one study the ^{81}Rb was absorbed on $ZrPO_4$ manufactured by BioRad, Applied Science, and ICN, and gave ^{81m}Kr elution yields of 82, 58, and 36%, respectively. A scanning electron microscope was used to

examine the gross surface characteristics of the zirconium phosphate in an attempt to interpret these results.

*This research was carried out at Brookhaven National Laboratory under contract with the U.S. Department of Energy and supported by its Division of Basic Energy Sciences.

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(5) J. C. Clark, R. D. Finn, J. A. Campbell, L. Bolomey and A. J. Gilson, 9th Intern. Hot Atom Symp., Virginia Polytechnic Institute and State University, Blacksburg, VA, Sept. 18-23, 1977. Abstract.

Paper No. B13

SPALLATION YIELDS OF Fe-52, Cu-67, Ga-67, AND Tl-201 FROM REACTIONS OF 800-MeV PROTONS WITH Ni, As, Pb, AND Bi TARGETS. P. M. Grant, H. A. O'Brien, Jr., B. P. Bayhurst, J. S. Gilmore, R. J. Prestwood, R. E. Whipple, and P. M. Wanek, Los Alamos Scientific Laboratory, Los Alamos, NM.

The principal mechanism for synthesis of radioactive isotopes at LAMPF is nuclear spallation induced by the medium-energy proton beam in thick targets. The experimental determination of thin-target cross sections and cumulative yields is an important segment of our program, as the data derived from these studies provide the basis for a comparative evaluation of spallation techniques with other methods of synthesis.

Thin targets of Ni, As, Pb, and Bi were irradiated with 800-MeV protons to an integrated intensity of 1 μA-hr, together with Al monitor foils. Beam intensity measurements were based on the yields and known cross sections of the following activities in Al: Na-24, 10.75 mb; Na-22, 16.3 mb; and Be-7, 6.4 mb. Radiochemical separations were performed on each target to recover elemental fractions of interest. Analyses of the various gamma-photon spectra were performed using the GAMANAL code.

The measured cross sections are reported as follows: target, product, chemical yield, and cross section. The following are the results obtained: Ni, Fe-52, 95.92%, 1.54±0.13 mb; As, Cu-67, 43.93%, 1.51±0.13 mb; As, Ga-67, 12.37%, 28.4±2.4 mb; Pb, Tl-201, 49.34%, 54.7±5.9 mb; and Bi, Tl-201, 22.76%, 58.5±6.7 mb. Calculated production yields based on these cross sections indicate that half-saturation end-of-bombardment yields of each of these nulides are in the range of 1 to 100 Ci for the LAMPF irradiation conditions; however, isotopic interferences in the case of Tl-201 may eliminate spallation reactions as a viable means of production.

Projected Yields of Radionuclides From LAMPF

(Assume I = 300 μA; Target Thickness = 2.3 cm)

Target	Product Nuclide ($T_{1/2}$)	EOB Yield at 0.5 Sat'n Factor
Ni	^{52}Fe (8.3 h)	8.3 Ci
As	^{67}Cu (61.7 h)	4.1 Ci
As	^{67}Ga (78.3 h)	76 Ci
Pb	^{201}Tl (73 h)	106 Ci
Bi	^{201}Tl (73 h)	97 Ci

800-MeV p^+ Cross Sections

Target	Chemical Yield	Isotope	σ (mb)	Type
Ni	0.9592	^{52}Fe	1.54 ± 0.13	C.Y.
		^{59}Fe	0.306 ± 0.048	C.Y.
	0.8577	^{43}Sc	7.02 ± 0.83	C.Y.
		^{44m}Sc	7.34 ± 0.65	I.Y.
		^{46}Sc	5.69 ± 0.49	I.Y.
		^{47}Sc	1.49 ± 0.13	I.Y.+ C.Y.
		^{48}Sc	0.249 ± 0.022	I.Y.
As	0.4393	^{61}Cu	7.01 ± 0.68	C.Y.
		^{64}Cu	15.4 ± 1.6	I.Y.
		^{67}Cu	1.51 ± 0.13	C.Y.
	0.1237	^{66}Ga	11.6 ± 1.0	C.Y.
		^{67}Ga	28.4 ± 2.4	C.Y.
		^{72}Ga	3.16 ± 0.27	I.Y.+ C.Y.
Bi	0.2276	^{200}Tl	83.8 ± 8.8	I.Y.+ C.Y.
		^{201}Tl	58.5 ± 6.7	C.Y.
		^{202}Tl	6.56 ± 0.69	C.Y.
Pb	0.4934	^{200}Tl	67.8 ± 7.1	I.Y.+ C.Y.
		^{201}Tl	54.7 ± 5.9	C.Y.
		^{202}Tl	18.2 ± 1.9	C.Y.

I.Y. = Independent Yield
C.Y. = Cumulative Yield

Paper No.B15

^{18}F PRODUCTION METHODS

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Fluorine-18 has been widely used for the detection of bone metastasis. Efforts are currently underway in many laboratories to extend the usefulness of F-18 for labelling - incorporation of F-18 into organic molecules of potential biological interest. The usefulness of F-18 as a tracer and label can be enhanced through improved methods of preparation of F-18 and a better delineation of its properties. The purpose of our investigation was to find an optimal and economical method for routine production of carrier-free F-18 either in a water solution or in the anhydrous form. This work has been done on the Heidelberg compact cyclotron providing 12 MeV deuterons and 28 MeV ^{3}He particles.

Among the nuclear reactions by which F-18 may be induced, the following three reactions are the most useful for F-18 production in high yields.

	Nucl.Reaction	Target material used
(1)	$^{20}Ne\ (d,\alpha)\ ^{18}F$	Ne gas
(2)	$^{20}Ne\ (^{3}He,\alpha p)\ ^{18}F$	
	$^{20}Ne(^{3}He,n)\ ^{18}Ne \xrightarrow{1,6\ s}\ ^{18}F$	Ne gas
(3)	$^{16}O\ (^{3}He,p)\ ^{18}F$	O_2 gas; H_2O
	$^{16}O\ (^{3}He,n)\ ^{18}Ne \xrightarrow{1.6\ s}\ ^{18}F$	Al_2O_3; Ta_2O_5

F-18 production rates, radioactive and inactive impurities and the chemical state of F-18 have been studied for the reactions mentioned above. Experimental parameters such as target fabrication are discussed. The fully automatically operated target system, using reaction (1), has been constructed and our six years' experience with routine production of F-18 in aqueous solution is described.

