Paper No. 1

BIOCHEMICAL ASPECTS OF RADIOPHARMACEUTICAL DESIGN

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Since life itself is an integrated sequence of chemical events it might be expected that biochemical criteria would be a *"sine qua non"* for the design of radiopharmaceuticals. However, serendipity has often played a larger role in the discovery of useful new radiopharmaceuticals than logical deduction based on normal or pathological biochemistry.

There are three main areas in which biochemical considerations may be applied to the design of radiopharmaceuticals for specific purposes, namely :

a. Labelled substrates, or substrate analogues, involved in normal or pathological biochemical pathways.

b. Labelled-drug-, or substrate-, carrier complexes designed to direct the radionuclide to a specific target tissue.

c. Antigen-antibody reactions.

The latter two aspects will not be discussed further as, although their potential, especially for immunological approaches, is exciting, research emphasis in these areas lies in fundamental studies of carrier molecules, antigens and antibodies rather than on new radiopharmaceuticals *per se*.

In the biochemical approach to radiopharmaceutical design it is first necessary to identify specific reactions, or reaction sequences, which, because they occur to a greater extent or at a faster rate in the desired target tissue, as compared to surrounding tissues, may be expected to result in significant differential uptake of a suitable labelled compound. The compounds selected for investigation may be :

i. Natural components of the chosen biochemical pathway, for example (C-11)amino acids for pancreas or tumour visualisation(1), (C-11)glucose for brain metabolism studies(2).

ii. Structural analogues of the natural components which follow all or part of the chosen pathway, such as (Se-75)selenomethionine, (F-18)6-fluorotryptophan for pancreas scanning(3) or (I-131)19-iodocholesterol(4) (Se-75)6-methyl-seleno-methyl nor cholesterol for adrenal studies.

iii. Inhibitors of enzymes essential for the chosen pathway, for example (I-123)3-iodo-SKF-12185 which has been proposed for adrenocortical scanning(5).

The choice of compound will be influenced by many factors including the availability of radionuclides and labelling procedures and especially by the nature of the information required from the procedure. If the primary requirement is a static image, or information on the transport to and uptake of a metabolite by an organ or tissue, the most appropriate agent may be a substance which follows the natural transport or uptake

pathways but is not metabolised in the tissue. Examples of such agents are (Tc-99m)pertechnetate for thyroid scanning and (C-11)1-aminocyclopentane carboxylic acid for tumour localisation(6). More stringent criteria apply when the agent is required to monitor the activity of a specific biochemical pathway. In this case the uptake and retention of the radiopharmaceutical must reflect the activity of the desired reaction sequence but it must not be involved significantly in non-related pathways. The effects of radionuclide incorporation on the biochemical behaviour of the selected compound may be important considerations. For example halogen-substituted amino acids or nucleotides may not be incorporated into protein or DNA to the same extent as their non-halogenated analogues(3,7). Such differences in behaviour are of especial significance when labelling is achieved by complex formation between a biochemical substrate and a cationic radionuclide. Frequently, complex formation involves reaction with the biochemically active sites with the result that the complex is either not utilisable or dissociates before significant localisation of the radionuclide has been achieved. This problem is illustrated by the failure of amino acid complexes to significantly increase the localisation of Zn-62 in the pancreas(8,9). A more dramatic illustration is provided by (Tc-99m)N(2,6-dimethyl-phenyl carbamoylmethyl)iminodiacetic acid (Tc-99m-HIDA) where the complex is cleared from the body through the hepato-biliary system while the uncomplexed drug is excreted through the kidneys(10).

The position of the radionuclide label within the substrate molecule may also be important in relation to the effectiveness of the radiopharmaceutical as a metabolic tracer. For example (F-18)2-deoxy-2-fluoroglucose is a substrate for hexokinase, but analogues with substituents on C1, C3 or C6 are not(11).

Until relatively recently the development of radiopharmaceuticals designed to exploit normal or pathological biochemical pathways has been restricted by the small number of gamma ray emitting nuclides suitable for incorporation into specific substrate molecules. However, the increasing availability of C-11, N-13, F-18, I-123 and Br-77 and the development of rapid labelling techniques offers many opportunities for the development of new types of radiopharmaceutical which can yield functional as well as anatomical information using the various types of imaging techniques now available.

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Paper No. 2

THE DESIGN OF RECEPTOR BINDING RADIOPHARMACEUTICALS

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The design of receptor binding radiopharmaceuticals can be greatly aided by the use of a simple two compartment equilibrium model to calculate the maximum target to blood ratio. Using the typical injected dose of C-11, Tc-99m, I-123, In-111, I-131 and I-125 in humans or in animals on a per kg basis, the concentration of the new derivative will be between 0.7 to 35 pM if distributed in the plasma (PV) and between 0.2 to 19 pM if distributed in the extracellular fluid (ECF). Tritium labeled compounds because of the long half life of tritium are 5670 pM in the PV and 1417 pM in the ECF if 3.6 μCi is injected in a 0.25 Kg rat. Receptor concentrations can usually be obtained from the literature although this concentration is an average value and may not reflect the higher effective concentration if the receptors are concentrated on the cell surface or in the cytosol. The affinity constants for the new derivatives can be obtained by in vitro analysis or by use of the literature value of the tritium labeled parent compound. The latter approximation will give the maximum value in an equilibrium situation since the derivatization will probably decrease the affinity constant.

Using this approach we have investigated three systems - estrogen receptors, *beta* adrenoceptors and muscarinic-cholinergic receptors. Using the law of mass action, the target organ to blood ratio can be calculated by assuming this ratio equal to the Bound (B) to Free (F) ratio.

$$\underset{(R_o-B)}{\text{Receptor}} + \underset{(L_o-B)}{\text{Ligand}} \rightleftharpoons \underset{(B)}{\text{Receptor Ligand Complex}}$$

$$B = + [1+KR_o+L_oK] \pm \sqrt{[1+KR_o+L_oK]^2-4K^2L_oR_o} \Big/ 2K$$

The maximum B to F ratios were calculated (Table 1). These theoretical maximum values can be compared with the experimental values obtained by injection of the appropriate dose in rats and guinea pigs (Table 2). The experimental B/F ratios approach the theoretical B/F ratios in the case of estradiol, ethynyl estradiol, propranolol, QNB in the guinea pig and QNB and MeQNB in the rat. Use of higher specific activity derivatives e.g. I-125 propranolol for 3-H propranolol does not alter the maximum ratio significantly. Ratios lower than the theoretical maximum result from protein binding, metabolism or nonequilibrium states. In contrast NE gives a ratio higher than theory predicts probably because NE is not binding to the beta adrenoceptor alone but is also concentrated by the Uptake I mechanism. Likewise I-125 TYR-PRAC gives a higher H/B ratio experimentally and therefore must be binding to proteins other than the beta adrenoceptor. In vivo displacement experiments using propranolol confirmed this hypothesis. The uptake of MeQNB in the guinea pig ventricle is higher than expected. From the Scatchard equation: $B/F = KR_o-KB$ it can be seen that the maximum B/F ratio is controlled by the product of the equilibrium constant and the receptor concentration. More than likely, the estimate of the receptor concentration in the guinea pig ventricle is low since coinjection of atropine showed that MeQNB was specifically bound to the cholinergic receptor. Both the theory and the experimental studies suggest that these three receptor systems can produce useful radiotracers. Judicious use of this simple model in conjunction with in vitro assays for affinity constants and nonspecific binding can greatly aid in the design of receptor binding radiotracers.

Table I

Compound	$[L_o]$ ECF nM	$[R_o]$ nM	Affinity Constant ($X10^{-9}$)	Max B/F
H-3 Estradiol	1.42	36	5.5 (1)	192
H-3 Ethynyl Estradiol	1.42	36	3.5	121
H-3 Propranolol	1.42	4	0.19 (2)	2.7
I-125 Propranolol	$18.9x10^{-3}$	4	0.19	3.6
H-3 Norepinephrine	1.42	4	0.01 (2)	0.039
I-125 TYR-PRAC	$18.9x10^{-3}$	4	0.002 (3)	0.008
H-3 QNB	1.42	10*	2.5 (4)	21.6
H-3 MeQNB	1.42	10	2.5	21.6

I-125 TYR-PRAC = 1-(3-iodo-4-hydroxy)phenethylamino-3-4-acetamido-phenoxy-propan-2-ol
H-2 QNB = quinuclidinyl benzilate
H-3 MeQNB = methyl quinuclidinyl benzilate
* Estimated from experiments performed in this laboratory

Table 2

Compound	Time (hr)	Target Organ	Target Blood
H-3 Estradiol	2	immature rat uterus	26
H-3 Ethynyl Estradiol	2	immature rat uterus	38
H-3 Propranolol	2	rat ventricle	.3
H-3 Norepinephrine (NE)	2	rat ventricle	8
I-125 TYR-PRAC	2	rat ventricle	19
H-3 QNB	2	rat ventricle	4
H-3 QNB	2	guinea pig ventricle	14
H-3 MeQNB	2	rat ventricle	4
H-3 MeQNB	2	guinea pig ventricle	28

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Paper No. 3

RADIOLABELED ENZYME INHIBITORS - ENHANCED LOCALIZATION FOLLOWING ENANTIOMERIC PURIFICATION

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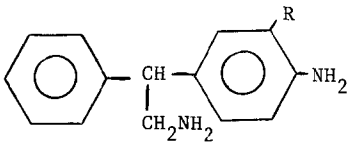
Dog adrenals have recently been imaged with I-123-(±)-2-(3-iodo-4-amino-phenyl)-2-phenethylamine(I), a reversible inhibitor of steroid 11β-hydroxylase (1). In view of the stereoselectivity of adrenocortical enzymes, studies were performed to determine if the enantiomers of I show different affinities for the adrenal cortex.



I: R = *I

II: R = H

Racemic-II was resolved by fractional crystallization of the diastereomeric *tartarate* salts. Conversion to the respective di-HCl salts gave [α]=-9.4 degrees for (-)-II and [α]=+9.6 degrees for (+)-II. The enantiomers were radiolabeled with I-125 in 90% yield by the chloramine-T method. The mild iodination conditions gave no racemization as determined by [α] measurements and N.M.R. analysis of the europium-camphorato complexes of the I-127 labeled compounds.

The tissue distributions of I-125 labeled (+)-and(-)-I were determined in 5 dogs each at 2 hours post-injection. The (-)-I form gave a 100% greater uptake in the adrenal cortex (3.47±0.12% Kg·dose/gm) and nearly a 3-fold increase in both the [adrenal cortex/liver] and [adrenal cortex/medulla] compared to the (+)-I enantiomer. The uptake values for racemic-I were intermediate between those obtained for the two pure enantiomers.

These results demonstrate the importance of enantiomer evaluation in maximizing the possible clinical utility of a chiral radiopharmaceutical.

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Paper No. 4

AGENTS FOR THE ARMAMENTARIUM OF REGIONAL METABOLIC MEASUREMENT IN VIVO VIA METABOLIC TRAPPING: ^{11}C-2-DEOXY-D-GLUCOSE AND HALOGENATED DEOXYGLUCOSE DERIVATIVES

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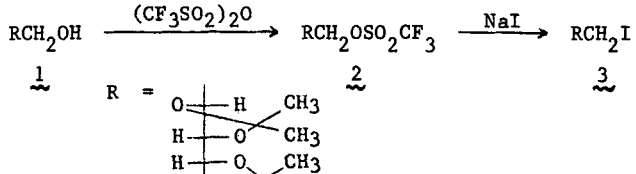
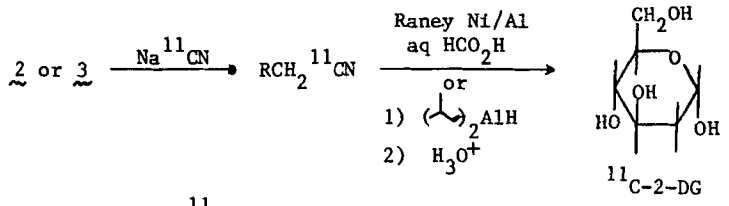
For over two decades, 2-deoxy-D-glucose (2-DG) has been used as a glucose analog in the study of various aspects of carbohydrate metabolism. The substitution of the hydroxyl group on C_2 of glucose with a hydrogen atom results in a molecule which isolates the hexokinase reaction and thus makes it possible to study the first step of glucose metabolism in the presence of other enzymes (1). Interest in labeled analogs of 2-DG which could be used to measure regional brain glucose metabolism was stimulated by the work of Sokoloff, Reivich and coworkers (2) and led to the development of ^{18}F-2-deoxy-2-fluoro-D-glucose (2-^{18}FDG) (3,4). The initial design of 2-^{18}FDG as a radiopharmaceutical was suggested by the work of Sols and Crane (1) and Bessell and coworkers (5) who demonstrated the relative insensitivity of the hexokinase reaction to structural modification at C_2. This is illustrated by comparing the relative kinetic constants of glucose, 2-DG, 2-FDG, 3-deoxy-3-fluoro-D-glucose (3-FDG) and 4-deoxy-4-fluoro-D-glucose (4-FDG) in the yeast hexokinase catalyzed phosphorylation reaction as determined by Bessell (5).

Substrate	K_m (mM)	Relative V_{max}
glucose	0.17	1.00
2-DG	0.59 ± 0.11	0.85
2-FDG	0.19 ± 0.03	0.50
3-FDG	70 ± 30	0.10
4-FDG	84 ± 30	0.10

Our interest in extending the availability of radiopharmaceuticals such as 2-^{18}FDG as well as in having the capability of doing serial metabolic studies at short time intervals has lead to new labeling studies. We report here (1) the development of a synthetic route to ^{11}C-2-DG, (2) the synthesis and instability of ^{131}I-2-deoxy-2-iodo-D-glucose (^{131}IDG), and (3) improvements in the synthesis of 2-^{18}FDG.

2-Deoxy-D-[1-^{11}C]glucose (^{11}C-2-DG): ^{11}C-2-DG was synthesized from Na^{11}CN by a reaction sequence similar to that used for the preparation of 2-deoxy-D-[1-^{14}C]ribose (6). D-(-)-arabinose was converted to 2,3:4,5-di-O-isopropylidene-D-arabinitol (1) in four steps with an overall yield of 39% using the method reported by Zinner and Kristen (7). Treatment of 1 with trifluoromethanesulfonic anhydride gave 2 which was either converted to the corresponding iodo compound, 3, or was used directly as a substrate in a cyanide displacement reaction.

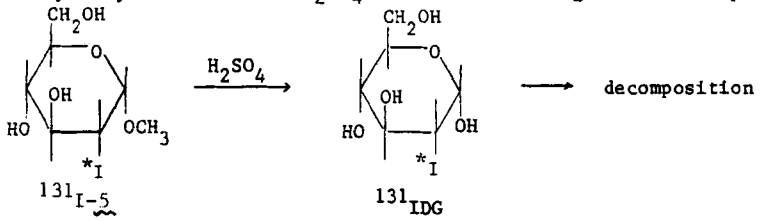




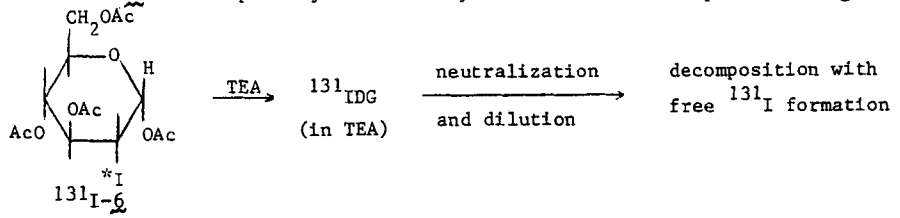
Cyclotron produced H^{11}CN was trapped in either an aqueous solution containing 10-20 µmole of carrier NaCN or, when near carrier-free product was desired, in 0.05 N NaOH. After evaporation of the water, a solution of 10-40 µmole of either 2 or 3 in DMF was added, and the mixture was warmed for 5 minutes. Workup provided the protected nitrile 4. This was converted to ^{11}C-2-DG in 50% yield in one step by a modification of the novel reduction method (aq. HCO_2H-Raney alloy (Ni/Al)) of van Es and Staskun (8). Alternatively, reduction of 4 with di-isobutyl aluminum hydride and hydrolysis gave ^{11}C-2-DG in lower yields. Current production procedures give radiochemical yields of > 30% in a synthesis time of 45 minutes from EOB providing 100 mCi of ^{11}C-2-DG over a specific activity range of near carrier-free to micromolar levels.

^{131}I-2-Deoxy-2-iodo-D-glucose (^{131}IDG): The synthesis of unlabeled IDG by the hydrolysis of methyl 2-deoxy-2-iodo-D-glucose (5) has been reported by Honda and Takiura (9). However, our attempts to repeat this synthesis resulted in partial formation of IDG followed by rapid extensive decomposition during hydrolysis.

The labeling of IDG with ^{131}I was attempted using two different routes from protected labeled precursors ^{131}I-5 and 6. ^{131}I-5 was prepared according to the synthesis of the unlabeled molecule described by Lemieux (10). ^{131}I-5 was hydrolyzed in 0.05 M H_2SO_4 at 100° according to Honda's procedure



and monitored by tlc (Silica G, $CHCl_3$:CH_3OH:H_2O (30:9:1), UV and radioactivity detection). Within 2 hrs the reaction mixture showed a 30% conversion to ^{131}IDG followed by rapid decomposition (competing with hydrolysis) within the next 30 min. A second approach to the synthesis of ^{131}IDG involved deacetylation of ^{131}I-2-deoxy-2-iodo-D-glucopyranose tetraacetate (^{131}I-6, prepared from ^{131}I-5) with triethylamine (TEA) at 25°C. Within 6 hrs ^{131}I-6 was completely and cleanly converted to a compound having the



same R_f as that produced by the partial acidic hydrolysis of ^{131}I-5. However, although this compound (presumably ^{131}IDG) was stable for at least 2 days in TEA solution it rapidly lost iodine on neutralization and dilution of the solution.