Experimental parameters of the internal target system using reaction (3) with water in the closed loop as the target material have been developed. F-18 production rates and recovery procedures are discussed.

The second part of our investigation describes recoil labelling experiments with F-18 and recovery of F-18 via various scavenging techniques during irradiation, particulary under anhydrous

conditions. For these studies all three nuclear reactions have been used. As target material for reaction (3) we used oxides of Al and Ta for production of Ne-18 which can be swept from the target with He. Different experimental conditions involving the use of liners from various materials was investigated. Different carrier gases, including H_2, NO, CH_4, COF_2 etc. have been used to produce other possible fluorinating agents labelled with 18-F. Our preliminary results provide encouragement for further development of these techniques.

Paper No.B16

^{3}He BOMBARDMENT OF MANGANESE FOR THE PRODUCTION OF ^{55}Co

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Among the radioactive cobalt isotopes, the neutron-deficient ^{55}Co, ^{56}Co, ^{57}Co and ^{58}Co can be used for medical applications. First, the internal radiation dose to the liver was calculated, assuming uniform distribution of 1μCi of each radionuclide in that organ, by the MIRD method[1] based upon published decay data [2]. The results, shown in Table I, indicate that ^{55}Co appears to be the most promising for medical use, and so the authors next investigated suitable conditions for the production of ^{55}Co by bombarding manganese targets with α- or ^{3}He- particles of up to 40 MeV from the IPCR cyclotron. The energy of gamma rays used for the measurements of activity are listed in Table II, together with threshold energies or Q values. Although the most abundant γ-ray of ^{55}Co is 930 keV, the 17% abundant 477 keV γ-ray was used to avoid interference from the 935 keV γ-ray of the ^{52}Mn by-product. Excitation functions for these nuclides have been constructed.

^{3}He was found superior to α-bombardment for ^{55}Co production; the (^{3}He,3n) reaction being understood to be the most important. The cross-section for this reaction has a maximum value of 44mb at 27 MeV. Thick target yield curves for ^{55}Co, ^{56}Co and ^{57}Co were constructed, from which the optimum bombardment conditions shown in Table III were derived.

The optimum conditions for production of ^{55}Co were bombardment of 91.7 mg cm^{-2} manganese targets by 37 MeV ^{3}He particles. The yield of ^{55}Co was then 119 μCi(μAh)$^{-1}$ with slight contamination by ^{57}Co and ^{56}Co. However, the activity due to formation of ^{56}Co is no more than 0.99% in the ^{55}Co product at EOB, and the formation of ^{57}Co is not significant.

(1) J. Nuclear Med. MIRD Method Pamphlet Supplement 4,5,6 (1972)
(2) Lederer C.M., Hollander J.M. and Perlman I., Table of Isotopes 6th Edition, 1967, and more recent Nuclear Data Sheets.

TABLE I : Internal radiation dose, uniform source in liver

	^{55}Co	^{56}Co	^{57}Co	^{58}Co	^{60}Co
*	0.02	3.79	0.468	0.516	37.12

* Internal radiation dose (rad $\mu Ci^{-1}h^{-1}$)

TABLE II : Characteristic gamma rays

Reaction	Threshold energy (MeV)	Gamma ray energy (keV)
$^{55}Mo(^{3}He, 3n)^{55}Co$	13.67	477 (16%)
$(^{3}He, 2n)^{56}Co$	3.02	847 (100%)
$(^{3}He, n)^{57}Co$	8.50 (Q-value)	122 (87%)
$(^{3}He,\alpha\ 2n)^{52}Co$	10.64	744 (87%)
$(^{3}He, \alpha)^{54}Mn$	13.84	835 (100%)
$(^{3}He,2p2n)^{54}Mn$	17.94	
$(^{3}He, 2pn)^{56}Mn$	0.45	1811 (29%)

TABLE III : The optimum irradiation condition

Energy (^{3}He) (MeV)	Target Thickness (mg cm^{-2})	^{55}Co ($\mu Ci\ \mu Ah^{-1}h^{-1}$)	^{56}Co (%)	I.R.D. (rad $\mu Ci^{-1}h^{-1}$)
23 - 37	91.7 (127 μ)	119	0.99	0.057
15 - 40	206 (286 μ)	229	1.75	0.195

Paper No.B17

EXCITATION FUNCTIONS AND PRODUCTION YIELDS FOR SOME RADIONUCLIDES OF IRON FROM THE ALPHA PARTICLE BOMBARDMENT OF NATURAL CHROMIUM

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In the recent compilations of excitation functions (1) it appeared that little attention has been paid to the ^{50}Cr $(\alpha,2n)$ ^{52}Fe reaction. In view of the as yet untapped potential of ^{52}Fe in nuclear medicine, the authors have attempted to measure the cross-sections for this reaction in order to show the optimum conditions for its production from an alpha bombardment. A "spinning wheel" type target (2) was used to measure the cross-sections for this and the corresponding alpha reactions, in the energy range of the threshold to a maximum of 28 MeV.

(1) a) Burrows T.W. and Burt J.S. BNL Report No. NCS-50640.
b) McGowan F.K. and Milner W.T. Atomic/Nuclear Data Tables. Academic Press N.Y.
(2) Watson I.A., Waters S.L., Bewley D.K. and Silvester D.J. Nuclear Instruments and Meths. 106, 231-235 (1973).

Paper No.B18

YIELD OF ^{73}Se AND ^{77}Br FOR VARIOUS REACTIONS AND THEIR CARRIER- AND/OR SALT-FREE SEPARATION

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Yield curves for ^{73}Se and ^{77}Br for various reactions were measured and compared. Target substances suitable for their production were selected, and their carrier- and salt-free separation was studied.

1. Excitation Curves and Thick Target Yields.
The excitation curves for the following reactions were measured up to proton, ^{3}He and α-particle energies of 52, 40 and 40 MeV, respectively, together with those for by-product formation reactions: (1) $^{75}As(p,3n)^{73}Se$, (2) $Ge(^{3}He,xn)^{73}Se$, (3) $Ge(\alpha,xn)^{73}Se$, (4) $Se(p,xn)^{77}Br$, (5) $^{79}Br(p,3n)^{77}Kr \longrightarrow {}^{77}Br$, and (6) $^{75}As(\alpha,2n)^{77}Br$. The results, expressed for the target of natural isotopic composition, are shown in the last page of our abstract. The thick target yields are then calculated; they are gived in Table 1. These values and related data reported by other workers (1~3) demonstrate clearly the superiority of the proton reactions for the present production purposes.