Our experiments suggest that the stability of ^{131}IDG has a strong dependence on the pH and perhaps the ionic strength of the solution. This probably reflects the chemical environment of the iodine atom which in the open form is part of an α-iodoaldehyde functionality which would be predicted to lose iodine. This instability at neutral (and physiological) pH has precluded a study of the biological properties of this radiopharmaceutical.

^{18}F-2-Deoxy-2-fluoro-D-glucose (2-^{18}FDG): The following modifications have been applied to the synthesis of ^{18}FDG which we reported earlier (3,4).

Step	Old Method	New Method	Advantage
separation of isomers	evaporation of reaction mixture and preparative glc	liquid chromatography of total crude reaction mixture	2-fold increase in yield; reduction in handling and time
neutralization of hydrosylate and $H^{18}F$ removal	ion exchange and Al_2O_3	evaporation and Al_2O_3	reduced handling

These changes have increased typical yields to 12-15% (based on ^{18}F delivered) and reduced synthesis time to 1¼ hrs. The synthesis produces 1 mg of ^{18}FDG with a specific activity of 10 mCi/mg for a typical patient delivery.

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Paper No. 5

SYNTHESIS AND ANIMAL DISTRIBUTION STUDIES OF ^{18}F-3-DEOXY-3-FLUORO-D-GLUCOSE

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There is considerable interest in fluorine-18 labeled derivatives of glucose as analogues for glucose metabolism (1). Of the possible substitution positions in glucose 3 appears the most attractive (Table 1)(2,3). Fluorination at C-6 prevents phosphorylation, at C-5 prevents ring closure and 1- and 4-fluoro-deoxy-glucoses are rapidly hydrolyzed in vivo (3). That a hydrogen bond acceptor is necessary at C-3 is shown by the values for 3-deoxy-D-glucose. Previous synthesis of 3-deoxy-3-fluoro-D-glucose required 6 days from preparation of the anhydrous fluorinating agent to isolation of the fluoro-sugar (4) and so are not suitable for use with fluorine-18 (half life = 110 min), so we developed a faster synthetic sequence (Figure 1).

Bombardment of neon containing 15% hydrogen with 6.3 MeV deuterons produces anhydrous carrier free fluorine-18 HF. This is passed over a silver wool plug impregnated with anhydrous cesium hydroxide which reacts with fluorine-18 HF to produce carrier-free fluorine-18 CsF. The silver wool plug containing the activity is added to a solution of 1,2-5,6-di-O-isopropylidene-3-trifluoromethane sulphonyl-α-D-allofuranose (I) in hexamethyl phsophoric triamide (HMPA), and heated to give 1,2-5,6-di-O-isopropylidene-3-deoxy-3-fluoro-α-D-gluco-furanose (II). Treatment of II with boron trichloride and water gives 3-deoxy-3-fluoro-D-glucose (III), which is purified on an ion exchange column. Yields on a typical run are 13 mCi absorbed on the silver wool from a 10 μA/hr bombardment, giving 4.5 mCi of carrier-free III 90 minutes from the end of bombardment. Cerebral studies from an intracarotid injection in a rhesus monkey gives a cerebral extraction fraction of 31.4%, compared to 30-32% obtained with carbon-11 glucose and Anger Camera images in a rhesus monkey and organ distribution studies in dogs give very similar values to those from carbon-11-D-glucose.

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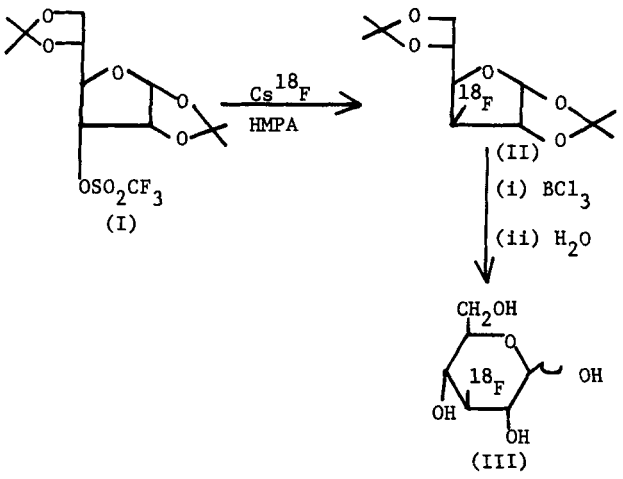

FIGURE 1

TABLE 1

Active Transport of Glucose and Analogues in Vitro

Sugar	K_i (2)	K_x (3)	V_{max} (3)
D-glucose	2.3	3.9	640
3-Deoxy-3-fluoro-glucose	2.5	2.3	600
2-Deoxy-2-fluoro-glucose	∞	-	-
3-Deoxy-glucose	24	15.3	340

Paper No. 6

2^1-FLUORO-2^1-DEOXYURIDINE

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Two important aspects of cancer treatment are early detection and evaluation of the subsequent treatment response. Radiofluorinated (^{18}F) nucleosides, which are incorporated into tumors with high selectivity, exhibit potential for tumor imaging and measuring the rate of tumor growth or supression before, during and after chemotherapy (1). It was therefore of interest to develop a fast and efficient synthesis of 2^1-fluoro-2^1-deoxyuridine (IV) since substitution of fluorine for hydrogen at the remote 2^1-position of 2^1-deoxyuridine may not change the biochemical behavior.

Reaction of 2,2^1-anhydrouridine (I, reaction scheme) (2) with dioxane hydrogen fluoride (II) afforded 2^1-fluoro-2^1-deoxyuridine (IV). Optimum chemical yields were obtained using 10 equiv. of II at 200° for 40 min as illustrated in the table. Protonation of the 2,2^1-anhydro ether linkage of I gives rise to the intermediate III which reacts further with cleavage of the anhydro bond as a result of nucleophilic attack by fluoride anion at the highly electrophilic C-2^1 position. In the absence of moisture ring opening of the 2,2^1-anhydronucleoside ring of III is stereospecific since the 2^1-*arabino* derivative V was not detected (3).

Dioxane hydrogen fluoride (II) is an attractive nucleophilic fluorinating reagent due to the facile generation of anhydrous hydrogen fluoride and the excellent solvating properties of dioxane. The potent nucleophilicity of II can be attributed to the existence of fluoride anion as "naked fluoride". In this respect II may prove to be a suitable, if not superior, alternative to tetra-*n*-butylammonium fluoride, alkalai metal fluorides in the presence of catalytic amounts of crown ethers or phase transfer reagents and fluoride labelled exchange resins for use in nucleophilic displacement reactions (4). Reactions of I with alkalai metal fluorides in polar organic solvents with or without 18-crown-6 using *p*-toluenesulfonic acid as a catalyst to protonate the 2,2^1-anhydro linkage did not proceed. Reactions employing tetra-*n*-butylammonium fluoride were equally unsuccessful.

Quantitative preparative high pressure liquid chromatography using a Merck Prepacked Size B silica gel 60 column with methanol:chloroform (1:1 v/v) as solvent at a flow rate of 4 ml/min and UV detection at 250 nm afforded IV (22 min) and unreacted I (36 min).

The total time (2-2.5 hr) expected for the preparation of ^{18}F-labelled IV includes preparation of II (1 hr), reaction time (45 min), purification (22 min) and preparation of a solution for use (15 min). This reaction is being studied in a teflon coated cylinder.

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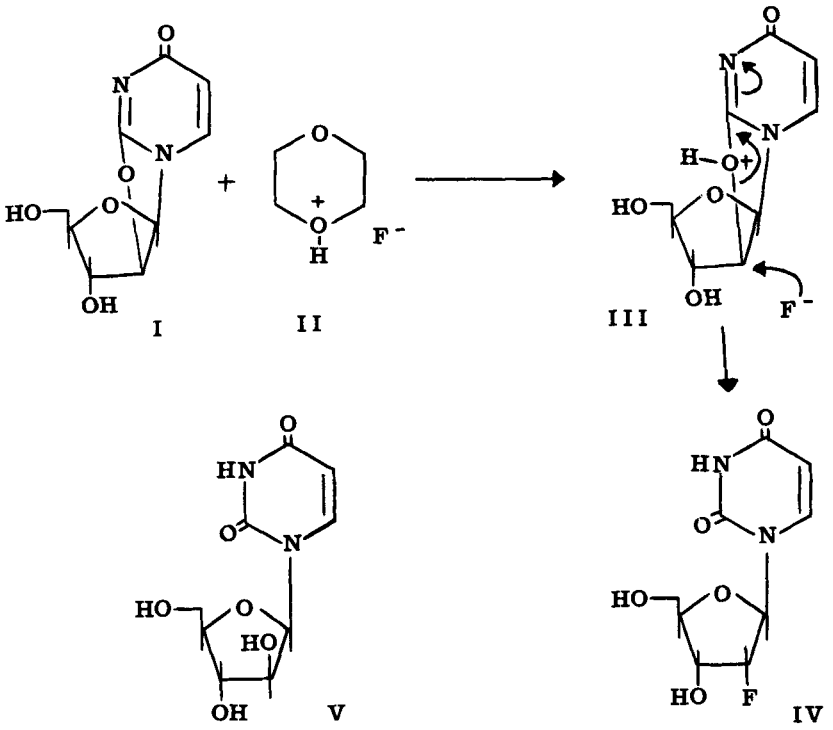

% Yield of 2^1-Fluoro-2^1-deoxyuridine (IV) from Reaction of Dioxane Hydrogen Fluoride (II) with 2,2^1-Anhydrouridine (I)[a]

mmol I	mmol II	Volume Dioxane(ml)	Reaction Time (min)	IV[b]	Recovered[b] I	Temperature Start[c]	End[c]
0.01	0.05	1.1	38	8	92	205	200
0.01	0.10	1.2	40	33	67	200	200
0.01	0.20	1.4	40	32	47	165	210

[a]The reactions were carried out in a teflon vial inserted inside a monel cylinder containing dioxane to equalize the pressure.

[b]Yields determined using quantitative preparative HPLC.

[c]Temperature of oil both at the start and end of the reaction.

Paper No. 7

THE PREPARATION OF ^{18}F-LABELLED 4-FLUOROESTRONE AND 4-FLUOROESTRADIOL.

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Estrogen derivatives labelled with a γ emitting radionuclide have been proposed as an aid to the diagnosis of estrogen dependant malignancies. Tritiated estradiol and its analogue hexestrol have been shown to concentrate in such tumours (1, 2). This process is thought to involve binding of the steroid to specific intracellular estrogen receptor proteins. It has been shown that there is a direct relationship between the presence of estrogen receptors in malignancy and remission of the tumour after endocrine abalation (3). Consequently it has been pointed out that a γ emitting derivative would be useful in detecting metastases and in following the course of abalation therapy (4, 5).

Most attempts to label estrogens both for this purpose and as potential prostate scanning agents have concentrated on the production of radioiodinated derivatives (4, 6, 7), but Vaalburg et al have reported the preparation of ^{11}C-ethynylestradiol (5). Since the number of steroid binding sites is small, high specific activities are required, and the difficulty in achieving this coupled with the instability of certain radioiodinated compounds in vivo have been the principal obstacles to progress. Fluorine-18 (half life 110 min. β^+) would appear to be an ideal label for this purpose since the introduction of fluorine into the molecule has been shown to increase hormonal activity in many cases. 4-Fluoroestradiol (4-fluoro-1,3,5(10)estratriene-3, 17β-diol) (IV) was selected since it has been reported to have 140% of the estrogenic activity of estradiol and the chemical synthesis described by Utne et al (8) could be adapted to labelling by known techniques. (Reaction Scheme 1). This route also yields 4-fluoroestrone (4-fluoro-1,3,5(10)estratriene-3-ol-17-one) (III). At present the method can only produce comparatively low specific activity material (100-500 μCi mg^{-1}) since a certain minimum quantity of the precursor (I) is required for labelling.

The diazonium tetrafluoroborate precursor (I) was prepared (8) in quantity and used as required, 15-25 mg being labelled using a recirculatory ^{18}F-neon target as described previously (9). It was then washed off the glass-fibre supporting disc with acetonitrile and the solvent evaporated under nitrogen. The residual labelled diazonium salt (I) was decomposed under vacuum at 165°C after which the temperature was raised to 200°C for 20 mins. This resulted in sublimation of the ^{18}F-4-fluoroestrone methyl ether (II) and minor amounts of other volatile compounds from the residue. (Analysis, TLC on silica gel with ethyl acetate/cyclohexane 20 : 80, R_f.(II) 0.4). The sublimate was then dissolved in chloroform (∿ 2 ml), 1 drop of boron tribomide was added and the solution was left to stand at room temperature for 10 mins. After addition of water (∿ 2 ml) the ^{18}F-4-fluoroestrone (III) was extracted into the chloroform layer which was then separated and dried over calcium chloride. ^{18}F-4-fluoroestrone may be separated at this stage by preparative TLC. (Silica gel with ethyl acetate/cyclohexane 30 : 70, R_f.(III) 0.35).

For the preparation (10) of ^{18}F-4-fluoroestradiol (IV) the chloroform solution was evaporated under nitrogen and the residue taken up in tetrahydrofuran (3 ml). Lithium aluminium hydride (∿ 20 mg) was then added and the solution refluxed with stirring for 30 mins. Sufficient water to hydrolyse the reaction mixture was added and the solution filtered. Most of the tetrahydrofuran was evaporated under nitrogen and the residue extracted into ethyl acetate (∿ 3 ml). This solution was dried over calcium chloride, evaporated under nitrogen to a volume of 0.5 ml., and the ^{18}F-4-fluoroestradiol (IV) separated by preparative TLC. (Silica gel with ethyl acetate/cyclohexane 40 : 60, R_f.(IV) 0.45).

The results for typical preparations are given in Table 1. Approximately 1.5 - 2 mg of (III) or (IV) are produced in the synthesis. Compounds (II, III, IV) were identified by mass spectroscopy (m/e 302 (M^+), 288 (M^+), and 290 (M^+) respectively). Analytical TLC using the same systems that were used preparatively showed the products to be >98% radiochemically pure by autoradiography. The chemical purity of the products is however in question. Estrone and estradiol have been detected by TLC among the products of this synthesis in addition to the fluoro derivatives. Presumably they are derived from reductive decomposition of the diazonium salt (11). Utne et al reported analogous results with the 2-fluoro isomer (8). This is being investigated further. ^{18}F-4-Fluoroestrone (III) may be prepared in about 180 mins. from the end of cyclotron bombardment while ^{18}F-4-fluoroestradiol (IV) takes about 240 mins.

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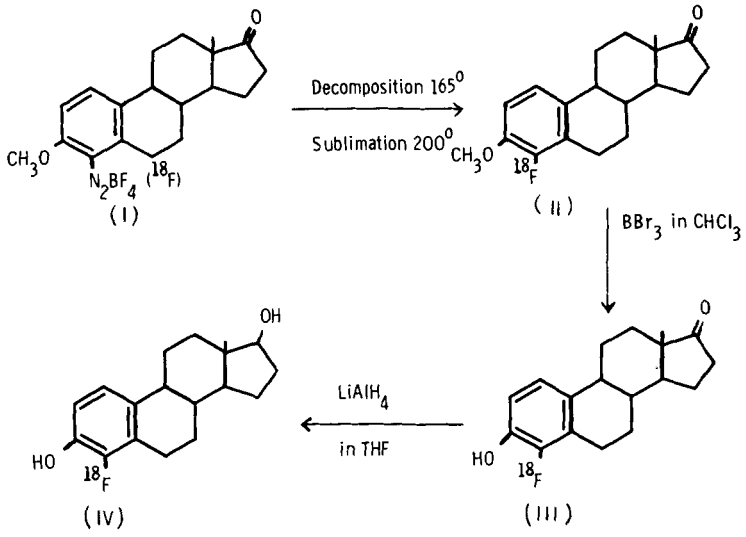
TABLE 1. Results

Wt.(I) mg.	% Activity in sublimate * †	Radiochemical yield (III) % †	Radiochemical yield (IV) % †
24	11.6	3.0	-
24	11.3	3.2	-
18	11.9	-	3.3

* Impure (II)
† Corrected for decay

REACTION SCHEME 1.



Paper No. 8

^{18}F-FLUORINATION BY K^{18}F-CROWN ETHER SYSTEM

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The introduction of fluorine into various positions of a certain steroid hormone has often resulted in enhancement of biological activity and ^{18}F-labelling of these compounds is useful for the development of positron imaging and an in vivo receptor assay of hormonal tissue in nuclear medicine.

We have investigated a covenient labelling synthesis with ^{18}F-labelled potassium fluoride - 18-crown-6 and (^{18}F)-21-fluoroprogesterone (I) with high specific activity (10 mCi/mg) has been prepared from 21-hydroxy-pregn-4-ene-3,20-dione methanesulfonate (II) in this method (1). The synthesis procedure is as follows: (^{18}F)-water was produced by α bombardment of water in a titanium target box with the ^{16}O(α,pn)^{18}F reaction. This irradiated water and carrier KF were dried up in a platinum crucible to give anhydrous K^{18}F. After refluxing of the K^{18}F, crown ether and (II) in various solvents, column chromatography gave (I) with radiochemical yield shown in Table I.