Table 1. Thick Target Saturation Activity (10^{9} dps/μA). (Taget: Element of Natural Isotopic Composition.)

Energy (MeV)	20	25	30	40	50
As + p → ^{73}Se	0	< 0.1	5.5	19	28
As + p → ^{72}Se	0	0	0	0.65	5.6
Ge + ^{3}He → ^{73}Se	0.11	0.20	0.30	0.50	
Ge + ^{3}He → ^{72}Se	< 0.01	0.065	0.25	0.33	
Ge + α → ^{73}Se	0.15	0.22	0.26	0.37	
Ge + α → ^{72}Se	0	0.015	0.060	0.19	
Se + p → ^{77}Br	3.4	6.6	8.9	12	20
Se + p → ^{76}Br	1.3	2.8	4.4	9.6	14
Se + p → ^{82}Br	1.1	1.1	1.1		
Br + p → $^{77}Kr \rightarrow {}^{77}Br$	0	0.2	2.2	9.5	14
As + α → ^{77}Br	0.3	0.9	1.7	2.6	
As + α → ^{76}Br	0	0	0.03	1.1	

2. Target Substances.
Various target substances presumably suitable were selected, and some of them examined experimentally. The choice given in Table 2 can be regarded as convenient.

3. Chemical Separation.
We tried various chemical separations of ^{73}Se and ^{77}Br, and found the following processes suitable.

Table 2. Convenient Target Substances.

Target Element	Beam Density ($\mu A/cm^2$)	Substance	Element Content(%)	M.P. (°C)
Ge	Always	Ge Metal	100	959
As	< 2	As_2O_3	76	315
	2~5	AlAs	74	>1500
	> 5	$NaAsO_2$-B_2O_3	(40)	(Glassy)
Se	< 1	SeO_2	71	Sub. 317
	1~3	Al_2Se_3	81	>1000
	> 3	Na_2SeO_3-B_2O_3	(40)	(Glassy)
Br	Always	NaBr	78	755

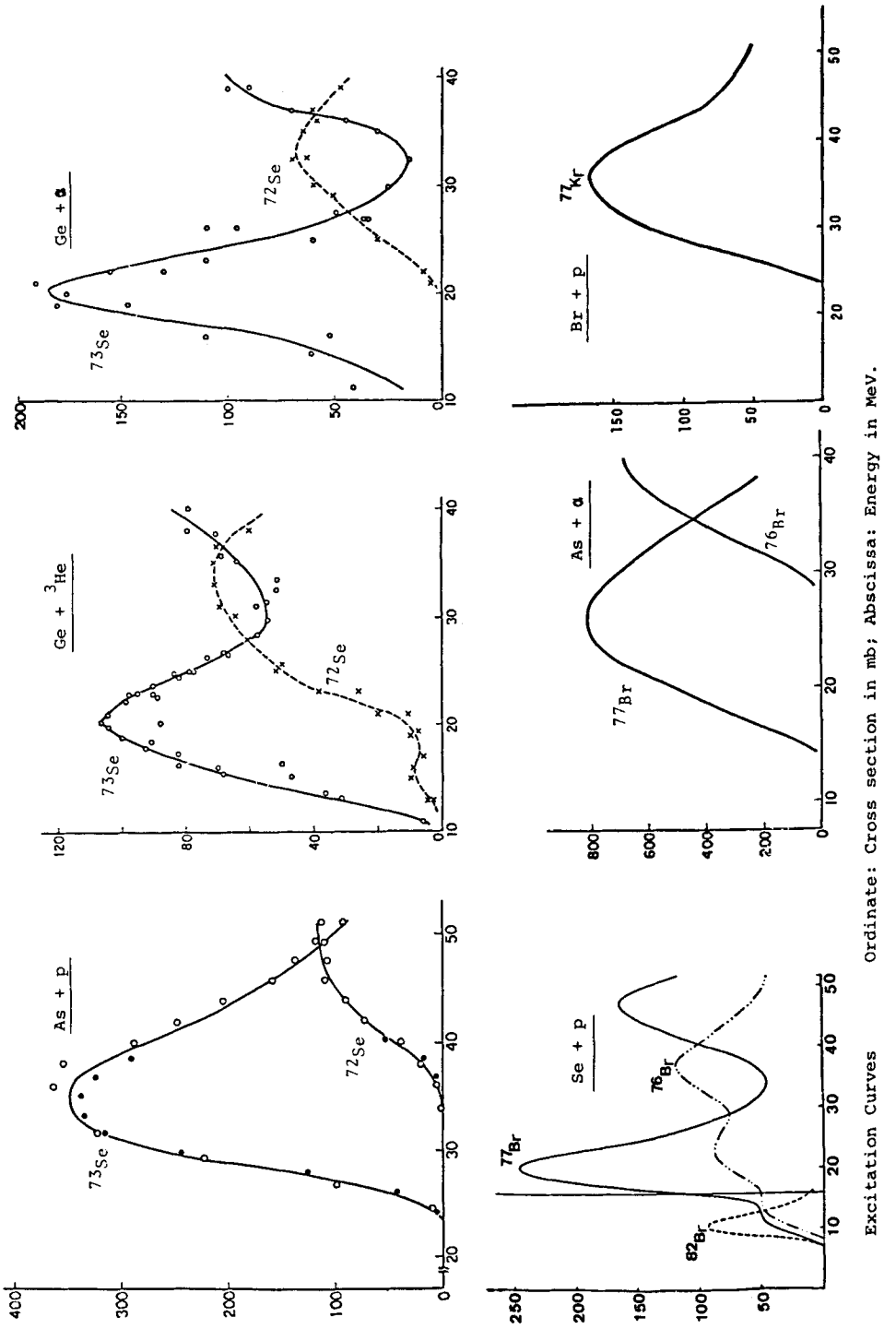
(a) ^{73}Se in red selenium with 70 μg carrier. 1) Target dissolution in acid (HCl for As_2O_3 and $NaAsO_2$-B_2O_3, HCl-H_2O_2 for AlAs, and HF-HNO_3 for Ge) containing 0.1 mg H_2SeO_3. 2) Reduction with hydrazine to precipitate red selenium. 3) Extraction of the selenium into CS_2. 4) Evaporation of the CS_2.

(b) ^{73}Se in carrier- and salt-free Se(IV). 1) Target dissolution in the acid containing 50 mg Na_2TeO_3. 2) Reduction with hydrazine to give ^{73}Se-Te coprecipitate, and its collection and washing. 3) Dissolution of ^{73}Se-Te in HBr-Br_2, and anion exchange separation with a Br-form resin column (Se is in the effluent, and Te in the column.). 4) Anion exchange treatment with an OH-form resin column (First comes out As, then Se is eluted, leaving Br in the column.). 5) Cation exchange treatment of the Se fraction.

(c) ^{77}Br in carrier- and salt-free Br^-. 1) Target dissolution in acid (H_2SO_4-H_2O_2 for AlAs and H_2SO_4 for the others), and treatment of the solution with $KMnO_4$ and with H_2O_2. 2) Distillation of ^{77}Br. 3) Successive treatment of the distillate with Fe powder, $BaCO_3$ and H-form cation exchange resin.

The separated ^{77}Br solution was analysed for As and Br by neutron activation and for Se by colorimetry with bismuthiol. It was found that the Fe powder and $BaCO_3$ treatment is very effective for the removal of trace quantities of As and Se but that care should be taken thoughout the process to avoid contamination with non-radioactive bromine.

(1) Stöcklin G., Int. J. appl. Radiat. Isotopes, 28, 131 (1977). Also the related references in it.
(2) Diksic M., Galinier J.L., Marshal H., and Yaffe L., ibid, 28, 885 (1977)
(3) Guillaum M., Lambrecht R.M., and Wolf A.P., "Selenium-73," Private communication.



Excitation Curves Ordinate: Cross section in mb; Abscissa: Energy in MeV.

Paper No.B19

PRODUCTION OF ^{77}Br AT A MEDICAL COMPACT CYCLOTRON

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^{77}Br ($T_{1/2}$ = 56 h) is produced via the nuclear reaction $^{75}As(\alpha,2n)^{77}Br$ as proposed by the Hammersmith group (1). In order to obtain high yields with a beam of relatively small diameter and a high current density the following technical improvements have been made:
i) the target material consists of copper arsenide (35-40% As, 120 mg/cm^2) which provides good thermal conductivity to the water cooled copper backing,
ii) the chemical separation is carried out by dry distillation analogous to that applied for ^{123}I separation from a ^{124}Te or $^{124}TeO_2$ target (for review cf. (2)). ^{77}Br is distilled in a helium stream at a temperature of 1100°C from the target. The arsenic from copper arsenide target and the ^{67}Ga produced by activation of copper also evaporate from the target under these conditions but are condensed at the cooler regions of the apparatus before the bromine trap. If trapped in alkaline solution, the product is obtained as carrier-free ^{77}Br-bromide with radionuclidic purity of > 99%.

(1) Helus F., Radiochem. Radioanal. Letters, 3, 45 (1970).
(2) Stöcklin G., Int. J. appl. Radiat. Isotopes, 28, 131 (1977).

Paper No.B20

PRODUCTION OF CARRIER-FREE $^{76,77}Br$

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^{76}Br and ^{77}Br produced by the decay of ^{76}Kr and ^{77}Kr seem to offer attractive properties for labeling organic molecules for biomedical use and for hot atom chemistry. We produce $^{76,77}Kr$ by proton irradiation of KBr pellets and isolate it by melting these pellets and trapping the krypton isotopes in liquid nitrogen cooled vessels with a yield of 70-90%.

An excitation curve is measured for the $^{79}Br(p,3n)^{77}Kr$ and $^{79}Br(p,4n)^{76}Kr$ reaction up to proton energies of 65 MeV. Earlier experiments showed thick target yields of 59 mCi/μAhr for ^{77}Kr and 1.1 mCi/μAhr for ^{76}Kr, when protons of 52 MeV were used.

An upper limit for the total mass quantity of bromine released directly from the KBr pellet was determined by the ^{77}Br and ^{80}Br (produced by the $^{79}Br(n,\gamma)^{80}Br$ reaction) activities found in the trapping vessels.

Excitation labeling experiments with $^{76,77}Br$ were performed with albumine, tyrosine and some other compounds.

Paper No.B21

LARGE-SCALE CYCLOTRON PRODUCTION OF HIGH-PURITY CARRIER-FREE IODINE-123

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The Crocker Nuclear Laboratory's Radioisotope Production program (1) which has been conducted since the early 1970's has developed a simple, reliable, safe, and highly efficient method for the production of high-purity ^{123}I.

The method is based upon the utilization of the $^{127}I(p,5n)^{123}Xe \rightarrow ^{123}I$ reaction (2). The target material consists of molten NaI irradiated with a 65.6 MeV external proton beam. The target material is maintained in the liquid state during the bombardment, providing reflux as required, with a continual stream of He passing through the target area. By maintaining the target temperature in the 650 $\pm$ 30 °C range, by a combination of beam intensity (μA) and an external adjustable flow of pre-cooled He, the ^{123}Xe radioactivity can be efficiently removed from the molten NaI target material. The He flow carries the ^{123}Xe radioactivity into a cold trap (-196°C) and then is purified by pumping it into the final decay vessel.

In order to maintain the radionuclidic purity of ^{123}I (>99.6% at TOC), the molten NaI target thickness in the radiation beam is predetermined to provide a certain reduction in the beam energy. However, it was observed that the target vessel swells slowly under beam conditions,therefore requiring special precautions to minimize target thickness variations. Experimental evidence confirming the effect of internal mechanical pressure (due to the NaI density variations $\pm$ 25%) on target swelling will be discussed. Several target designs were tested under high-beam intensity conditions. A target loading and a target bombarding procedure was developed and found to be highly effective in minimizing the target swelling. These procedures are presently being utilized in our routine biweekly production of ^{123}I (Table 1).