The several experiments under the different conditions (volume of (^{18}F)-water, amount of carrier KF and crown ether) showed solvent effect and carrier KF effect. The carrier effect is that the reduction of used KF quantity gave poor radiochemical yield, which was regarded to be due to the presence of a certain amount of non reactive ^{18}F-metal complex from the target material. In order to make clear this method and obtain the compound with higher specific activity, the following three factors were investigated:

(1) Solubility of KF in various solvents containing of crown ether
(2) Reactivity of F^- anion in various solvents
(3) Influence of unknown metal-^{18}F complex

Chloroform and acetonitrile solubilized KF in the presence of crown ether more than DMF, DME, acetone and benzene (Table II). As shown in Table III, the order of the radiochemical yield of (II) was chloroform > benzene > acetonitrile > DMF > acetone. Thirdly, even when sufficient amount of crown ether was used for the labelled K^{18}F, ^{18}F activity was not dissolved completely and the ^{18}F-solubility against the various amount of KF showed the titration curve, of which inflection point is equal to the quantity of non reactive ^{18}F species.

According to the above mentioned facts, chloroform is the best solvent in this method, but in this solvent about 5% of 21-chloroprogesterone was produced as by-product (estimated by NMR quantitative analysis). In the other solvents, the proper selection of the labelling condition would give appreciable radiochemical yield. Furthermore, in this method there is a limit of specific activity of ^{18}F-labelled compound due to the metal complex. Getting rid of this problem would give a compound with ultra high specific activity (several mCi/ g).

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Table I : Radiochemical yield of (I)

Run	Solvent (0.5ml)	$K^{18}F$ (μmole)	18-Crown-6 (μmole)	(II) (μmole)	Yield (%)
1	chloroform	5.9	50	5	26
2	chloroform	1.5	83	10	7
3	acetone	2.5	90	10	6
4	DMF	6	50	5	6.6

* 2 - 3 hr under reflux

Table II : Solubility of KF in 18-Crown-6 solution

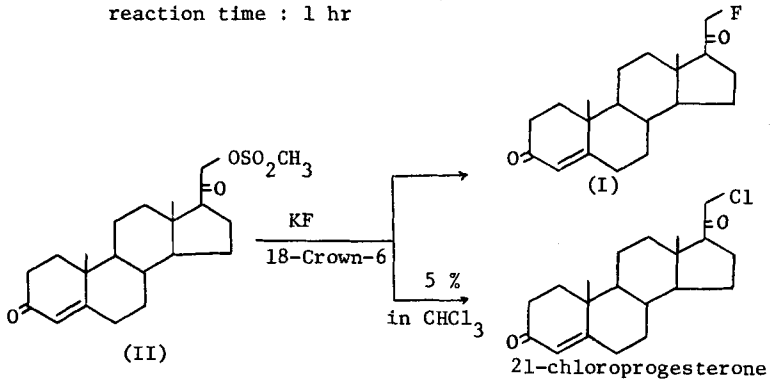
Solvent	Solubility (mM/L)	Solvent	Solubility (mM/L)
chloroform	45	benzene	0.5 - 1.8
acetonitrile	16.3	DMF	2.2
acetone	1.23	DME	2.2

* 18-Crown-6 cocentration : 100 mM/L
total amount of used KF : 500 mg/12 ml of (^{18}F)-water

Table III : Radiochemical yield of (I) in the same concentration of $K^{18}F$ dissolved in 18-Crown-6 solution

chloroform	20.9 %	benzene	20.0 %
acetonitrile	17.3 %	DMF	12.8 %
acetone	12.2 %		

* $K^{18}F$: 1 μmole , (II) : 30μ mole
solvent volume : 2 ml , temperature : 70° - 90°
reaction time : 1 hr



Paper No. 9

FLUORINE-18 LABELLED TRACERS FOR THE INVESTIGATION OF NEUROTRANSMITTERS

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The investigation of the neurotransmitters dopamine, norepinephrine and 5-hydroxytryptamin in the intact human brain is hampered by the lack of a simple and atraumatic method. We set out to develop a procedure by which the rate of formation of a neurotransmitter ("turnover") can be measured. Metabolic derangements and abnormal turnover rates of neurotransmitters have been implicated with Parkinsonism, schizophrenia and depression (1,2). The procedure employs the labelled precursor concept. Dopamine, norepinephrine and 5-hydroxytrytamin are made in the brain from their respective amino acid precursors 3,4-dihydroxyphenyl alanine(DOPA),3,4-dihydroxyphenyl serine and 5-hydroxytryptophan. Unlike the amines, the amino acid precursors can enter the brain from blood. When the precursor labelled with a gamma-emitting isotope is injected into the blood and the radioactivity is monitored over the head it would be possible to extract information on the transport of the amino acid across the blood-brain-barrier, the production of the neurotransmitter by the brain and its removal from the brain.

We have successfully applied this concept to the investigation of dopamine using the labelled precursor analog [F-18]5-fluoro-DOPA (3). The biological behaviour of this analog resembles remarkably that of native DOPA: [F-18]5-Fluoro-DOPA is decarboxylated to [F-18]5-fluoro-dopamine (4) which was found to bind to the dopamine receptors and stimulates the dopamine-sensitive adenylate cyclase. [F-18]Fluoro-dopamine induces the typical circling behaviour in rats in which unilateral lesions of the nigro-striatal pathway have been made (5).

When injected intravenously into baboons [F-18]5-fluoro-DOPA accumulates in the brain and the pattern of accumulation can be influenced in a predictable fashion by α-methyl-dopa, reserpine, pargyline or haloperidol. Hence, [F-18]fluoro-DOPA can be used to trace the cerebral metabolism of dopamine.

Quantitative data on transport of fluoro-DOPA across the blood-brain-barrier and its conversion in the brain to fluoro-dopamine was next obtained: Young cynomolgus monkeys were injected simultaneously with [F-18]fluoro-DOPA and [In-113m]indium-transferrin as a blood marker. The changes in F-18 and In-113m radioactivity with time were monitored continuously from the whole head and arterial blood. F-18/time curves have been derived that represent the [F-18]fluoro-DOPA in the arterial blood (feed to the brain) and the accumulation of F-18 in the brain. The fate of [F-18]fluoro-DOPA has been represented by a three-compartment model (F-18 in blood; F-18 in the capillary walls, blood-brain-barrier; F-18 in neurons). The impulse response of this model was derived mathematically. It has been convolved with the F-18 blood curve to produce a response that best fits the observed F-18 accumulation curve in the brain. Thus, the fractional rate constants of the bidirectional transfer across the blood-brain-barrier and the production of [F-18]fluoro-dopamine (=dopamine) could be obtained.

The success with the tracer [F-18]fluoro-DOPA will encourage synthetic efforts and applications for other ring-radiofluorinated neurotransmitter precursors, such as L[F-18]3,4-dihydroxyfluoro-phenylserine for norepinephrine and L[F-18]5-hydroxy-fluoro-tryptophan for 5-hydroxytryptamin.

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Paper No.10

TECHNETIUM CHEMISTRY

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Recent aspects of the chemistry of technetium will be presented. The emphasis will be on well-characterized coordination complexes in the oxidation states V, IV, III, II, and I. Comparisons with the chemistry of the corresponding rhenium compounds will be made where possible.

Paper No.11

PREPARATION OF CHROMATOGRAPHICALLY PURE TECHNETIUM-99m(IV) FOR NUCLEAR MEDICINE

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Technetium-99m is today the most widely used radiopharmaceutical in nuclear medicine[1]. Sodium pertechnetate-99m is injected routinely for thyroid and brain scintigraphy while its salts or complexes, in the reduced oxidation state of the element, are employed for the visualization of other organs of the body[2]. Technetium-99 has been shown[3] to exist in the oxidation states ranging from -1 to +7.
Although the importance of the lower oxidation states of technetium-99m in nuclear medicine was emphasized as early as 1965 by Harper and coworkers[4], the preparation of simple technetium-99m salts in these oxidation states of the element has so far not been reported. The usual reducing agent , stannous chloride, commonly used for the preparation of technetium-99m radiopharmaceuticals in the reduced oxidation state has some undesirable properties: (1) it is a strong reducing agent, (2) it forms metal-metal bonds with the element it reduces[5], and (3) it complicates the distribution of technetium-99m in the body[6]. The reduction of pertechnetate-99 ion by hydrohalic acids has been extensively studied and the preparation of chromatographically pure technetium-99(V) and technetium-99(IV) described[3]. In the present work the reduction of pertechnetate-99m ion by concentrated hydrochloric acid has been studied chromatographically and the stability of the reduced product studied.
The reduction of pertechnetate-99m ion by concentrated hydrochloric acid is very rapid in comparison to that of pertechnetate-99 ion under the same condictions. Thus, while the reduction of the solution of pertechnetate-99 ion to technetium-99(IV) by concentrated hydrochloric acid at room temperature was not complete even after aging for 2 years, the pertechnetate-99m ion is reduced to chromatographically pure technetium-99m(IV) after 10 hours. Although the reduction of the pertechnetate-99m ion proceeds through technetium-99m(V) as an intermediate step, the isolation of this intermediate species was not possible because of

the rapidity of the reaction. At 70 °C the reduction to technetium-99m(IV) is accomplished after heating the solution for 3 hours. At 80 °C considerable loss of technetium-99m(IV) takes place due to volatilization. The solution of technetium-99m (IV) is stable in hydrochloric acid and in the physiological saline which permits its injection for the study of its physiological behaviour.
The distribution of the technetium-99m (IV) chloride complex in healthy and Morris hepatoma-3924-bearing rats is being studied.

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Paper No.12

SOLID PHASE REDUCTANTS FOR THE PREPARATION OF Tc-99m RADIOPHARMACEUTICALS.

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Several Sn(II)-saturated chelating resins have been used for the preparation of Tc-99m-labeled agents. Since the reductant does not compete with the radioisotope for available binding sites, this method should be useful where quantities of the compound to be labeled must be severely restricted due to short supply or toxicity.

Chelating resins used in this study included Corning ethylene diaminetriacetic acid bonded to controlled pore (550 Å) glass beads (ED3A-CPG550), 8-hydroxyquinoline-CPG550, and the chelating resin Biorad Chelex 100. The reductant is prepared by equilibrating a solution of excess stannous glucoheptonate in acetate buffer with the chelating resin. The resin is then washed with buffer and distilled water, dried, and stored under nitrogen. A similar preparation of Sn(II)-Chelex 100 was recently described by Basmadjian, et. al. (1).

In order to produce a labeled agent, a mixture of the desired compound and Tc-99m-pertechnetate is added to the reductant and equilibrated for 5 minutes. Experiments with Sn-113 show that elution of Sn from the resin is below 0.1 μg Sn.

Following this method, 0.5 mg of Sn(II)-saturated ED3A-CPG550 was used to label 1 mg of human serum albumin (HSA) with 10 mCi of Tc-99m. Chemical and biological properties of the resulting Tc-99m-HSA were identical to those of Tc-99m-labeled HSA prepared from kits containing 25 mg HSA and 0.1 mg stannous chloride dihydrate. Similarly, "Sn(II)-free" Tc-99m-labeled pyrophosphate, methylene diphosphonate (MDP), glucoheptonate, and HIDA have been prepared and shown to be identical to corresponding Sn(II) containing preparations.

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TABLE 1. Sn-CAPACITY OF IMMOBILIZED REDUCTANTS
Data From Experiments Using Sn-113

Chelating Resin	Sn-Capacity, μg Sn/mg Dry Resin*	n
ED3A-CPG550	2.0 ± 0.6	15
8HQ-CPG550	2.8 ± 0.2	4
Chelex 100	2.6 ± 0.5†	4

*Mean ± standard deviation.
† Based on wet weight of resin (~ 70% H_2O by weight).

TABLE 2. Tc-99m LOSSES ON IMMOBILIZED REDUCTANT
Tc-99m-pertechnetate (~ 10 mCi) and variable quantities of HSA were equilibrated with Sn(II)-ED3A-CPG550 to demonstrate competition of resin for Tc-99m.

Sn(II)-ED3A-CPG550, mg	HSA, mg	% TcO_4^- on Resin
100	1	84
20	1	70
5	1	30
1	1	14
0.5	1	10
20	20	8
20	10	20
20	5	35
20	1	70

TABLE 3. COMPARISON OF "Sn(II)-FREE" AND Sn(II)-CONTAINING Tc-99m HSA

Lot Number	FP1069/67-7	CARDIOLITE™, Lot 8002
HSA, mg	1.0	25.0
Reductant	0.5 mg Sn(II)-ED3A-CPG550	0.10 mg $SnCl_2 \cdot 2H_2O$
% Free TcO_4^-	4.0	0.0
Biodistribution in Mice, 3 Hours After IV Injection, % Injected Dose/Organ		
Blood*	35.0	37.3†
Liver	11.8	8.3
Kidneys	10.7	11.4
Stomach	0.4	0.4
Intestines	5.2	6.2

*Based on 5% body weight.
† Mean of 3 animals.

Paper No.13

THE PREPARATION AND CRYSTAL STRUCTURE OF OXOTECHNETIUM bis (THIOMERCAPTOACETATE) AND ITS RELATIONSHIP TO RADIOPHARMACEUTICALS LABELED WITH Tc-99m.

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No fully characterized discrete coordination compounds of technetium having sulphur donor atoms have been previously reported. Several radiopharmaceutical preparations involving potential sulphur donor ligands, however, have been proposed or are in current clinical use (1-6).

We report the synthesis, isolation and characterization of the novel five coordinate Tc(V) compound $(Bu_4N)[TcO(SCH_2COS)_2]$ (complex I). A single crystal X-ray structural determination of complex I was performed (7); the structure of the anion is shown in Figure 1. The technetium is coordinated to an oxygen and four sulphur atoms in an idealized square pyramid, with the sulphur atoms forming the basal plane and the oxygen the apex. The metal-oxygen distance is 1.672(8) angstroms, and the average technetium to sulphur distance 2.320(3) angstroms. The metal atom lies 0.791 angstroms above the basal plane.

The complex was synthesized by the reduction of TcO_4^- ion with a large molar excess (> 100) of aqueous thioglycolate (SCH_2COO^-) at pH 7.5, and isolated as the tetrabutylammonium salt.

The formation of this and related compounds (8) shows conclusively that (+5) oxotechnetium complexes are stable in aqueous media. Furthermore, the avidity of the technetium for an impurity in commercially available thioglycolate solutions suggests that considerable caution be exercised in assuming that a particular Tc-99m labeled radiopharmaceutical is simply a complex of the intended ligand.

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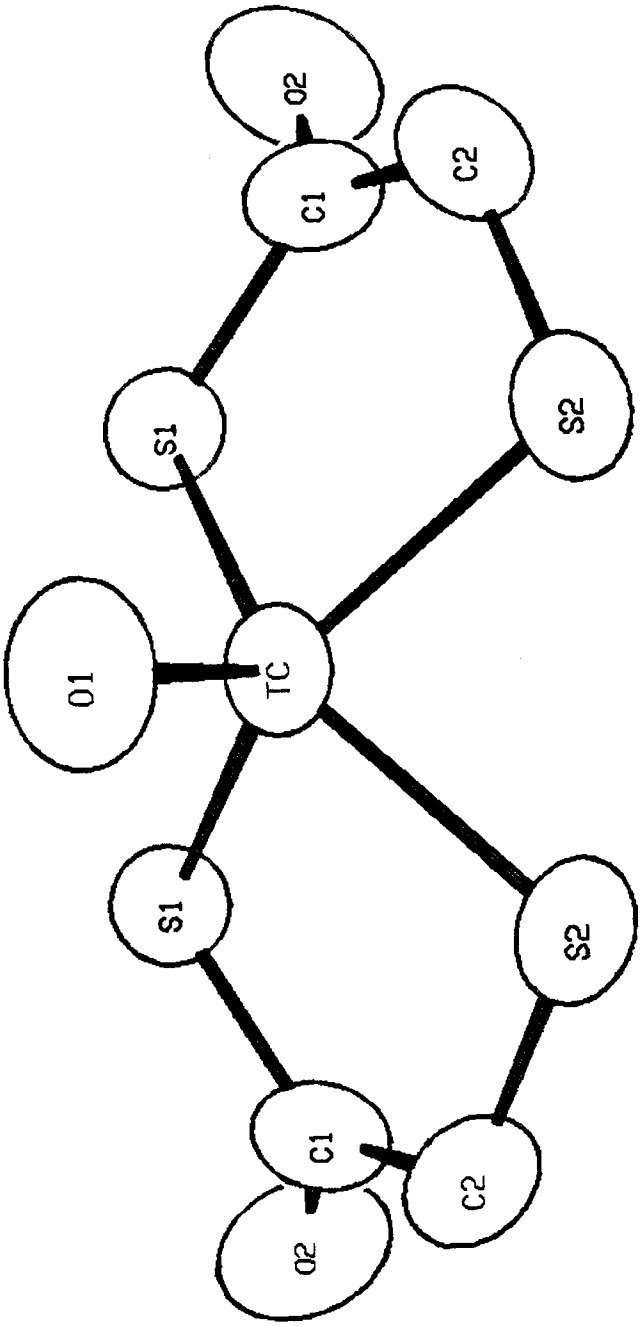
O2
C1
C2
S1
S2
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TC
S1
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C1
C2
O2

Paper No.14

THE DEVELOPMENT OF SELENIUM-75 CHOLESTEROL ANALOGUES

A. L. M. Riley
The Radiochemical Centre, Amersham, England.

Novel selenium-75 labelled cholesterol derivatives were prepared as part of a development programme assessing the value of selenium-75 labelled steroids as radiopharmaceuticals. General synthetic methods employed (1) for the labelling of steroids with selenium-75 include:

(i) Insertion of selenium-75 by action of [^{75}Se]-selenium dioxide on α, β unsaturated keto steroids

$$R\ CH_2CH_2CO\ CH = R' \xrightarrow[\text{heat}]{SeO_2, CH_3COOH} [R\ CH = \underset{\underset{Se^*}{|}}{CH}\ CO\ CH = R']_2$$

(ii) Displacement of suitable groups with selenium-75 labelled nucleophiles

$$RR'Y \xrightarrow{R''Se^-} R\ R'\ Se\ R'' + Y^-$$

(iii) Indirect labelling by coupling selenium-75 containing moieties to the steroid nucleus.

e.g. $RR'C{=}N\,O\,CH_2COOH \xrightarrow[(2)\ NH_2(CH_2)n\ Se\ R'']{(1)\ \text{mixed anhydride formation with iso-butylchloroformate}} R\,R'\,C{=}N\,O\,CH_2\ CON(CH_2)_nSe\ R''$

RR' ≡ the remainder of the steroid molecule

Y ≡ halo, tosyloxy, mesyloxy

R'' ≡ alkyl

It has already been demonstrated that radioiodine labelled cholesterol analogues are valuable for imaging the adrenal glands by gamma scintigraphy (2,3). However it was considered that their selenium-75 labelled counterparts would have a number of advantages including improved stability, lower β-particle emission and a better range of photon energies.