^{123}I is available as a ^{123}I-NaI or as a ^{123}I-ICl aqueous solutions in specific concentrations up to 150 mCi/ml and 200 mCi/ml, respectively. The radiochemical purity of both preparations exceed 98 %. Chemical stability measurements indicated no variations in solutions stored without special precautions for up to 60 hr from end of processing. ^{123}I sodium o-iodohippurate is also available biweekly in isotonic solutions with >98 % radiochemical purity. Chemical stability on the ^{123}I-Na o-IH solution showed no measurable variations for up to 60 hr from end of processing. The ^{123}I-Na o-IH is prepared by an isotope-exchange reaction (3).

A comparison of the high-purity ^{123}I with the ^{123}I made directly by the $^{124}Te(p,2n)$ and $^{122}Te(d,n)$ reactions will also be discussed. The radiation dose estimates to humans from administration of 100 μCi of the different purity preparations is given in Table 2. A further analysis of the radionuclidic purities, thyroid doses, and useful photon yields at different TOC will also be discussed (Table 3).

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(2) Wilkins S.R., Shimose S.T., Hines H.H., Jungerman J.A., Hegedeus F., and De Nardo G.L., Int.J.Appl.Radiat.Isotop. 26, 279 (1975)
(3) Zielinski F.W., Holly F.E., Robinson G.D., Bennett L.R., Radiology, 125, 753 (1977).

TABLE 1. Summary of U.C. Davis ^{123}I Target's Performance

Target (Runs)	T°C	Beam Intensity (μA) Average	Maximum	Yields (mCi/μAhr) Average	Maximum	(mCi) Max. Yield (1 hr run)
11mm (46)	640±20	18±1	23±1	12±2	15±1	270
13mm (68)	650±20	16±1	20±1	15±2	18±1	345
15mm (136)	680±20	15±1	18±2	18±3	21±2	441

TABLE 2. Radiation Dose (mrads) estimates to humans from administration of 100μCi (at TOC) of ^{123}I sodium iodide.[1]

Target Organ	^{123}I (p,5n) Thyroid Uptake (%)			^{123}I (p,2n) Thyroid Uptake (%)			^{123}I (d,n) Thyroid Uptake (%)		
	(5)	(15)	(25)	(5)	(15)	(25)	(5)	(15)	(25)
Liver	2.9	2.9	2.8	4.5	4.9	5.3	4.6	4.8	5.2
Ovaries	3.6	3.4	3.1	5.1	4.8	4.4	5.1	4.8	4.4
Red Marrow	3.0	3.0	3.0	4.2	4.6	5.1	4.1	4.4	4.7
Stomach Wall	25.0	23.0	21.0	35.6	32.7	29.8	36.7	33.7	30.7
Testes	1.3	1.2	1.2	2.1	2.0	2.0	2.2	2.0	2.0
Thyroid	295.0	927.0	1611	1127	3359	5679	1052	3196	5376
Total Body	2.5	2.8	3.1	4.2	5.5	6.9	4.0	5.0	6.1

Note 1. These calculations are based on MIRD. Dose Estimates Report No. 5 J. Nucl. Med., Vol. 16,(9)857(1975). Dose calculations do not include ^{24}Na which is present in the (p,2n) and (d,n) products.

TABLE 3. Comparison of (p,5n), (p,2n) and (d,n) produced ^{123}I for a nominal 100μCi (at TOC) of Sodium Iodide at Expiration Dates

Products	(p,5n)			(p,2n)*			(d,n)*		
Time After Calibration	36 hr			48 hr			36 hr		
Total Radioactivity (μCi)	15.26			8.17			14.48		
Radionuclidic Purity (%)	97.49 (^{123}I)			95.43 (^{123}I)			78.38 (^{123}I)		
	2.51 (^{125}I)			4.57 (^{125}I)			21.10 (^{124}I)		
							0.52 (^{24}Na)		
							82.18 (^{123}I)		
							4.93 (^{124}I)		
							5.84 (^{126}I)		
							2.46 (^{130}I)		
							4.04 (^{131}I)		
							0.55 (^{24}Na)		
Thyroid Dose (MRADS) (%)	(5)	(15)	(25)	(5)	(15)	(25)	(5)	(15)	(25)
	92	219	509	74	123	415	737	2176	3659
							706	2128	3539
Useful Photon-Yield (%)	98.3			98.1			75.7		75.3

*Data obtained from commercial manufacturer's product specifications.

Paper No.B22

RUTHENIUM-97 : HALF-LIFE, YIELD MEASUREMENTS AND ISOLATION FROM MOLYBDENUM TARGETS.

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Comar and Crouzel (1), Wenzel et al. (2), and earlier Subramanian and McAfee (3), have all drawn attention to the characteristics of ^{97}Ru which may make it an attractive label for radiopharmaceuticals. For our studies, ^{97}Ru was made, together with shorter-lived ^{94}Ru and ^{95}Ru, traces of 39d ^{103}Ru, and large amounts of Tc radionuclides, by bombarding natural molybdenum targets with α-particles of 21.8 MeV or 30 MeV. Targets consisted of Mo foils, MoO_3 pellets or Mo sprayed on stainless steel backing plates.

Recovery of ^{97}Ru was achieved in two ways; one using "wet" chemistry, and the second a differential sublimation procedure. For the former, foil or sprayed Mo targets were simply dissolved by immersion in warm 10 v/v H_2O_2. The resulting solution was brought to pH 14 and Tc radionuclides were extracted with MEK, then ^{97}Ru was extracted into pyridine following oxidation to Ru VIII by NaOCl (4). Separation by differential sublimation was achieved using a two-oven system originally developed as a ^{99m}Tc generator (5) and shown schematically in Fig.1.

The half-life of ^{97}Ru was determined using a γ-spectrometer to follow the decay of the 215-keV photopeak over a period of 340 h. Computer analysis of the data gave a half-life value of 68.13 ±0.14 h.

Experimental thick-target yields are shown in Table 1, and are compared with the theoretical yields obtainable from pure ^{95}Mo targets, based on data measured by Graf and Münzel (6).

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(3) Subramanian G. and McAfee J.G., J. Nucl. Med. 11, 365 (1970)
(4) Wyatt E.I., in "Recent Radiochemical Separation Procedures for As, At, Be, Mg, Ni, Ru and Se" (Ed. K.V. Marsh) NAS-NS-3059 Washington DC, USA (1974) p52.
(5) Helus F. and Maier-Borst W., J. Labelled Compds. and Radiopharmaceuticals, 13, 190 (1977)
(6) Graf H.P. and Münzel H., J. Inorg. Nucl. Chem. 36, 3647 (1974)

* On temporary leave of absence from MRC Cyclotron Unit, Hammersmith Hospital, London W12 OHS, U.K.