Route (ii) was selected as a general method for the preparation of seleno-cholesterols. This involved the preparation of a non-radioactive halocholesterol precursor (3,4) followed by substitution of the halo atom with the appropriate alkylseleno or arylseleno group. Purification of the reaction mixtures was accomplished by preparative layer chromatography, the required products being located by autoradiography. Acetoxy derivatives were prepared from the sterols by treatment with pyridine-acetic anhydride mixtures.

All the compounds prepared showed high affinity for the rat adrenal. Compound 1 was subsequently developed as an adrenal scintigraphic agent.

	Compound	Concentration in rat adrenals % kg dose per gram 6 days
1	6β-methyl-[^{75}Se]-selenomethyl 19-norcholest-5(10)-en-3β-ol	14.4
2	6β-methyl-[^{75}Se]-selenomethyl-19-norcholest-5(10)-en-3β-ol-acetate	12.5
3	6β-butyl-[^{75}Se]-selenomethyl-19-norcholest-5(10)-en-3β-ol	7.5
4	6β-cyclohexyl-[^{75}Se]-selenomethyl-19-norcholest-5(10)-en-3β-ol	3.6
5	6β-benzyl-[^{75}Se]-selenomethyl-19-norcholest-5(10)-en-3β-ol	5.2
6	6β-phenyl-[^{75}Se]-selenomethyl-19-norcholest-5(10)-en-3β-ol	7.3
7	19-methyl-[^{75}Se]-selenocholesterol	5.6
8	19-methyl-[^{75}Se]-selenocholesteryl acetate	6.3

Apart from the proven utility of 1 as an adrenal scintigraphic agent in man, selenium-75 cholesterol analogues may be of value as tracers for cholesterol in the human body.

References

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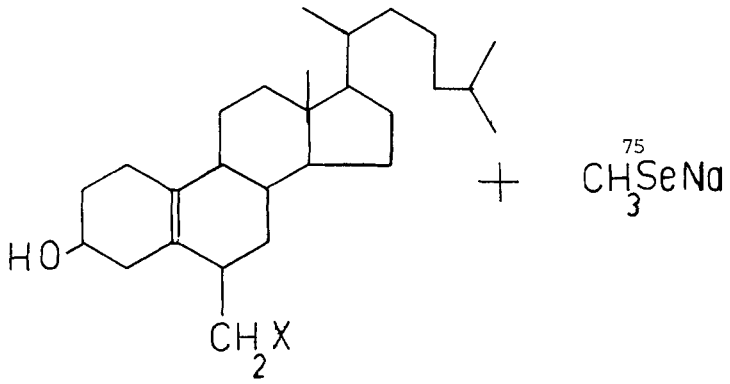
(2) Beierwaltes W.H., Lieberman L.M., Ansari A.N. and Nishiyama H., Visualization of human adrenal glands in vivo by scintillation scanning, J.Amer.Med.Assoc., 216, 275 (1971).
(3) Kojima M., Maeda M., Ogawa H., Nitta K. and Takayuki I., New adrenal scanning agent, J.Nucl.Med., 16, 666 (1975).
(4) Dannenberg H., Neumann H.G. and Dannenberg von Dresler D., Liebigs Ann. Chem., 674, 152 (1964).

Synthesis of 6β-methyl-[^{75}Se]-selenomethyl-19-norcholest-5(10)-en-3β-ol

1. $^{75}Se \xrightarrow[\text{anhydrous ammonia}]{Na} Na_2{}^{75}Se_2 \xrightarrow[\text{anhydrous ammonia}]{CH_3I} (CH_3{}^{75}Se)_2 + NaI$

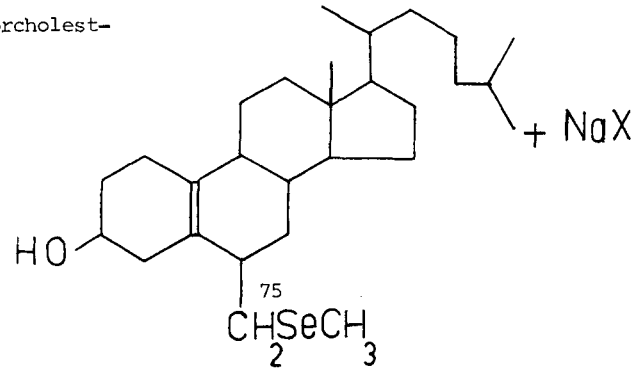
$$\xrightarrow[\text{anhydrous ammonia}]{Na} CH_3{}^{75}SeNa + NaI$$

2.



6-β-iodomethyl-19-norcholest-5(10)-en-3β-ol

inert atmosphere
inert solvent



X ≡ halogen

Paper No.15

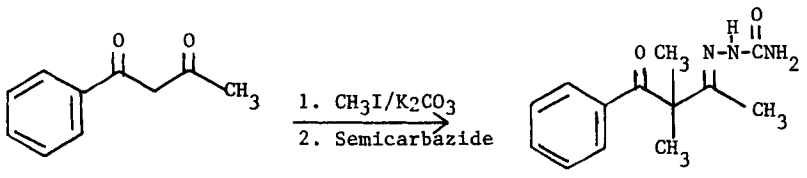
POTENTIAL ADRENAL IMAGING AGENTS. SYNTHESIS AND BIODISTRIBUTION OF 1,2,3-SELENADIAZOLES.

Robert N. Hanson and Michael A. Davis, Joint Program in Nuclear Medicine, Harvard Medical School, Boston, MA 02115.

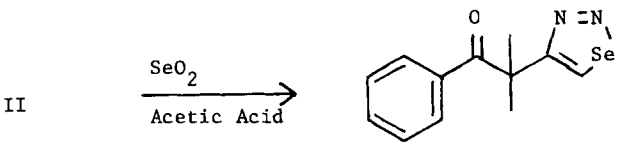
The currently used adrenal imaging agents such as ^{131}I-19-iodocholesterol and ^{131}I-6-β-iodomethyl-19-norcholesterol (NP-59) are structurally related to cholesterol. They have several clinical disadvantages including *in vivo* deiodonation and delayed adrenal localization (1,2). Recent studies by Beierwaltes, et al. have demonstrated that inhibitors of corticosteroid biosynthesis display more rapid and quantitatively greater uptake in the adrenal cortex than do these steroid derivatives (3). Although ^{3}H-metyrapone and ^{3}H-metyrapol possess excellent uptake properties, no γ-emitting analogs of these compounds have been reported. Structure-activity studies by Napoli and Counsell indicated that 11β-hydroxylase inhibitory activity was retained when the pyridine adjacent to the carbonyl was replaced by a phenyl ring and was lost when the pyridine adjacent to the gem-dimethyl was so replaced (4). We have chosen to replace the pyridine ring adjacent to the gem-dimethyl with another electron deficient ring system, the 1,2,3-selenadiazoles into which ^{75}Se can be introduced (5).

The desired products are prepared starting from the appropriate 1,3-diketones. Our initial study utilized benzoylacetone (I) which was converted to the dimethyl product in 80% yield with methyl iodine and potassium carbonates. The condensation with semicarbazide proceeds at the less hindered carbonyl to give the monosemicarbazone (II) in 60% yield. The use of one equivalent of selenium dioxide in acetic acid gives a 30% yield, after recrystallization, of the selenadiazole (III), with the remainder of the mixture being unreacted starting material. On a small scale (20μmoles) introduction of $H^{75}SeO_3^-$ into the last step provides the ^{75}Se-labeled material in 10-20% radiochemical yield and 98% radiochemical purity. The initial biodistribution of this product, administered i.v., has been examined in normal rats.

(1) Maeda M., Kojima M., Ogawa H., et al., Steroids 26, 241 (1975).
(2) Counsell R.E., Ranade V.V., Blair R.J., et al., Steroids 16, 317 (1970).
(3) Beierwaltes W.H., Wieland D.M., Ice R.D., et al., J. Nucl. Med. 17, 998 (1976).
(4) Napoli J.L. and Counsell R.E., J. Med. Chem. 20, 762 (1977).
(5) Lalezari I. and Shafree A., Tet. Lett., 5105 (1969).



I II (48%)



III (30%)

^{75}Se-III 30 mCi/mmol

Paper No.16

POTENTIAL Te-123m RADIOPHARMACEUTICALS: A SIMPLE METHOD FOR THE INTRODUCTION OF Te-123m INTO ORGANIC COMPOUNDS

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University of Oklahoma Health Sciences Center, College of Pharmacy, Oklahoma City, Oklahoma 73190.

There is a need for a photon emitting radionuclide that can be introduced into organic molecules so that the entire spectrum of organic compounds will be available as potential radiopharmaceuticals. Tellurium-123m possesses good physical properties for γ-camera imaging (84% of 159 KeV) with a half life (117d) sufficiently long to allow extensive synthesis, and is capable of forming covalent bonds.

Tellurium-123m has been incorporated into steroids by Knapp and Callahan (1) utilizing a cumbersome method which generates disodium ditelluride *in situ* by the reaction of sodium and tellurium in liquid ammonia (2). We wish to report a simple method for the incorporation of tellurium into organic molecules which can be completed in less than three hours using metallic tellurium and sodium borohydride ($NaBH_4$) in absolute ethanol. This method is suitable for radioactive tellurium synthesis. The sequence of the reaction is:

$$\underset{}{Te} + NaBH_4 \xrightarrow[\Delta\ /N_2]{EtOH} \underset{I}{NaTe\text{-}TeNa} \xrightarrow{NaBH_4} \underset{II}{NaTeH} \xrightarrow{NaBH_4} \underset{III}{H_2Te}$$

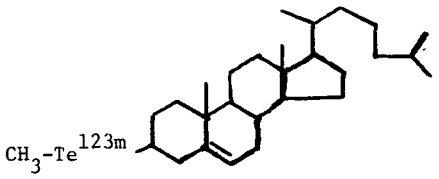
(deep-red) (colorless) (gas)

The generation of intermediates I and II *in situ* is dependent on the amount of $NaBH_4$ and the reaction was not carried out under stoichiometric conditions since the $NaBH_4$ reacts with the solvent (EtOH) under reflux. When excess $NaBH_4$ is used, the reaction equilibrium favors the formation of the gas, hydrotellurol (H_2Te, III), which is not desirable for this particular radiochemical synthesis. Precautions should be taken to avoid the evolution of the radioactive gas during the synthesis by: 1) the addition of sodium ethoxide at the beginning of the reaction, and 2) allowing the reaction to proceed only to the solubilization of the metal with the concurrent appearance of a deep-red color. An aqueous iodine solution is used as a trap for any radioactive tellurium gases that are formed. Thus, the desired reactive intermediate disodium ditelluride (I) is generated *in situ* and used in nucleophilic displacement reactions similar to the incorporation of Se-75 into organic molecules (3,4) as shown:

$$\underset{I}{NaTe\text{-}TeNa} \xrightarrow{R\text{-}Y} \underset{IV}{R\text{-}Te\text{-}Te\text{-}R} \xrightarrow{NaBH_4} \underset{V}{RTeH} \xrightarrow{NaOEt} \underset{VI}{RTeNa} \xrightarrow{R'\text{-}Y} \underset{VII}{R\text{-}Te\text{-}R'}$$

The dialkyl ditelluride (IV) formed from the nucleophilic displacement of a good leaving group (Y in R-Y) is reduced with $NaBH_4$ to produce the alkyl tellurol (R-TeH, V). Upon the addition of sodium ethoxide, compound V is converted to the nucleophile VI, which reacts with R'-Y to form the stable dialkyl telluride (R-Te-R', VII).

This procedure was used to synthesize 3-methyl-telluro-cholesterol (VIII), and its Te-123m analog as a potential adrenal scanning agent. Cholesterol-3-p-tosylate (R=cholesterol, Y=p-tosylate) was used in the reaction sequence above.



VIII

The non-radioactive compound was characterized by NMR, IR, mass spectroscopy, atomic absorption and elemental analysis, and behaves the same as the Te-123m analog in chromatographic analysis.

(1) Knapp, Jr., F.F. and Callahan, A.P., J. Nucl. Med., 18, 610(1977).
(2) Chen, M.T. and George, J.W., J. Organometal. Chem. 12, 401(1968).
(3) Basmadjian, G.P., Hetzel, K.R., Ice, R.D., Int. J. of Appl. Rad. and Isotopes, 26, 695(1975).
(4) Sarkar, S.D., Ice, R.D., Beierwaltes, W.H., Gill, S.P., Balachandran, S.B. and Basmadjian, G.P., J. Nucl. Med., 17, 212(1976).

Paper No.17

THE EFFECTS OF NUCLEAR AND SIDECHAIN MODIFICATIONS ON THE ADRENAL CONCENTRATION OF STEROIDS LABELED IN THE SIDECHAIN WITH Te-123m*†

F. F. Knapp, Jr., K. R. Ambrose and A. P. Callahan. Nuclear Medicine Technology Group, Health and Safety Research Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830.

Earlier investigations have demonstrated (1) the chemical synthesis of Te-123m labeled 3β-hydroxy-24-nor-23-(isopropyl telluro)-5α-cholane (I). The adrenals of rats and rabbits have been clearly imaged following intravenous administration of this new agent and detailed tissue distribution, excretory and metabolic studies with this compound have been reported in rats (2).

The general method that was developed for the introduction of Te-123m into the sidechain of (I) can be adapted for the preparation of a wide variety of steroids. These methods are thus uniquely suited for an investigation of the effect of steroid structure on the ability of the rat adrenal to concentrate such substances. Various structural modifications of the steroid nucleus and sidechain were envisioned which could give a general indication of the structural features which determined the uptake of such compounds by the adrenal. In addition, the results of such studies could possibly be used to design a Te-123m labeled steroid that would exhibit maximal adrenal uptake at early time intervals. The general synthetic strategy involved the following transformations:

$$Te + Na \rightarrow Na_2Te_2 \rightarrow R_2Te_2 \rightarrow R\text{-}Te\text{-}Na + Br\text{-steroid} \rightarrow \text{steroid-}Te\text{-}R.$$

Bile acids were used as convenient starting materials and were converted to the requisite halogenated steroid substrates by either of the following routes:

$$\text{Steroid}(CH_2COOH) \xrightarrow[\text{Hünsdiecker}]{\text{Modified}} \text{Steroid}(CH_2Br)$$

$$\text{Steroid}(CH_2COOH) \xrightarrow{\text{Reduction}} \text{Steroid}(CH_2CH_2OH) \xrightarrow[CBr_4]{(Ph)_3P} \text{Steroid}(CH_2CH_2Br).$$

The halogenated steroid substrates were then coupled with selected alkyl tellurols (R-Te-H) which were generated by sodium borohydride reductive cleavage of dialkyl ditellurides. In this manner a group of telluro steroids encompassing a variety of structural features was prepared:

3α-Hydroxy-24-nor-23-(isopropyl telluro)-5β-cholane (II) (cis A/B ring juncture)

3β-Hydroxy-17β-[(isopropyl telluro) methyl]-androst-5-ene (III) (short sidechain)

3β-Hydroxy-24-nor-23-(octyl telluro)5α-cholane (IV) (long, hydrophobic sidechain)

3β-Methoxy-24-(isopropyl telluro)-chol-5-ene (V) (hydrophobic C-3 substituent)

3β-Hydroxy-24-(isopropyl telluro)-chol-5-ene (VI) (nuclear 5(6)-double bond).

These steroids were analyzed by ultraviolet, infra-red, nuclear magnetic resonance and low and high resolution mass spectral methods. The physical and chemical properties of the synthetic products were consistent with the proposed structures. The Te-123m labeled steroids were prepared and purified by silicic acid column chromatography. Detailed tissue distribution and excretion experiments and rectilinear scanning and camera studies were then performed in female Fisher strain rats. The results of these investigations indicated a complex relationship between steroid structure and the relative rates of entry and exit of the Te-123m labeled steroids from the various body compartments. Steroids (II) and (III) showed essentially no adrenal uptake while steroids (IV) and (V) accumulated very slowly in the adrenals. Steroid (VI) showed a slightly greater adrenal uptake than (I) at all time intervals studied. Both (I) and (VI) showed significant medullary concentration and medullary/cortical ratios (calculated from % dose/gm tissue data) in female Fisher rats were as follows: (I), 2.34 ± 0.78; (VI), 2.07 ± 0.24.

The combined results of these studies have clearly demonstrated that a combination of structural features are required for maximal adrenal uptake of steroids containing the tellurium heteroatom in the sidechain. In the series of compounds investigated these requirements include: a planar (all trans) ring structure, a free C-3 hydroxyl group, and a sidechain of moderate length.

(1) Knapp F. F. and Callahan A. P., J. Nucl. Med. 18, 610 (1977).

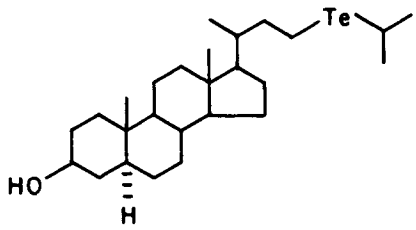
(2) Knapp F. F. and Ambrose K. R., J. Nucl. Med. 18, 600 (1977).

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

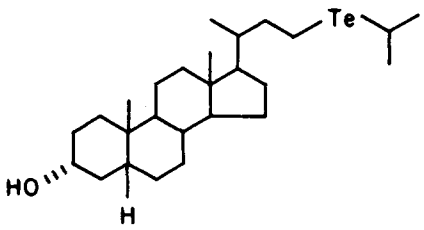
Adrenal/Liver and Adrenal/Blood Ratios After Intravenous Administration of Te-123m Labeled Steroids†

Te-123m Labeled Steroids	Adrenal/Liver			Adrenal/Blood		
	1 Day	3 Days	7 Days	1 Day	3 Days	7 Days
I	26.9	36.8	63.5	43.7	53.2	53.0
II	4.9	7.2	12.7	11.2	11.7	15.2
III	1.8	2.2	1.7	4.3	3.9	1.9
IV	4.9	9.6	24.7	8.3	12.4	28.8
V	5.0	9.8	12.5	23.1	29.7	26.7
VI	27.1	47.9	66.9	54.8	96.1	106.4

†Ratios calculated from (% dose/gm of tissue) and (% dose/ml of blood) data.



I



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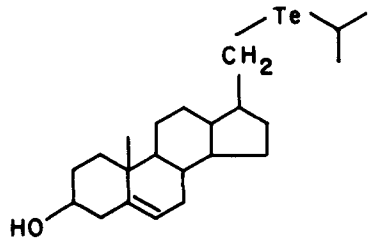
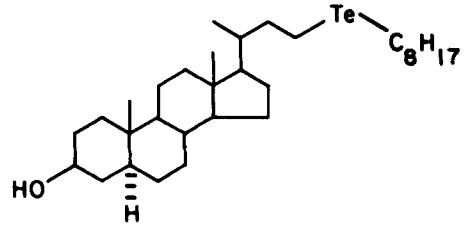
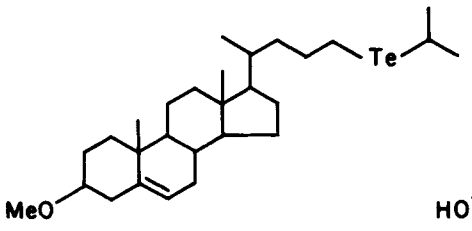


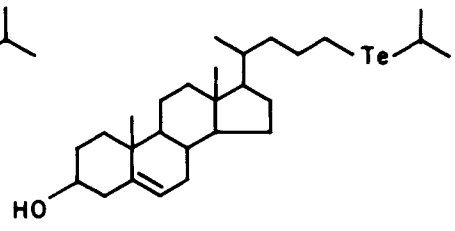
III



IV



V



VI

Paper No.18

BILE ACID ANALOGUES LABELLED WITH SELENIUM-75 OR TELLURIUM-123m

G.S. Boyd, M.V. Merrick, R. Monks, and I.L. Thomas.
The Radiochemical Centre, Amersham, Buckinghamshire, England.

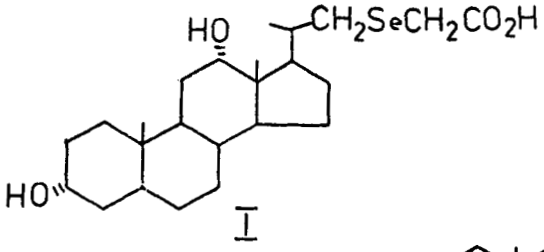
It is possible that bile acid analogues labelled with suitable gamma emitting nuclides could have important *in vivo* and *in vitro* applications in measurements of function in the entero-hepatic system.

19-methyl-[^{75}Se] seleno-cholesterol, which was found to be unsuitable as an adrenal visualisation agent in humans, was used for the biosynthesis of a mixture of C_{19} ^{75}Se-labelled bile salts. The cholesterol analogue was administered to a rabbit using a bile fistula, and the bile was subsequently collected and treated chemically to give a crude mixture of ^{75}Se-bile acid conjugates and natural bile salts. The mixture was further purified by preparative thin-layer chromatography. The product behaved as a mixture containing amino acid conjugates of ^{75}Se bile acids as shown by its chromatographic behaviour and its behaviour as a substrate for the enzyme cholylglycine hydrolase.

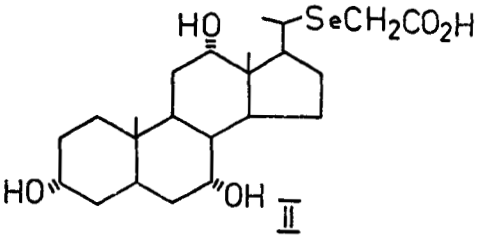
Subsequently a selected range of bile acid analogues were chemically synthesised, labelled in the C_{17} side chain with ^{75}Se or ^{123m}Te. The following are examples: 3α,12α-dihydroxy-22-(carboxymethyl-[^{75}Se] seleno)-23,24-bis nor-5β-cholane,(23-[^{75}Se]selena-25-homodeoxycholic acid) (I); 3α, 7α,12α-trihydroxy-20-(carboxymethyl-[^{75}Se]seleno)-5β-pregnane, (22-[^{75}Se] selenacholic acid) (II); 3α,7α-dihydroxy-23-(β-carboxyethyl-[^{75}Se]seleno)-24-nor-5β-cholane (III); 3α-hydroxy-24-(carboxymethyl-[^{75}Se]seleno)-5β-cholane (IV); 3α,12α-dihydroxy-23-(carboxymethyl-[^{123m}Te]telluro)-24-nor-5β-cholane (V).

Compounds of the type illustrated by I, III and IV were produced by the reaction of an appropriate iodo- or bromo-derivative of the suitably substituted modified 5β-cholane with an ω-selenocyanatocarboxylic acid (or its ethyl ester) and sodium borohydride in ethanol or ethanol/tetrahydrofuran at 0°C (Scheme 1). The products obtained were subjected to alkaline hydrolysis to afford the bile acid analogues, which were purified by preparative thin layer chromatography on silica gel. Compound II was obtained by reaction of 3α,7α,12α-triformoxy-20-iodo-5β-pregnane with disodium diselenide in 1-propanol at ca 90° for 3 hours. The dipregnane diselenide thus obtained was not isolated but was subjected to reductive cleavage with sodium borohydride and the resulting selenol was alkylated with ethyl bromoacetate. 22-[^{75}Se] selenacholic acid (II) was isolated after alkaline hydrolysis. Following a similar procedure, the ^{123m}Te derivative (V) was obtained by the synthesis of disodium ditelluride in liquid ammonia, its reaction with iodoacetic acid, cleavage of the resulting ditelluride with alkaline dithiothreitol and reaction with 3α, 12a-diformoxy-23-iodo-24-nor-5β-cholane in dimethylformamide at 60°. The structures of the products were confirmed using IR and NMR spectroscopy.

Work on labelling bile acid analogues in alternative positions, and biological evaluation of the compounds, is in progress.


HO
CH2SeCH2CO2H
HO

I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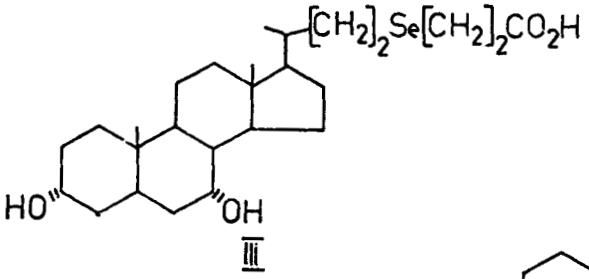
SeCH2CO2H
HO
OH

II


[CH2]2Se[CH2]2CO2H
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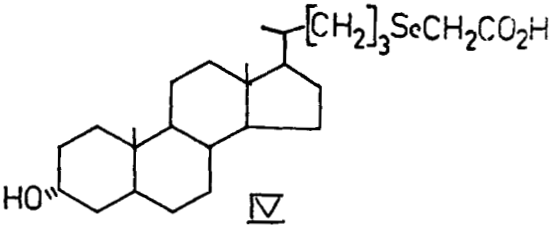
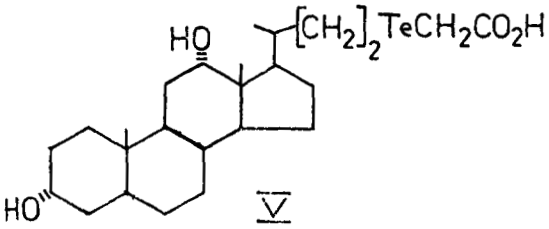
OH

III

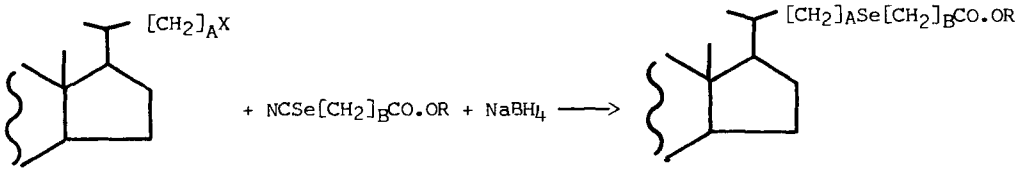

[CH2]3SeCH2CO2H
HO

IV


HO
[CH2]2TeCH2CO2H
HO

V

Scheme 1



Where X = Br or I; R = H or C_2H_5

A = 1, 2 or 3; B = 1 or 2

Paper No.19

SELENIUM-75 LABELED HYPOTAURINE FOR MYOCARDIAL AND PANCREATIC IMAGING

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Selenohypotaurine has been labeled with Se-75 to study its localization in pancreatic and heart tissue. The labeled amino acid was prepared by reducing selenous acid with sodium borohydride, reacting the product with 2-bromoethylamine under nitrogen and finally oxidizing with aqueous bromine. The labeled product was separated by cation exchange and monitored throughout by HPLC.

Se-75 hypotaurine was injected into mice and distributions in 8 tissue types and blood determined at 15 and 60 min. The uptake at 60 minutes in percent administered dose per gram was found to be 1.28 ± 0.17 (heart), 2.81 ± 0.26 (lung), and 5.47 ± 0.65 (liver) and was significantly different from that of Se-75 selenous acid.

Pancreas/liver ratios (per gram) were determined in rats for both Se-75 labeled selenohypotaurine and selenomethionine. At 30 min. post injection the ratios were 2.59 ± 1.30 and 2.45 ± 1.29 respectively.

Images obtained at 30-60 min. post injection on a gamma camera in rabbits injected with either agent were comparable in pancreatic visualization; the heart was clearly visible with selenohypotaurine. These results suggest that Se-75 selenohypotaurine may be useful for myocardial imaging, in addition, because of its ease of preparation, Se-75 hypotaurine may be a useful alternative to selenomethionine for pancreatic imaging.

(This work supported in part by ERDA (E(11-1)-4115)).

Paper No.20

SITE OF IODINATION OF BLEOMYCIN

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Bleomycin is a mixture of structurally similar basic glycopolypeptides (MW≈1400) which exhibit antineoplastic activity. It chelates efficiently with divalent cations and this property has been used to label it with gamma emitting metal nuclides as a potential tumor-localizing radiodiagnostic agent. Bleomycin contains no tyrosyl residues but it contains one β-hydroxyhistidyl residue and one pyrimidine residue; however the later is methylated at carbon 5, the potential site for electrophilic halogenation.

The iodination of bleomycin was first reported by Renault et al (1), who used the chloramine-T method. They found that the iodinated bleomycin dehalogenated rapidly. From previous studies of chloramine-T iodination chemistry we had reason to believe that other methods of iodination could yield more stable histidyl-iodine bonds (2) and reported the preparation of stable *I-bleomycin by the iodine monochloride reaction (3).

While we presumed that the imidazole ring of the centrally located β-hydroxyhistidine was the site of iodination, our attempts to prove this by several different proteolysis/hydrolysis methods were inconclusive. The iodine-bleomycin bond invariably hydrolyzed more rapidly than the peptide bonds, even at neutral pH. We therefore turned to ^1H nuclear magnetic resonance spectrometry as a probe to identify the reaction site. These measurements and their interpretation are described in this report.

Iodine monochloride (300 µl, 0.011 mmoles of ICl in 0.01M DCl/D_2O) was neutralized with 0.1N NaOD in D_2O. A few µCi of tracer iodine was added followed immediately by 15 mg of bleomycin sulfate. The solution was stirred 1 hr at room temperature, followed by the addition of 50 µl D_2O containing 1% DSS internal standard. The resulting pH was carefully measured and was somewhat below neutral, owing to the acidity of bleomycin sulfate. An aliquot of the reaction mixture was analyzed by electrophoresis (15 cm cellulose polyacetate strips, pH 8 buffer, 300 V, 8 min) to separate iodide from covalently bound iodine. The ^1H nmr spectra were obtained from a JEOL Fourier Transform nmr spectrometer optimized for proton spectra.

The ^1H nmr spectrum of bleomycin contained four major peaks in the aromatic region, one each from the #2 and #5 positions of the imidazole group and two from the two thiazole groups. The chemical shifts of the two thiazole protons were 8.22 and 8.05 ppm at neutral pH and were not significantly affected by changes in pH; however, they were affected by the neighboring terminal amine residues which varied with the different forms of bleomycin. The bleomycin which we used was 70% A_2 and 30% B_2 and we detected two distinct peaks for each thiazole proton. They were separated by about 0.02 ppm and had relative areas of about 2:1. The imidazole protons were single peaks because they are far from the regions of chemical variability of bleomycin. They are, however, affected by changing pH, the #2 proton being the most strongly affected. The relative areas of the imidazole proton peaks were 49.9% and 44.2% for the #2 and #5 positions, respectively, compared to the total area for the two thiazole protons from bleomycin.

When 0.011 mmoles of bleomycin reacted with 0.011 mmoles of ICl, the relative areas of the #2 and #5 imidazole protons decreased to 35.5% and 34.4% normalized to the thiazole area. These are reductions of 28.8% and 22.2% from their size in bleomycin and suggest that an average of 0.5 of the two imidazole protons have reacted with iodine. Electrophoresis of the same

sample showed 40% of the radioactivity as free iodide ion and 60% covalently bound, implying that an average of 0.6 iodine atoms were bound per bleomycin molecule. In three experiments within the pH range 4.5 to 7.0, the areas for both proton peaks were affected approximately equally and there was agreement between electrophoretic yields and nmr peak areas.

Having determined that iodination causes a reduction in size of both imidazole proton resonances, we wished to determine whether the product was diiodobleomycin or two different isomers of monoiodobleomycin. Iodination within aromatic rings may influence the chemical shift of neighboring protons. The chemical shift for the #2 protons of histidine and bleomycin were within 0.05 ppm of each other at pH 4.5, whereas the chemical shift for the corresponding proton of 5-iodohistidine was 0.5 ppm lower. Assuming that this relationship holds for the iodinated β-hydroxyhistidine within bleomycin, one would expect to see the peak for the #2 proton of monoiodobleomycin move ≈0.5 ppm lower than the corresponding peak for unmodified bleomycin at the same pH. Since no peak was present in this region and since both imidazole proton peaks decreased in size by the same amount when bleomycin reacted with ICl, we conclude that the product is primarily a diiodoimidazole derivative rather than an equimolar mixture of both monoiodinated derivatives.

These results represent a variation from iodination of histidine, perhaps caused by an activating effect of the hydroxy group alpha to the aromatic ring. The covalent binding of ICl to bleomycin is all accounted for by reaction with the imidazole ring. The reaction of chloramine-T with bleomycin undoubtedly involves a different site of electrophilic attack which we have not yet identified (4). This study emphasizes that there are distinct chemical differences in the various reactions for electrophilic iodination and that these differences become more significant when labeling less reactive (ie. non-tyrosyl) residues.

This research was funded in part by Cancer Research Funds of the University of California and by the American Cancer Society (PDT-94A).

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Paper No.21

SYNTHESIS AND BRAIN UPTAKE OF ^{123}I-LABELLED 4-IODO-2,5-DIMETHOXYPHENYLISOPROPYLAMINE

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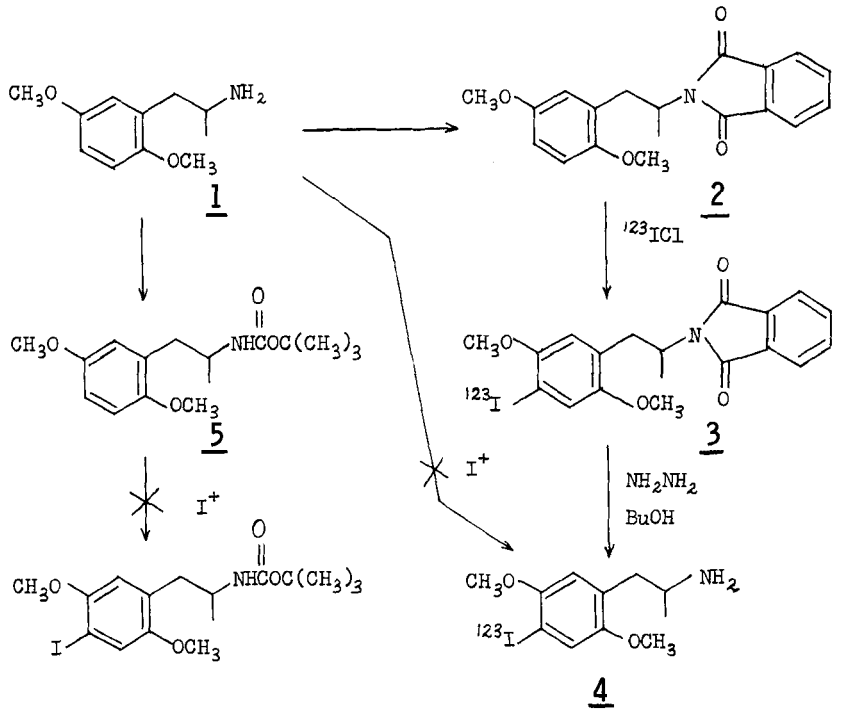
4-Bromo-2,5-dimethoxyphenylisopropylamine (DOB) is known to be a centrally-active agent in man (1) and has been found, through labelling experiments employing ^{77}Br and ^{82}Br, to be actively taken up in the brain and lung of human subjects (2). The relatively high energies of the gamma radiation of these two isotopes limit their usefulness as visualization agents in scintigraphic studies. Of the available isotopes of iodine, ^{123}I emits a 159 keV gamma ray, ideal for imaging purposes, and has a ratio of useful gamma rays to tissue dose some 50 times greater than that of ^{131}I, an important consideration for studies in human subjects. These properties prompted the synthesis of the iodine analog of DOB, 4-iodo-2,5-dimethoxyphenylisopropylamine (DOI, 4) using ^{123}I. However, its relatively short half-life (13 hr) required an appropriately rapid synthetic method, the subject of this report.