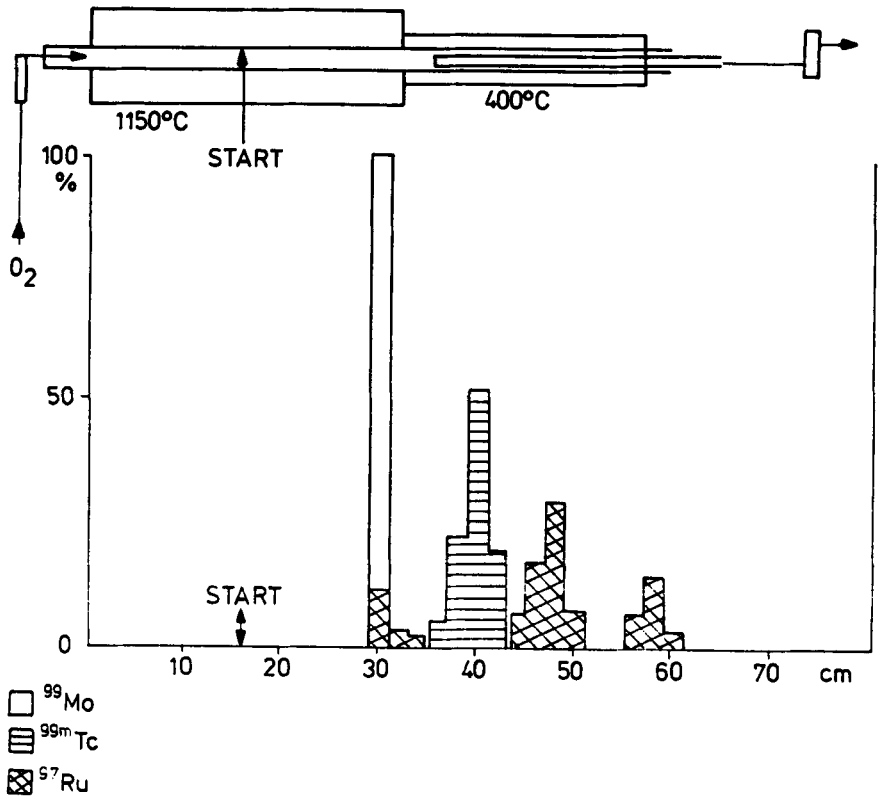
TABLE 1.

THICK-TARGET YIELDS OF ^{97}Ru

Incident α-particle energy	22 MeV	30 MeV
Yield of ^{97}Ru:-	$\mu Ci(\mu Ah)^{-1}$	$\mu Ci(\mu Ah)^{-1}$
(i) Calculated from $^{95}Mo(\alpha,2n)$ reaction[a]	8.4	68
(ii) Comar and Crouzel[b]	19	-
(iii) This work[b]	26.8	75.3

(a) Assuming bombardment at a current of 1 μA for 1 hour.
(b) Conditions: See text of full paper.

Figure 1.



Schematic view of the experiment set up and distribution of Ru, Mo and Tc radionuclides after separation

Paper No.B23

BIOCHEMISTRY AND ORGAN DISTRIBUTION OF METALLOCENE DERIVATIVES LABELLED WITH ^{103}Ru

Martin Wenzel, J. Andrew Taylor, Michael Schneider and Jürgen Macha, Pharmazeutisches Institut, Freie Universität Berlin (Germany).

Radioactive metallocene derivatives can be synthesized by an exchange reaction between the inactive ferrocen-derivative and $RuCl_3$ labelled with the γ-emitter ^{103}Ru or $^{59}FeCl_3$ (1).

The ^{103}Ru labelled ruthenocen-derivatives (I) were injected to mice and rats and their biochemical metabolism and organ-distribution were studied.

Results:

By comparison between ferrocene and ruthenocene or acetyl-ferrocene and acetyl-ruthenocene it could be demonstrated that the ruthenocene is more stable than the ferrocene ring system (2,3). The organ distribution of the ^{103}Ru labelled ruthenocene derivatives depends on the side chain added to the ring system (5).

For example carboxyl and sugar derivatives showed a high affinity for the kidney (2), acetyl-ruthenocene for adrenals (4). Some lipophilic ruthenocene-derivatives had tumor/muscle ratios up to 6:1.

(1) Langheim D., Wenzel M. and Nipper E., Chem.Ber.108, 146-154 (1975).
(2) Wenzel M., Nipper E. and Klose W., J.nucl.Med. 18, 367-372 (1977).
(3) Taylor A.J. and Wenzel M., Naturwissenschaften 64, 273 (1977).
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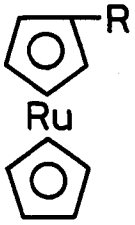

Paper No.B24

CYCLOTRON PRODUCTION OF CARRIER-FREE RHODIUM-101m, TARGET CHEMISTRY AND BIOLOGICAL RESEARCH.

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The investigation of rhodium isotopes and their potential application in nuclear medicine has been prompted by the recognition of the excellent physical properties of ^{101m}Rh and by the anti-neoplastic properties shown by some Rh-metal complexes (1). It decays with a 4.26 day half-life by electron capture (93%) and isomeric transition (7%), with the emission of 307 keV (88%) and 545 keV (6%) gamma-rays.

Several methods have been proposed for the production of ^{101m}Rh (2,3). At U.C. Davis, the reaction: $Pd(p,xn)^{101}Ag(10.8m) \rightarrow {}^{101}Pd(8.3hr) \rightarrow {}^{101m}Rh$ (4.26d.) is utilized to produce carrier-free ^{101m}Rh by irradiating a natural Pd target with 65.6 MeV protons. The thick-target yields (μCi/μAhr) for ^{101}Pd and ^{101m}Rh (direct and indirect reaction channels) (Table I) were measured for proton energies from 40 to 65 MeV (Figure 1). The yield of ^{101m}Rh was measured as 0.9 mCi/μAhr at 33.7 hours EOB, when a 23 MeV thick natural Pd target was irradiated with a 63 MeV incident energy proton beam.