The direct iodination of 2,5-dimethoxyphenylisopropylamine 1 was tried employing a variety of procedures. The attempted direct halogenation of the amine salt of 1 with I^+ or ICl, a procedure which is successful with



elemental bromine (1) and chlorine (3) leads to preferential oxidation of the amine function. Amine derivitization as the acetamide provided protection against oxidation, but deacetylation of the iodinated intermediate could not be achieved with an acceptable yield and speed (4). Protection of the amine was also possible with the easily removable tertiary butyl carbamate, but this "t-BOC" derivative could not be successfully iodinated under neutral conditions, employing the usual forms of I^+. More acidic iodination conditions invariably hydrolyzed the carbamate linkage of 5 preferentially, leading to decomposition.

The phthalide group was found to be sufficiently stable to allow iodination of the ring of 2 directly with iodine monochloride to provide the amide 3 which could be quickly hydrolized with hydrazine in butanol without isolation. The title compound 4 was then obtained by hydrolysis, as the hydrochloride salt with a radioisotopic incorporation efficiency of 10%. The identity of the incorporated halide (iodide rather than chloride) was established by chemical ionization mass spectroscopy.

Injection of 4 into a monkey was followed by imaging and quantitation of uptake in brain with a scintillation camera. The brain uptake half-time was 8 sec, and at later times an unusual concentration was seen in the eyes. This compound may have application in the study of mental disorders, as it is an analog of both dopamine and amphetamine; it may also have clinical potential for imaging of normal brain tissue.

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Paper No.22

RADIO-IODINE LABELLED 4-AMINO-7-IODOQUINOLINES FOR MELANOMA DETECTION.

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ANALOGUES of the antimalarial chloroquin have received attention since 1968 as potential melanoma imaging agents(1,2) but have apparently not yet entered routine clinical usage in spite of encouraging clinical trials in man(3). Since the in vivo degradation of chloroquin is known to be extensive(4) a range of iodine compounds, designated IQ1—10, has been synthesised for iodine-125/131 exchange labelling. Some of these iodo-compounds were chosen to correspond to chloroquin or SN 9584 metabolites as shown in Scheme 1, others were analogues derived empirically.

A 4-stage screening procedure was envisaged for these compounds, namely : (i) measurement of the binding to synthetic melanin. (ii) in vitro uptake by melanoma and amelanotic (HeLa) cells in culture. (iii) in vivo studies in melanoma-bearing mice. (iv) metabolic studies in rats. From these experiments it is hoped to select candidate compounds for studies in man.

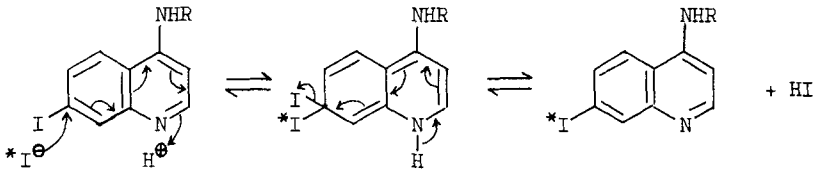
Two synthetic routes to such labelled iodoquinolines are known, via isotopic exchange (*I for I)(5) or via a diazonium salt (*I for NH_2 effectively)(6). The former method was chosen because it gave adequate quantities of labelled material for any of the stages (i—iv). 4-Chloro-7-iodoquinoline was synthesised in bulk(7) and converted to the various 4-(substituted)-amino compounds either by reaction for 1hr/170°C with an excess of the amine (8 mol)(7) or by reaction with the amine in phenol at 160—170°C (IQ1 6hr, IQ7 1hr)(8). The diquinolyl-compound IQ5 was an unexpected product from the reaction of the 4-chloroquinoline with propylene diamine. 4-Ethoxy-7-iodoquinoline, IQ10, was obtained by reaction of sodium ethoxide (1 mol) with the 4-chloroquinoline. Vacuum sublimation was generally a vital stage in the purification of the title compounds.

The title compounds were labelled by exchange with radioiodide ($^{125}I^-$ or $^{131}I^-$) in dimethylacetamide, acetic acid (1 : 3 vols,~0.2ml) for 18hr at 80—90°C. The product was isolated by base-extraction into ether, followed by preparative TLC on a 5 X 10cm 'analytical' plate of silica gel (UV_{254}) eluted with EtOAc, 2-PrOH, NH_4OH d = 0.88 (79 : 15 : 6-vols) or other eluants as detailed under Table 1. The radiochemical yield from 3—10mg and 5—10mCi of starting materials was 10—30% after purification.

For compounds IQ2—4, the TLC-system was successfully modified for HPLC operation with an eluant gradient. A 50 X 0.3cm column of 200—400mesh silica gel was eluted at 1ml/min with a linear gradient lasting 20min from 100% solvent A to 100% solvent B (Fig 1)

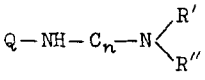
Solvent A.....AmOH, 2-PrOH, H_2O (79 : 15 : 6-vols)
Solvent B.....AmOH, 2-PrOH, NH_4OH (79 : 15 : 6-vols)

The mechanism of the exchange is interesting and seems to be connected with the extraordinary in vivo stability of these iodo-compounds. When the reaction was attempted in the presence of oxidising agents, etc to promote the formation of I^+, no exchange occured. In the absence of such species and in the presence of aprotic solvents and acid, exchange occured at a slow but useful rate. The mechanism appears to involve attack of the quinoline by I^-, facilitated by protonation of the ring(-1) nitrogen atom.

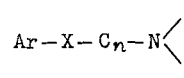


The normal mechanisms of *in vivo* deiodination could involve enzyme-mediated displacement by H or OH, and the former process should be related to the ease of exchange by I^+. In these quinolines the latter process has been shown not to occur. We suggest that the small amount of radioactivity (~1% ID) that localises in the thyroid following intravenous (iv) injection of one of these compounds could arise in fact from nucleophilic-type displacement of I by OH *in vivo*.

When compounds IQ1–5 were tested in the biological screens, some information suggesting a structure-selectivity relationship* was obtained(9). Compound IQ1 showed low uptake into melanoma cells *in vitro* (screen ii) but high uptake into melanoma cells *in vivo* at ~2hr post iv-injection (screen iii). Compounds IQ2–5 showed a high uptake *in vitro*, and at 2–4day *in vivo*. The common structural element appears to be



R′	H	alkyl	H
R″	alkyl	alkyl	aryl

The structural element 

occurs in other compounds that have shown positive uptake in the biological screens (i–iii) when carried out by other workers, e.g. chlorpromazine(10), quinine and an amphetamine analogue(11). The fact that melanin and/or melanocytes bind a range of (mainly) basic compounds other than chloroquin does not appear to be widely regarded.

* Named after the structure-activity relationship of pharmacology.

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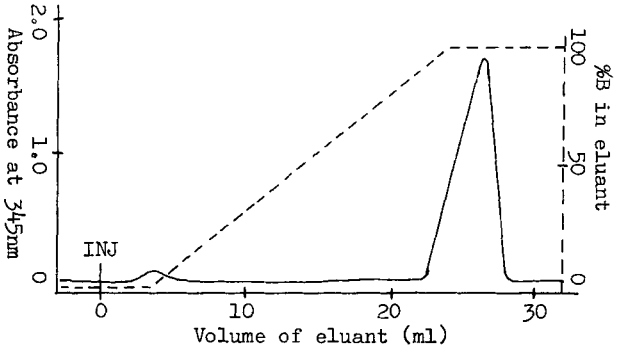
Table 1 : 4-(R)-substituted-7-iodoquinolines.

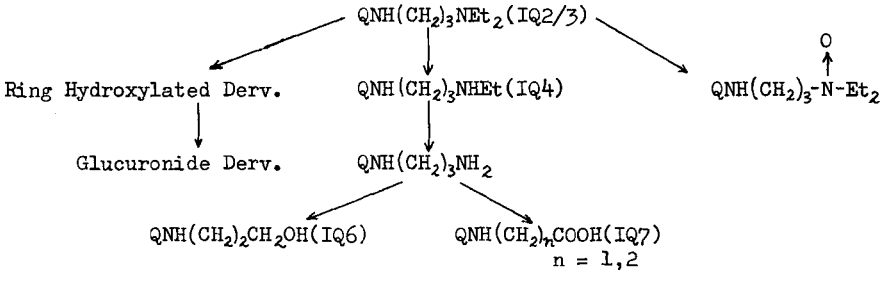
Compound	R	M.Pt.	Formula	Requires(%) C	H	N	Found(%) C	H	N	R_F
IQ1	$-NH_2$	201-203°	$C_9H_7N_2I$	40.0	2.6	10.4	40.1	2.6	10.2	0.7(a)
IQ2	$-NH(CH_2)_2NEt_2$	132-134°	$C_{15}H_{20}N_3I$	48.8	5.5	11.4	49.0	5.5	11.3	0.6
IQ3	$-NH(CH_2)_3NMe_2$	101-103°	$C_{14}H_{18}N_3I$	47.3	5.1	11.8	47.2	5.1	11.6	0.3
IQ4	$-NH(CH_2)_3NHEt$	91-92°	$C_{14}H_{18}N_3I$	47.3	5.1	11.8	47.5	5.1	11.8	0.2(b)
IQ5	$-NH(CH_2)_3NH-$	115-140°	not obtained analytically pure							
IQ6	$-NHCH_2CH_2OH$	204-205°	$C_{11}H_{11}N_2IO$	42.1	3.5	8.9	42.4	3.5	8.6	0.5
IQ9	$-NHCH_2CH_2Ph$	180-182°	$C_{17}H_{15}N_2I$	54.6	4.0	7.5	54.8	4.0	7.4	0.9(c)
IQ10	$-OC_2H_5$	68-69°	$C_{11}H_{10}NIO$	44.2	3.4	4.7	44.1	3.3	4.6	...(d)

R_F values for TLC-system silica gel eluted with EtOAc, 2-PrOH, NH_4OH d = 0.88 (79 : 15 : 6-vols) except for the following :
(a) R_F 0.5 with eluant EtOAc, 2-PrOH, 1.7% NH_4OH (79 : 15 : 6-vols)
(b) R_F 0.5 with eluant 2-PrOH, NH_4OH d = 0.88 (19 : 1-vols)
(c) R_F 0.7 with eluant $CHCl_3$, EtOAc, 2-PrOH, EtN_3 (50 : 25 : 20 : 5-vols)
(d) R_F 0.7 with eluant $CHCl_3$, EtOAc (9 : 1-vols)

Fig 1 : Analytical Liquid Chromatography of Compound IQ3 after Purification by Preparative-TLC. Column 50 X 0.3cm silica gel (200−400mesh) eluted with solvents A and B (see text). Linear gradient as shown. Flow rate 1ml/min.



Scheme 1 : Metabolic Degradation of a Chloroquin analogue (SN 9584). The iodo-analogues prepared are shown thus (X). Q = 7-chloro/iodo-quinolyl residue.



Paper No.23

ADRENAL AFFINITY AND PLASMA LIPOPROTEIN BINDING OF RADIOHALOGENO DERIVATIVES OF CHOLESTEROL

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For studying the effect of the chemical structure on biological properties of halogeno steroids, we have undertaken to prepare the following radiohalogeno derivatives of cholesterol and to compare their adrenal affinity and plasma protein binding in rats and mice: [1] 131I-19-Iodocholest-5-en-3β-ol (CL-19-I), ^{131}I-6β-Iodomethy-19-norcholest-5(10)-en-3β-ol(NCL-6-I), their 3-acetate (CL-19-I-3-Ac, NCL-6-I-3-Ac), NCL-6-77Br, [2] Cholesteryl-3-^{18}F, 77Br, and ^{131}I (CL-3-X), ^{14}C-CL-3-I. Preparation methods of these compounds have been reported (1,2,3), and tlc method was used to determine the radiochemical purity of the labelled compounds.

The comparison of adrenal uptakes between NCL-6-I and NCL-6-Br, and between CL-3-F, Br, and I showed that iodo compounds accumulated in adrenal more actively than bromo and fluoro compounds. Except for CL-19-I and CL-3-F, the adrenal uptakes of all the other compounds increased steadily until 2 or 3 days, thereafter the peak concentration was almost retained in adrenal. On the contrary, the adrenal uptake of CL-19-I and CL-3-F reached the peak within 2 hr after injection, then decreased gradually.

In contrast to Counsell et al's result (4), 3-acetate of CL-19-I showed almost the same adrenal uptake as CL-19-I in C3H mice. Unexpectedly, CL-19-I-3-Ac was rapidly deiodinated in vivo. The adrenal uptake of 3-acetate of NCL-6-I was also nearly equal to NCL-6-I, which was about ten times higher than that of CL-19-I and CL-19-I-3-Ac after 5 days in rats and mice. Therefore the 3-hydroxy group of an iodo-sterol is not indispensable to adrenal accumulation and 19-nor structure has higher affinity for adrenal than 19-methyl structure (5). In accordance with Yu et al's report (6), the adrenal uptake of CL-3-I or CL-3-Br 2days after injection in mice was 2.2 times or 1.9 times that of CL-19-I, respectively.

In view of the long-term retention of iodo steroids in adrenal, there is some possibility that iodo steroids would be metabolized in adrenal and the liberated iodine would be fixed covalently to unsaturated fatty acids enzymatically. We prepared double labelled iodo steroids, ^{125}I-CL-3-I and ^{14}C-CL-3-I, and compared their retention curve in adrenal of mice up to 30 days. The result, that the clearance of ^{125}I activity from adrenal was significantly faster than that of ^{14}C activity, made this retention mechanism to be a minor factor.

By means of low-speed centrifugation and dialysis of the blood of injected rats, it was shown that all the compound examined were bound to red blood cell and plasma proteins. Density gradient ultracentrifugation study revealed that such compounds as CL-19-I, NCL-6-I, their 3-acetates, and CL-3-I were bound to neither albumin nor high density lipoprotein, but mainly bound to low density and very low density lipoproteins. This remarkable affinity of helogeno steroids thus far examined to β-lipoprotein is the common feature, which is quite different from natural cholesterol. The ratio of RBC-bound activity to plasma-bound activity was quite variable

among compounds, i.e., RBC to whole blood ratio of CL-3-F was about one third of that of CL-3-I and about one fifth of that of NCL-6-I (Table). We propose the following working hypothesis that the β-lipoprotein binding is a necessary condition for adrenal affinity of a compound and RBC uptake of it, which may be mainly determined by the binding constant of a compound with the lipoprotein, is a sufficient condition. In this case, measuring of β-lipoprotein and RBC binding of a compound may be useful as a criterion for adrenal scanning agents in vitro.

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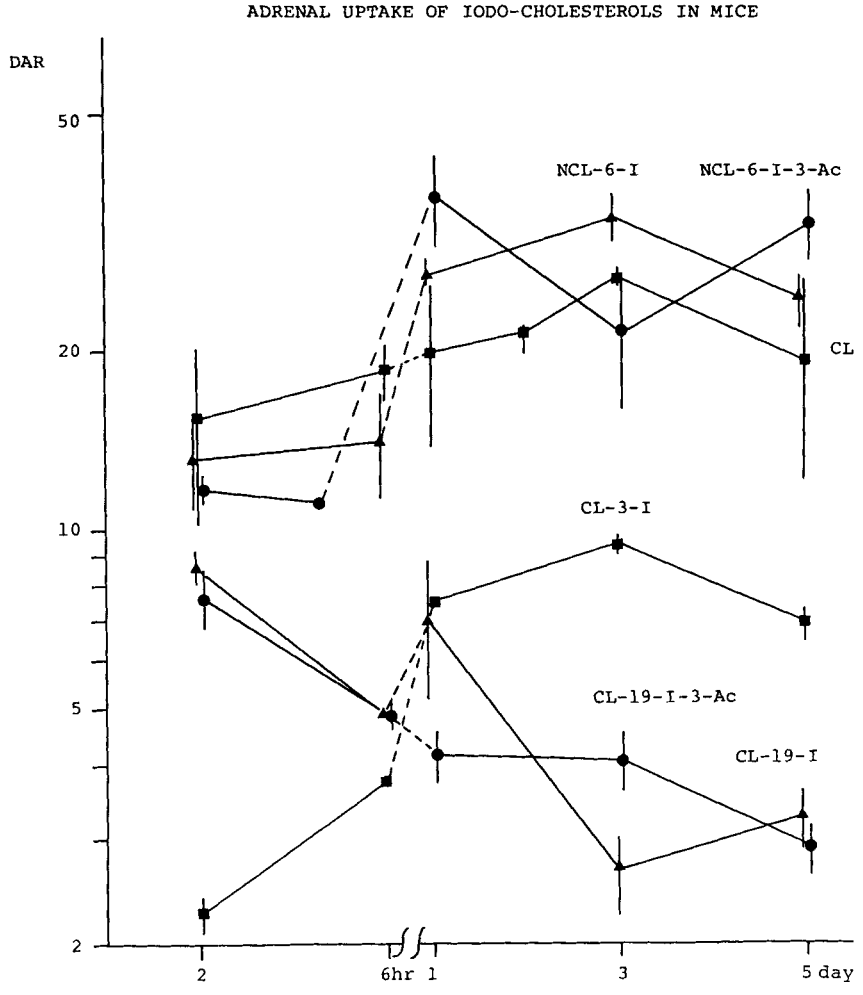
Table of RBC and Plasma Protein Binding of Halogeno Steroids in Wistar Rats.