Several chemical procedures were investigated to purify the ^{101m}Rh from the target material and from the proton-induced Pd and Ag radioisotopes. A procedure which has proven to be simple, reliable and suited for remote manipulations is described. After an in-growth period of 30-32 hours, the Pd target is dissolved in aqua regia, evaporated to dryness and redissolved in 2N HCl. The Pd (∿430mg) is precipitated with dimethylglyoxime. After filtration the Ag radioactivity induced in the target is precipitated as AgCl (K_{sp} 1.78×10^{-10}) by addition of $AgNO_3$ 0.1N solution. In the filtrate, a Rh-Pd final chemical separation is accomplished by ion-exchange chromatography by taking advantage of the differences in their complex forming behavior. In the 2N HCl solution, anionic chloro-complexes of Rh and Pd and cationic aquo-chloro complexes of Rh are formed. The solution is loaded onto a Dowex 1x8 anion-exchange column where the anionic complexes are retained. The anionic Rh-chloro complexes are eluted with 4N HCl with better than 90% efficiency, prior to Pd breakthrough. The solution containing the Rh is then evaporated to dryness and finally dissolved with 0.01N HCl. The final solution contains 99.5% ^{101m}Rh with $^{100}Rh(21h)$ being the only detectable radionuclidic impurity.

As an initial investigation of the potential of ^{101m}Rh for use in nuclear medicine, $RhCl_3$ and $Rh(AcO)_3$ were injected into tumor bearing mice and rabbits to measure organ distribution (Table 2). Blood analysis (gel chromatography) indicated that 75% of the ^{101m}Rh was bound to plasma protein, indicating the need of stronger coordination complexes for labelling ^{101m}Rh.

(1) Giraldi T., Sava G., Bertoli G., Mestroni G., and Zassinovich G., Cancer Res., 37, 2662 (1977).
(2) Evans J.S., Kashy E., Neumann R.A., and Petry R.F., Phys. Rev., 138, 39 (1965).
(3) Scholtz K.L., Sodd V.J., and Blue J.W., Int. J. Appl. Radiat. Isotop., 28, 207(1977).

TABLE 1. Q-Values (MeV) and Threshold Energies (MeV) for the Direct and Indirect Reactions Leading to the Formation of ^{101m}Rh.

Target Nuclide (% Abundance)	Reaction	Product Nuclide	Q-Value (MeV)	Threshold Energy (MeV)
^{102}Pd (1.0)	p,2n	^{101}Ag	-15.8	-15.9
^{104}Pd (11.0)	p,4n	^{101}Ag	-33.4	-33.7
^{105}Pd (22.2)	p,5n	^{101}Ag	-39.6	-40.0
^{106}Pd (27.3)	p,6n	^{101}Ag	-49.7	-50.2
^{102}Pd (1.0)	p,pn	^{101}Pd	-10.6	-10.7
^{104}Pd (11.0)	p,p3n	^{101}Pd	-28.2	-28.5
^{105}Pd (22.2)	p,p4n	^{101}Pd	-35.2	-35.6
^{106}Pd (27.3)	p,p5n	^{101}Pd	-44.8	-45.3
^{108}Pd (26.7)	p,p7n	^{101}Pd	-60.6	-61.2
^{104}Pd (11.0)	p,α	^{101m}Rh	+ 2.9	+ 2.9
^{105}Pd (22.2)	p,αn	^{101m}Rh	- 4.2	- 4.3
^{106}Pd (27.3)	p,α2n	^{101m}Rh	-13.8	-13.9
^{108}Pd (26.7)	p,α4n	^{101m}Rh	-29.5	-29.8

TABLE 2. Tumor/Tissue Ratios for ^{101m}Rh complexes in Mice Bearing Transplanted Mammary Adenocarcinomas.

Time after Injection (Hours)	Tumor/Blood* (mean ± average deviation)	Tumor/Liver*
4	0.82 ± 0.22	0.19 ± 0.06
24	5.36 ± 3.7	0.13 ± 0.02
48	2.31 ± 0.33	0.16 ± 0.05

* Data for $^{101m}RhCl_3$ and $^{101m}Rh(AcO)_3$ have been combined because of no observable difference in the biologic distribution of the two chelates.

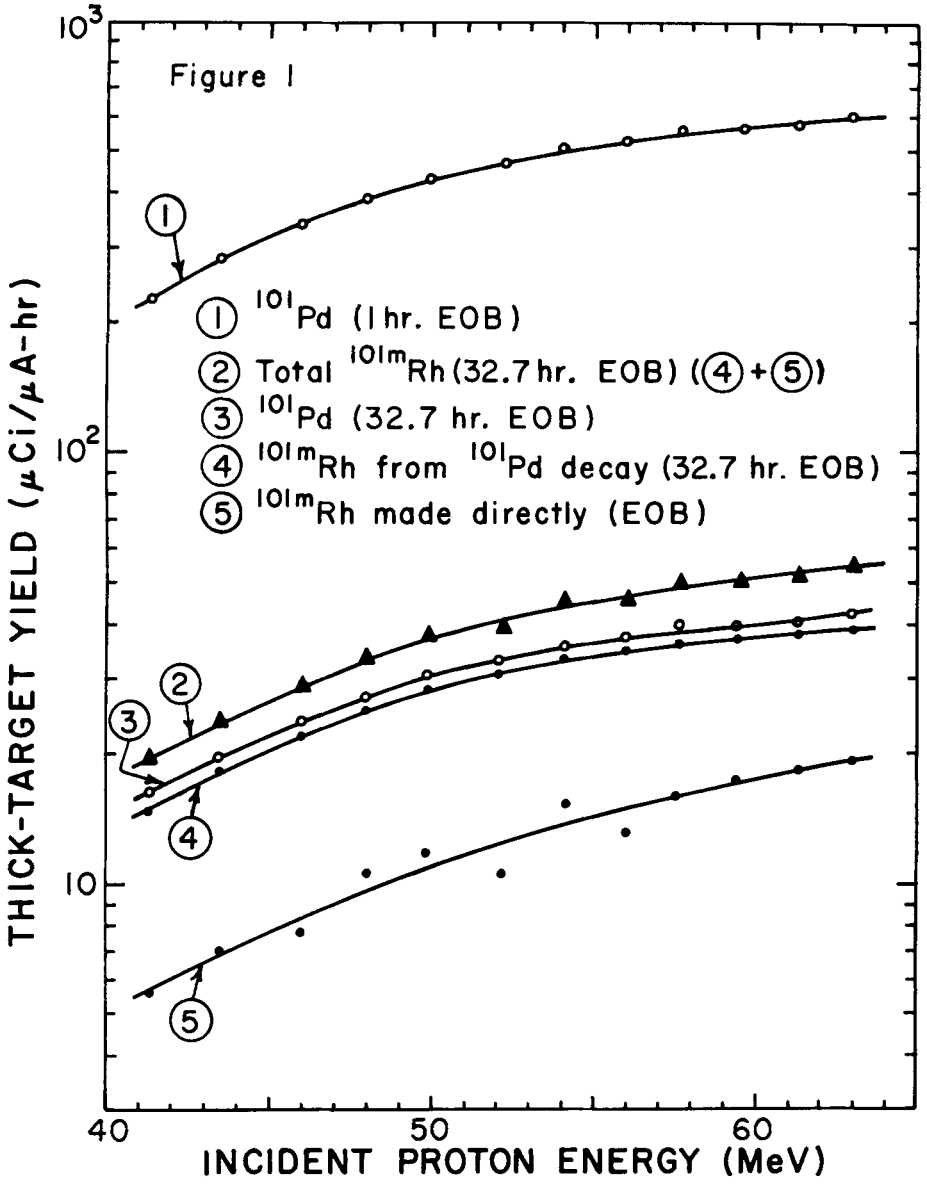
Figure 1
① ^{101}Pd (1 hr. EOB)
② Total ^{101m}Rh (32.7 hr. EOB) (④+⑤)
③ ^{101}Pd (32.7 hr. EOB)
④ ^{101m}Rh from ^{101}Pd decay (32.7 hr. EOB)
⑤ ^{101m}Rh made directly (EOB)
THICK-TARGET YIELD (μCi/μA-hr)
10^3
10^2
10
40
50
60
INCIDENT PROTON ENERGY (MeV)