	RBC	Plasma Lipoprotein				
		RES.	HDL	LDL	IDL	VLDL
CL-19-I**	61%	2.7%	2.0%	19%	9.8%	5.9%
NCL-6-I**	59	2.1	2.5	19	11	6.6
NCL-6-I-3-Ac**	29	3.6	2.8	34	18	13
CL-3-F#	9.4	(total plasma=91%)				
CL-3-I*	34	2.0	5.3	32	11	17
CL-3-OH**	77	1.0	9.8	8.5	3.0	2.0

RBC, red blood cell; RES, residue (d>1.21); HDL, high density lipoprotein (d=1.21-1.063); LDL, low density lipoprotein (d=1.063-1.019); IDL, intermediate density lipoprotein (d=1.019-1.006); VLDL, very low density lipoprotein (d<1.006).
Dialyzed plasma was used for CL-19-I, NCL-6-I and NCL-6-I-3-Ac.
* 25 min ** 2 hr # 3 hr after injection.


ADRENAL UPTAKE OF IODO-CHOLESTEROLS IN MICE
DAR
50
20
10
5
2
NCL-6-I
NCL-6-I-3-Ac
CL
CL-3-I
CL-19-I-3-Ac
CL-19-I
2
6hr 1
3
5 day

Paper No. 24

A KINETIC STUDY OF ISOTOPIC IODIDE EXCHANGE IN O-IODOHIPPURIC ACID

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This laboratory recently reported a method for the rapid labelling of o-iodohippuric acid (OIH) using high specific activity NaI-125(1). A kinetic study of this exchange reaction is presently in progress to determine rate constants, reaction order, and activation energy, with the elucidation of the reaction mechanism the ultimate objective.

Varying concentrations of OIH and KI-131 in aqueous solution were preheated separately and mixed in a glass vial. The reaction medium was shielded from light, maintained at constant temperature and pH, and free of oxygen. Portions of the reaction mixture were withdrawn at different times, and these samples were rapidly cooled to 0°C to prevent further reaction progress. A chemical separation of inorganic iodide from the organic species was then performed, followed by the quantitative analysis of ^{131}I in the experimental fractions via Ge(Li) γ-ray spectrometry. The relative fractions of radiolabelled O*IH and active $*I^-$ were calculated from the measured activities (A).

The logarithmic form of the isotopic exchange law (2) for this reaction is

$$-\ln(1-F) = \frac{R([OIH]+[I^-])t}{[OIH][I^-]} \qquad (1)$$

where

$$F = \frac{A(O*IH)_t}{A(*I^-)_{t=0}} \cdot \frac{[OIH]+[I^-]}{[OIH]} \qquad (2)$$

and denotes the fraction exchange at time t. R is the actual rate of exchange of iodine atoms, while [OIH] and $[I^-]$ represent total reactant concentrations in molarity. When the exchange is 50% complete, F = 1/2 and $t = t_{1/2}$ by definition. Substitution and rearrangement of eq. (1) gives

$$R = \frac{[OIH][I^-]\ 1n2}{([OIH]+[I^-])t_{1/2}} \qquad (3)$$

A plot of ln(1-F) vs. t is used to determine $t_{1/2}$, and R is then calculated from eq. (3).

If the exchange reaction is first order in both [OIH] and $[I^-]$, i.e., an S_N2-type reaction, then $R = k_2[OIH][I^-]$, and the graph of R/[OIH] vs. $[I^-]$ would be linear, pass through the origin, and have a slope equal to the specific reaction rate k_2(3). If the rate-determining step of the exchange is an initial ionization of OIH however, the reaction is S_N1 and independent of $[I^-]$, and $R = k_1[OIH]$. In this case, the plot of R/[OIH] vs. $[I^-]$ would be a straight line parallel to the $[I^-]$ axis with the intercept on the other axis equal to k_1. If the exchange has contributions from both paths, then $R = k_2[OIH][I^-] + k_1[OIH]$ and the graph of R/[OIH] vs. $[I^-]$ would result in a straight line with the slope and intercept equal to k_2 and k_1, respectively.

At the time of this writing, experimentation on this project is still incomplete. Several runs have been performed at a temperature of 75°C, and preliminary analysis of the data indicates that they are consistent with an S_N2 pathway for the exchange of I^- with OIH. A rate constant k_2 = 0.62 ± 0.12 1/mole-hr has been extracted from these experiments, but work is continuing at 75° to improve the precision of this measurement. Data will also be taken at various other temperatures to better establish the type of mechanism of this reaction, as well as to determine its activation energy from an Arrhenius plot (4). Should the finding of an S_N2 mechanism remain valid upon further experimentation, this result would contradict a previous study (5), which found an overall first order rate dependency of this exchange reaction.

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Paper No.25

RECENT DEVELOPMENTS WITH SOME SHORT-LIVED CYCLOTRON-PRODUCED RADIONUCLIDES.

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The variety in the applications of the short-lived isotopes ^{15}O (T=2.05 min) ^{13}N (T=10 min) and ^{11}C (T=20.4 min) for diagnostic purposes seems unlimited. Because they are tracers for 3 of the main components of biological matter, their use covers the fields of physiology, biochemistry, toxicology and pharmacology.

For the production of these radioisotopes and the design of labelling procedures the radiopharmaceutical chemist has a preponderant role and his knowledge should cover hot atom chemistry, biochemistry and organic chemistry as well.

In the development of his work the chemist is faced with such conflicting imperatives as speed and purity, high chemical yields and low concentrations, high specific activities and isotopic dilution.

The unique properties which make these radioisotopes so important for the future development of nuclear medicine are the following :

- Short half-life, which means low radiation dose delivered to patients or possibility of repeating the tests within a short time.
- Positron disintegration, which means 511 KeV γ rays emission in coincidence opening the way to tomographic detection and quantitative measurements.
- High specific activity, which means small amounts of material to be injected to the patient. This fact is of great importance when labelling high molecular weight radiopharmaceuticals or toxic products.

During the few past years, automated on-line procedures combining nuclear reactions, hot atom and conventional chemistry and rapid purification allow the production of ^{15}O, ^{13}N and ^{11}C and their incorporation in simple molecules which may either be used directly for medical application or incorporated by enzymatic or organic reactions into more complex radiopharmaceuticals.

1) $C^{15}O$, $C^{15}O_2$, $^{15}O_2$ and $H^{15}O_2$ are now currently used for metabolic studies such as blood flow measurements, lung studies, water distribution, blood pool determination and local oxygen consumption by brain tissue. This last parameter opens a completly new area since the information obtained is directly related to the metabolism of brain cells.

By continuous inhalation of $C^{15}O_2$ and $^{15}O_2$ the radioactivity is supplied to the brain as labelled water or oxygen and the pictures recorded give a good representation of blood flow, water diffusion and oxygen extraction.

2) Nitrogen 13 is advantageous for lung ventilation and perfusion studies as N_2 in gas form or in solution. Its low solubility in biological fluids and the high-energy gamma radiation emitted ensure quantitative and local measurements of lung functional activity.

Nitrogen 13 as labelled ammonia is taken up by many tissues chiefly the myocardium and brain. Its distribution in these organs may be indicative of

blood perfusion. It has also been reported that in the brain $^{13}NH_3$ is rapidly incorporated in amino-acids, mainly into glutamine amide groups.

$^{13}NH_3$ has also been used for fast, within a few minutes, amino-acids labelling by enzymatic synthesis. This new method is promising since it opens the way to measure transport mechanisms across biological membranes in vivo.

3) Carbon 11, owing to its longer half-life, allows more complex molecules to be synthesized. Using ^{11}CO, $^{11}CO_2$, $H^{11}CN$, $I^{11}CH_3$, $H^{11}CHO$ etc ... as precursors a number of new radiopharmaceuticals are under development, covering amino-acids, amines, sugars, proteins, hormones, fatty acids and drugs.

Generally one or two radioactive steps permit the synthesis to be obtained in less than one hour including purification by high-pressure liquid chromatography and sterilization. Automated procedures are necessary to guarantee radioprotection of the manipulators and reproductibility of the quality of the product.

One of the most interesting studies which might be undertaken with these ^{11}C labelled products is the in vivo visualization and quantification of specific receptors which represent the site of action of a number of hormones.

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Paper No.26

^{11}C-METHYLLITHIUM - A NEW SYNTHETIC TOOL IN RADIOPHARMACEUTICAL CHEMISTRY

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Methyllithium is a widely used synthetic reagent in organic chemistry. This highly reactive organo-metallic compound offers unique possibilities for one step incorporation of ^{11}C-labelled methyl-groups into organic or bio-organic molecules such as steroid hormones.

As starting material was used carrier-free (no carrier added) ^{11}C-methyl iodide, which was synthesized from $^{11}CO_2$ (1). Conversion of CH_3I into methyllithium by metallic lithium in ether proceeds smoothly when the concentration of the methyl iodide exceeds 0.04 mmol/ml. However, with lower concentrations, which are necessary in synthesis of compounds of high specific activity, the reaction is irreproducible and gives low yields. The halogen-lithium interconversion turned out to be a more fruitful approach. In 1963 Applequist and O'Brien (2) treated ethyl iodide with an equal molar amount of iso-propyllithium at -70°C in ether. At equilibrium, which was reached within a few minutes, 60% of ethyl iodide was converted to ethyllithium, while no Wurtz coupling could be detected. On the basis of their results we investigated the exchange reaction of ^{11}C-methyl iodide with n-butyllithium (equation 1).

$$^{11}CH_3I + \text{n-BuLi} \rightleftharpoons {}^{11}CH_3Li + \text{n-BuI} \qquad (1)$$

From the carbanion stability an equilibrium constant of $K > 1$ is expected. The n-BuLi reacts with carrier free $^{11}CH_3I$ and gives a high percentage of ^{11}C-methyllithium. To achieve the exchange a current of nitrogen or helium carries the $^{11}CH_3I$ into a solution of 0.15 mmole n-BuLi in diethyl ether or tetrahydrofuran which is kept at -70°C by external cooling. The exchange reaction is allowed to proceed for 10 minutes. The extent of exchange was determined by quenching the reaction mixture at -70°C with water. Methyl iodide and methane were introduced and the mixture was warmed up and kept at 50°C (reflux and stirring) for 5 minutes. Gas chromatographic analysis showed that more than 99% of the radioactivity in the gas phase was associated with the methane peak, while no $^{11}CH_3I$ could be detected. From these data it is concluded that the conversion of $^{11}CH_3I$ is virtually quantitative.

Of the many potential applications the addition of $^{11}CH_3Li$ to estrone methyl ether (I) is described as an example.

To the ethereal solution of carrier free $^{11}CH_3Li$ and the residual n-BuLi at -70°C 0.15 mmole of (I) was added, followed by 0.15 mmole of $LiClO_4$. The resulting mixture was stirred under helium at room temperature for 30 minutes. A 20% aqueous NH_4Cl solution was added, and the steroids were taken up in chloroform. High pressure liquid chromatography (silica gel; chloroform:hexane = 1:1) was used to remove starting material (I) and 17α-n-butyl-3,17β-estradiol 3-methyl ether (III). Carrier free carbon-11 labeled 17α-methyl-3,17β-estradiol 3-methyl ether (II) was obtained in a radiochemical yield (corrected for decay) of 10-15%. According to the thin layer chromatogram (silica gel; chloroform:hexane:methanol = 40:10:1) the radiochemical purity of the labeled steroid (II) was better than 93%. The whole procedure takes approximately 90 minutes. Although the reaction of (I) with excess MeLi in ether results in complete conversion to (II) within 30 minutes, the addition with carrier free $^{11}CH_3Li$ is far from complete in the same time. The effect of temperature, solvent and catalyst has to be examined more closely.

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O
CH3O
I
11CH3Li
n-BuLi
HO 11CH3
CH3O
II
+
HO n-Bu
CH3O
III

Paper No.27

THE SYNTHESIS AND DEVELOPMENT OF RADIOACTIVE QUATERNARY AMMONIUM COMPOUNDS FOR IN VIVO ASSESSMENT OF CARTILAGE

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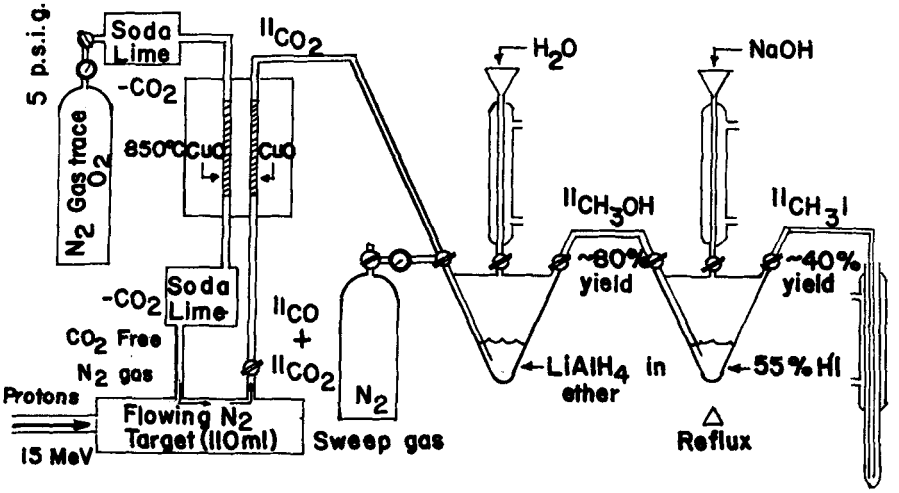
The development of an imaging technique to assess the status of cartilage in diseased joints in various forms of arthritis might provide a non-invasive method to obtain otherwise unavailable information. The localization of N-methyl-^{14}C- and ^{3}H-hexamethonium and N-methyl-^{14}C-decamethonium in avascular cartilage of the mouse was reported by Asghar and Roth (1,2) in 1970 and in 1971 by Wassermann (3). This localization in cartilage, demonstrated by autoradiographic technique, had been overlooked when tissue distribution studies were done (4,5). Interest in the biologic distribution of quaternary ammonium compounds was prompted by their action as neuromuscular blocking agents (6) which became known when tubocurarine chloride was isolated (7) and shown to contain two quaternary nitrogen atoms. The curare-like blocking action was found to be a property of onium ions in general. Asghar and Roth (8) subsequently showed that polymethylene bistrimethylammonium compounds bind strongly to chondroitin in vitro. Levine (5), using chemical methods, found the biologic localizations of hexamethonium and its monoquaternary derivative, 1-triethylammonium-6-dimethylamine, to be similar. This investigator did not assay cartilage although she had previously reported the combination of monoquaternary compounds with intestinal mucus.

Hexamethonium and its monoquaternary compound have been labeled with ^{11}C essentially using the method employed by Zaimis (9), i.e., refluxing the appropriate diamine dihydrochloride in methanol with methyl iodide. The $^{11}CH_3I$ is produced by a modification of the method reported by Comar et al. (10). The synthetic process is shown diagrammatically:



One end of the diamine precursor, 1,6-bis(dimethylamino)hexane, reacts quantitatively with $^{11}CH_3I$ (< 100 μg) to form the ^{11}C-monoquaternary compound, which is extracted into water, washed with ethyl ether to remove any unreacted diamine, and evaporated to dryness. The dry monoquaternary compound is dissolved in methanol, and an excess of CH_3I is added to form the diquaternary compound. The latter is prepared for injection by evaporation to dryness to remove methanol and unreacted CH_3I, washed with ethyl ether and dissolved in normal saline. Radiochemical purity was determined by chromatography on Silica-Gel-G-TLC with methanol:6N HCl (9:3). The R_f values in this system are: hexamethonium, 0.33; monoquaternary compound, 0.45; diamine precursor, 0.60. The iodide moves with the solvent front. Time for complete preparation, end of bombardment to injection of the subject, is one hour and yields about 3 mCi (∿ 30%) from approximately 80 mCi of $^{11}CO_2$.

Good images have been produced with both the monoquaternary and diquaternary ^{11}C-compounds. The monoquaternary preparation cleared the blood and was excreted more rapidly than ^{11}C-hexamethonium in a study conducted with the same human. This finding is at variance with data reported by Levine (5) who found lower blood levels in the rat and a faster urinary excretion in the dog for hexamethonium than for the monoquaternary compound when 10-30 mg/Kg of nonradioactive materials were used. Opposite findings for the human may indicate either a species difference, or a mass effect from the larger chemical amounts as compared to the few hundred micrograms of the ^{11}C-compounds.

It appears probable that a longer-lived radionuclide could be successfully introduced into these two, or other neuromuscular blocking compounds, and still give good localization in cartilage. An ^{131}I derivative of decamethonium, found to retain the pharmacologic activity, has been prepared by refluxing with Na ^{131}I (11). If the iodine atom remains attached to the compound in vivo, a radioiodinated compound would obviously extend the usefulness to clinical facilities that do not have cyclotrons.

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Paper No.28

[N-$^{11}CH_3$]-MORPHINE

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The advent of the medical cyclotron and more recently the positron emission transaxial tomograph (PETT) has stimulated research in developing positron emitting radiopharmaceuticals for metabolism and imaging studies. Work in a number of laboratories has demonstrated the reliability of labeling a variety of compounds with carbon-11 (1-7).

The chemistry and pharmacology of opiate alkaloids have been studied extensively but to date in vivo studies in humans has been difficult (8-10). In this work the synthesis of [N-$^{11}CH_3$]-morphine (III) is described.