Paper No.B25

LYMPHOCYTE LABELING WITH ^{111}IN: THE EFFECT ON LABELED CELLS.

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^{111}In-oxine has been used for labeling a variety of hematopoietic elements, including lymphocytes (1). The published method has been simplified by omitting the prior formation and solvent extraction of the indium oxinate. The desired amount of oxine in ethanol was added to $^{111}InCl_3$ in normal saline and the final pH adjusted to 7.2 using NaOH. This solution was added to the cell suspension (total volume of 2 ml), and incubated for 30 minutes at room temperature. Subsequently, S-MEM supplemented with 10% fetal calf serum was added and the incubation mixture diluted to 10 ml. The cell suspension was washed three times with the same medium and the labeling efficiency determined. With a total added weight of oxine $\geq$5 µg, (2.5 µg/ml incubated; $20x10^6$ cells) between 60 and 70% of the radioactivity was normally found to be associated with the cells, although some variability was observed.

In order to test the effect of oxine on cell viability, normal human lymphocytes were isolated from peripheral blood using Ficoll-Hypaque density sedimentation. The cells were treated with various oxine concentrations according to the labeling procedure, but without the addition of $^{111}InCl_3$. Subsequently, the cells were incubated for 48 hours at 37°C in standard medium (RPMI-1640 containing 20% human serum), and viability determined by trypan blue exclusion. No effect on viability was found at oxine levels up to 5 µg/10^6 cells (100 µg oxine added; $20x10^6$ cells; total volume of 10 ml).

The potential radiotoxicity of ^{111}In was examined at various activity levels, holding the total oxine level at 10 µg (ie 5 µg/ml; 0.5 µg/10^6 cells) during labeling, and determining the viability as described before. In this case, marked cellular toxicity was seen, even in incubations performed with low levels of ^{111}In ($\leq$ 0.03 µCi/10^6 cells).

The in vivo distribution of lymphocytes labeled with ^{111}In was also compared to that of ^{51}Cr-labeled lymphocytes, and these in turn to the distribution of pure ^{111}In-oxine. Rat spleen lymphocytes, after passage through a tissue sieve, were isolated in a single-cell suspension, by Ficoll-Hypaque density sedimentation, and injected intravenously into syngeneic animals after labeling. Significant differences were seen between the in vivo distribution of the ^{111}In- and ^{51}Cr-labeled cells.

From this work, it can be concluded that ^{111}In-labeling resulted in toxicity with respect to lymphocytes, although the experimental design so far does not differentiate between toxicity due to oxinates of metal impurities which may be present in the ^{111}In solution and radiotoxicity due to the In-111 itself.

(1) Rannie G.H., Thakur M.L., Ford W.L.; Clin. Exp. Immunol. 29, 509 (1977).

TABLE 1. LYMPHOCYTE VIABILITY DETERMINED AS A FUNCTION OF OXINE CONCENTRATION, 48 HOURS AFTER INCUBATION. NO ^{111}In ADDED

Total Wt. of Oxine Added (μg)	No. of Cells ($x10^{-6}$)	% Viability
0	20	90
1	20	87
5	20	90
15	20	91
50	20	90
100	20	86

TABLE 2. LYMPHOCYTE VIABILITY DETERMINED AS A FUNCTION OF ^{111}In ACTIVITY 48 HOURS AFTER INCUBATION

^{111}In Activity μCi/10^6 Cells	% Viability
0	95
0.03	83
0.16	63
0.8	65
1.4	43
1.5	40
1.8	21
2.1	15
2.2	9
3.3	7
3.4	7
4.0	7
4.4	4
5.5	5

TABLE 3. IN VIVO DISTRIBUTION OF LABELED LYMPHOCYTES 24 HOURS AFTER INJECTION IN SYNGENEIC RATS

Injectate	Total Activity (μCi)	No. Cells x 10^{-6}	%ID per Organ				Liver/ Spleen Ratio
			Liver	Spleen	Lungs	Bone	
^{111}In-lymphocytes	0.12	40	41	18	2.5	20	2.3
	0.4	40	39	17	2.0	13	2.3
	0.6	40	41	20	2.3	18	2.1
	1.5	40	43	19	2.1	22	2.3
	15	40	65	10	1.6	9	6.5
^{51}Cr-lymphocytes	25	40	18	28	0.4	40	0.6
^{111}In-oxine	40	-	15	1.2	1.9	13	12.5

ANNOUNCEMENT

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