Prior to labeling morphine with carbon-11 cold chemistry reactions were performed to quantitate and optimize the reaction procedures. Colorimetric determinations by Nash assay were performed to determine the conversion yield of CO_2 to H_2CO (90%). Yields on the reductive methylation reaction were determined by ultraviolet spectroscopy (22%). The identity and purity of the H_2CO and morphine were readily identified by gas chromatography.

Following the procedures of Christman, et al and Maziere, et al (1,2) carbon-11 CO_2 was produced by the $^{14}N[p,\alpha]^{11}C$ reaction. Subsequent reduction and hydrolysis of $^{11}CO_2$ over $LiAlH_4$ in tetrahydrofuran and oxidation of $^{11}CH_3OH$ over silver wool at 450°C produced the desired carbon-11 H_2CO (I).

Compound (I) was used in a reductive methylation reaction (Scheme 1) to produce [N-$^{11}CH_3$]-morphine (III) from normorphine (II).

The identity of Compound (I) and (III) were determined by radiogas chromatography and radio thin layer chromatography respectively. The total time for the synthesis was one hour from end to bombardment.

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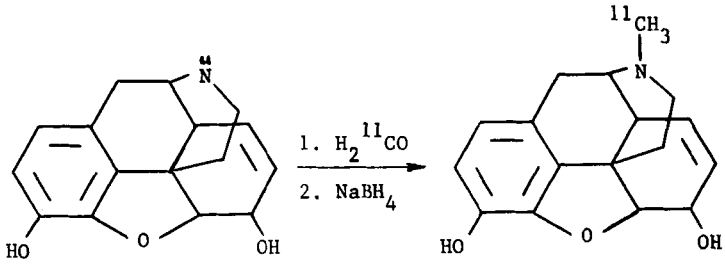
Scheme 1



(II) (III)

Paper No.29

ENZYMATIC SYNTHESIS OF C-11 PYRUVIC ACID, LACTIC ACID AND L-ALANINE.

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Lactic acid is a normal metabolite produced in tissues during periods of oxygen deprivation. Lactic acid is readily utilized by the myocardium and an externally detectable form of lactic acid would permit in vivo studies of myocardial metabolism. An enzymatic synthesis for C-11 lactic acid was devised and is performed in two steps: 1) production of C-11 pyruvate from C-11 CO_2 and acetyl CoA in the presence of an enzyme extracted from Clostridium acidi urici 2) Conversion of C-11 pyruvate to C-11 lactic acid by lactic dehydrogenase. C-11 pyruvate may also be converted to C-11 L-alanine in the presence of glutamic acid by the enzyme glutamic-pyruvic transaminase.

The incubation mixture contained the following substances in the indicated amounts in a total volume of 2 ml: C-11 carbon dioxide, 30-40 mCi; ferredoxin, 2×10^{-6} mmole; ferredoxin reductase, 0.1 unit; $NADPH_2$ 8×10^{-2} mmole; acetyl CoA, 2×10^{-2} mmole; acetyl phosphate, 2.9×10^{-2} mmole; phosphotransacetylase, 25 units, cysteine, 5×10^{-3} mmole; potassium phosphate buffer, 17.6×10^{-2} mmole; NADH, 1.25×10^{-3} mmole; lactate dehydrogenase, 50 units; pyruvate-ferredoxin oxidoreductase, approx. 1 mg. The C-11 carbon dioxide was produced in a (p,α) reaction at the UCLA Biomedical cyclotron. After anaerobic incubation at 37°C for 10 minutes, the sample was passed through an AG 50W x 8 resin column which had been equilibrated with 0.1N sodium acetate buffer, pH 2.3. The effluent was then passed through an AG 1 x 8 resin column (7 x 60 mm) which had been equilibrated with HCL at pH 3 and then washed with double distilled water. A control sample from which pyruvate-ferredoxin oxidoreductase and lactate dehydrogenase were omitted was run in parallel with the preparation sample through the incubation and column treatment procedures. Both samples were then gassed with dry carbon dioxide until the control sample had lost all of its labeled CO_2. The pH of the sample containing the synthesized C-11 lactate was then adjusted to 7 and the concentration of NaCl was made physiological. The column, CO_2 flushing, and pH adjustment treatments required about 10 minutes. A yield of about 3-5% (corrected for decay) was obtained. The identity of the C-11 lactate was established in a separate synthetic sample which contained C-14 CO_2 instead of C-11 CO_2. The C-14 labeled material produced in that sample was found, upon paper chromatography, to correspond to standard lactic acid. Each of the C-11 and C-14 labeled products was also identified by its behavior in various chromatographic columns, where it acted like standard lactic acid.

Tissue distribution studies in rabbits have demonstrated a 7:1 myocardium to lung ratio for C-11 lactic acid. The C-11 label is suitable for quantitative tomographic imaging. The pathway for this synthesis of lactic acid may also be utilized to produce C-11 pyruvic acid and C-11 L-alanine.

SYNTHESIS OF ^{11}C-LACTIC ACID.

$$^{11}C\text{-}CO_2 + \text{ACETYL-CoA} + \text{FERREDOXIN(reduced)} \xrightarrow{\text{pyruvate-ferredoxin oxidoreductase}} {}^{11}\text{C-PYRUVATE} + \text{CoA} + \text{FERREDOXIN(oxidized)} \quad \ldots\ldots (1)$$

$$^{11}C\text{-PYRUVATE} + \text{NADH} \xrightarrow{\text{lactate dehydrogenase}} {}^{11}\text{C-LACTIC ACID} + NAD^+ \quad \ldots\ldots (2)$$

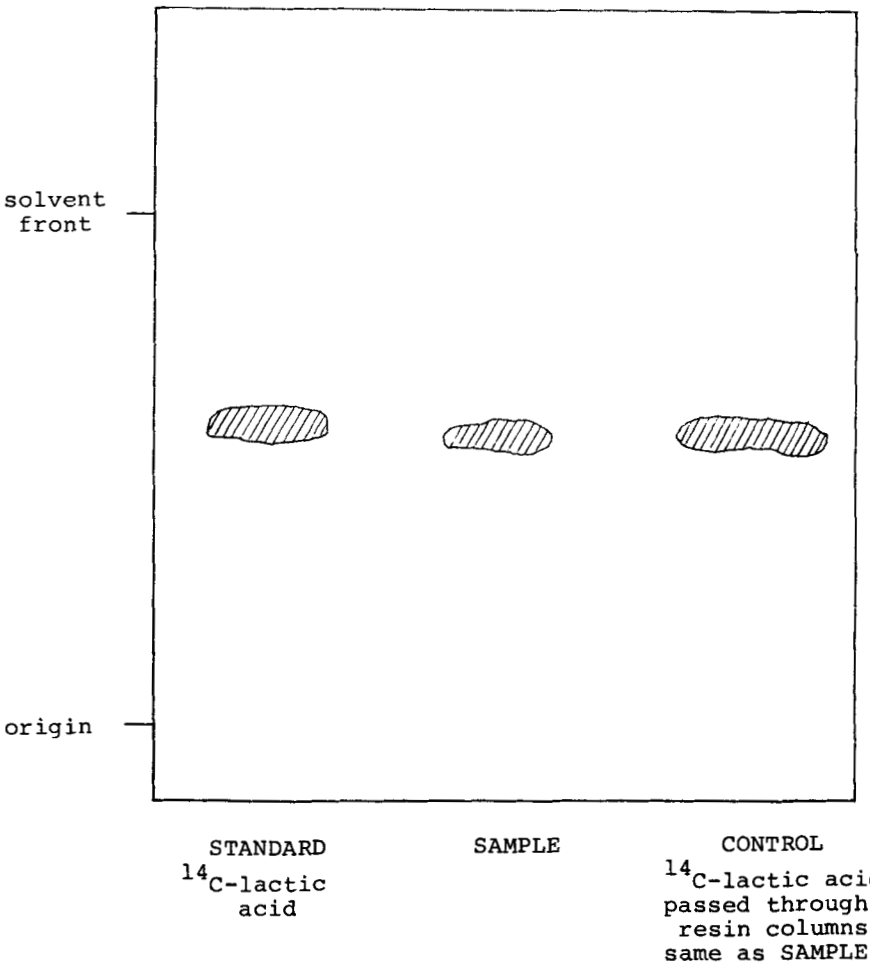
DISTRIBUTION OF ^{11}C-LACTIC ACID IN RABBITS.

(5 minutes post-injection)

TISSUE	AVG. COUNTS/30 seconds/gm.WET TISSUE. (corrected for decay)
HEART	38400
LUNG	5600
KIDNEY	34150
LIVER	20400
SPLEEN	43300
MUSCLE	9850
SKIN	15550
BLOOD*	26800

*upper limit of normal for serum lactate in rabbits is 4-5X that of man. (90mg% vs 20mg%)

RADIOAUTOGRAPH OF SYNTHESIZED ^{14}C-LACTIC ACID.



solvent system, amyl alcohol:5N HCOOH (1:1).

Paper No.30

PREPARATION OF ^{77}Br AND ^{76}Br L-DOPA AND 5-HYDROXYTRYPTOPHAN ANALOGUES

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The turnover rate of neuro-transmitters *in vivo* is a useful measure of neuronal activity. Measurements of this type can be of aid in basic studies of neuronal function in addition to providing a potential diagnostic tool for clinical use. Our object has been the synthesis of brominated analogues of two of the important transmitters (1), Dopamine and Serotonin. Since these compounds do not penetrate the blood-brain barrier (1), the metabolic precursors, which are analogues of L-Dopa and 5-hydroxytryptophan (5HTP), were synthesized and allowed to be decarboxylated *in vivo*. The method of synthesis utilized the enzyme, chloroperoxidase (2). The enzyme was incubated in a sodium phosphate buffer at pH 2.8 with the amino acid substrate in the presence of H_2O_2 and $^{76}Br^-$ or $^{77}Br^-$ at 37° for 0.5 hrs. At the end of this period the analogues were purified by cation exchange columns of Dowex-50 using pH gradient elution; this was followed by anion exchange with columns of Dowex Al to remove traces of Br^-. The resulting products were tested for purity by use of thin layer chromatography, and in a few selected cases by use of an HPLC column containing a strong cation exchange resin. The yields varied between 5% and 25% and were strongly dependent on pH and age of the enzyme solutions. It was found to be mandatory to assay the enzymatic activity before use (3).

Studies of tissue distributions in the rat indicated that ∿0.5%/g of the analogues did pass through the blood-brain barrier. The ratio of Br specific activity in the caudate nuclei to that in the cerebellum was 1.25 + .4 for injections of the Br-DOPA analogue and 0.23 ± .1 for injections of the Br-5HTP analogue as would be expected from the physiological distributions of the dopamine and serotonin neurones in the brain.

A rabbit was injected with ^{76}Br-DOPA and imaged using a Searle planar emission tomographic camera set for the β^+ annihilation radiation. The data indicate slight uptake of the ^{76}Br activity in the brain consistent with the tissue distributions in rats. When a rat, having a right sided unilateral lesion transecting the axons of the dopamine neurones, was injected with ^{77}Br-DOPA it was observed by use of a γ-camera and pinhole collimator that the transmission of activity to the right striatum was less than to the left. Subsequent sacrifice of the animal and measurement of the concentration in the tissues showed a 12.5 ± 3% left-right imbalance. Chemical isolation of the various DOPA metabolites in the cerebellum of a rat sacrificed 45 minutes post injection of ^{77}Br-DOPA indicated

that 25% of the ^{77}Br was present as ^{77}Br-DOPA, 25% as ^{77}Br-dopamine, and 50% as ^{77}Br-DOPAC (3,4-dihydroxyphenylacetic acid). These results lead us to conclude that these analogues are decarboxylated and appear to enter the active transmitter pool.

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*Work performed under the auspices of the Division of Biological and Environmental Research of the Department of Energy.

^{77}Br-5HTP and ^{77}Br-DOPA

Distribution in Rats

	%		%/gram	
Organ	^{77}Br-5HTP	^{77}Br-DOPA	^{77}Br-5HTP	^{77}Br-DOPA
cortex	.072	.12	.082	.13
caudate	.0056	.018	.049	.15
septum	-	.003	-	.083
diencephalon	.026	.043	.096	.13
cerebellum	.055	.031	.21	.12
brainstem	.021	.04	.092	.16
liver	3.1	4.3	.35	.48
heart	.25	.58	.24	.36
lung	.40	.67	.43	.62
spleen	.17	.48	.29	.38
kidney	2.1	2.6	.91	1.0

Radiobrominated 5-HTP and DOPA distributions in rats killed one hour after injections (authors results, 1977).

Paper No.31

STUDIES ON PRACTICALLY CARRIER-FREE LABELLING WITH BROMINE-76,77

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Bromine-76 and -77 have suitable nuclear properties for in vivo application. In addition, the C-Br bonds are more stable than the corresponding C-I bonds. Bromine-76,77 radiopharmaceuticals are therefore beginning to receive some attention.

Besides the most commonly employed direct production by the $^{75}As(\alpha,2n)^{77}Br$ reaction we have also used the indirect method via $^{76,77}Kr$-precursors obtained from the $^{79,81}Br(d,xn)^{76,77}Kr$-process. The latter reaction allows the application of decay-induced labelling via the $^{76}Kr(EC)^{76}Br$ and $^{77}Kr(EC,\beta^+)^{77}Br$ decay, analogous to the ^{123}Xe-gas exposure labelling for iodine-123. In order to explore the practical value of the $^{76,77}Kr$-gas exposure bromination we have carried out some systematic studies on the reactivity and selectivity of this excitation labelling method. On the other hand, new bromination methods starting with directly produced carrier-free bromide have also been studied.

In the gas phase $^{76,77}Kr$ gas exposure of monosubstituted benzenes leads to less than 1% hydrogen substitution with an almost complete lack of selectivity. In the liquid phase, on the other hand, somewhat higher yields of H-substitution are observed reaching about 10% in activated systems such as phenol and anilin. Depending on the type of substituent the isomer distribution exhibits an increased selectivity in the liquid phase (anilin: 48:2:50). Within the experimental error no significant difference is observed between labelling via $^{76}Kr(EC)^{76}Br$ and $^{77}Kr(EC,\beta^+)^{77}Br$. These findings are very similar to those observed in decay induced iodination and astatination.

The rather low yields of brominated products exclude a general application of decay induced bromination. Therefore attempts have also been made to apply brominating reagents allowing the introduction of radiobromine in aromatic and benzylic or allylic positions. In an approach to utilize carrier-free bromide we have used imides for preparing bromination reagents. In this case, the position of bromination can be chosen by the type of imide, i.e. its different electron attracting substituents. Using toluene as model substrate we found that N-Cl-succinimide in the presence of ^{77}Br-bromide only gives rise to benzylbromide (20% after 8 h at room temperature). N-Cl-tetrafluorosuccinimide, however, leads to 30% bromotoluene (after 4 h at room temperature) while the benzylbromide yield is less than 0.5%. The reasonably good yields together with the high selectivity (ortho: meta: para = 40:0:60) and the lack of side chain bromination suggests an electrophilic substitution mechanism. The results so far available seem to indicate that N-Cl-succinimides and -phthalimides are promising reagents for brominations starting with carrier-free $^{76,77}Br$-bromide.

Paper No.32

RADIOIODINATED BETA ADRENERGIC ANTAGONISTS AS POTENTIAL MYOCARDIAL IMAGING AGENTS.

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The use of radiolabeled cardioselective beta adrenergic antagonists offers potential for the development of myocardial imaging agents. Our program was based on (1) utilization of known cardioselective drugs as the starting materials; (2) selection of analogs that retain specificity and can readily be converted to their iodo derivatives; and (3) preparation of these derivatives such that the iodine moiety would be reasonably stable in vivo. The medicinal chemistry literature indicated that practolol and acebutolol demonstrate cardioselectivity both in animals and in man (1,2). Both drugs also have 4'-N-benzoyl analogs which retain cardioselectivity, and an iodine placed on the analogs as an iodobenzoyl group (as in hippuran) would be more stable than an iodine ortho to a phenolic hydroxyl.

Our initial study involved the conversion of practolol and acebutolol to their meta-iodo-benzoyl derivatives (I and II, respectively) in 35-45% overall yield (3). For comparative purposes, the 4'-iodo derivative of the non-selective beta antagonist propranolol (III) was prepared in 40% yield. All of the iodinated derivatives were labeled by ^{125}I-iodide exchange using the melt procedure. Radiochemical yields of 35-70% were obtained with specific activities in the range of 33-83 mCi/mmol (96% radiochemical purity). Biodistribution studies were carried out in normal rats, with groups of animals being sacrificed at 15 minutes and 1 hour. The results indicated that the radioiodinated derivatives of practolol and acebutolol possessed greater myocardial uptake and higher heart/lung and heart/blood ratios than ^{125}I-iodopropranolol. This suggested that our assumption to look only at the cardioselective drugs may be valid.

Our second study involved the synthesis of the ortho- and para-iodobenzoyl derivatives of acebutolol (IV and V) in 35-50% yield. Radioisotopic exchange was effected either by the melt method or by autoclaving in a pH 6.0 buffer. The organ distribution of the three iodobenzoyl acebutolol derivatives was compared to that of thallium-201 in normal rats.

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Table 1. Percent Injected Radioactivity in the Tissues of Rats Fifteen Minutes after Intravenous Administration of Iodinated Beta Adrenergic Antagonists

	^{125}I-Practolol		^{125}I-Acebutolol		^{125}I-Propranolol	
	%ID/g	%ID/organ	%ID/g	%ID/organ	%ID/g	%ID/organ
Liver	1.76±0.37*	18.94±1.45	2.40±0.31	20.19±1.91	0.74±0.12	7.30±0.63
Spleen	1.33±0.31	0.80±0.11	0.97±0.09	0.52±0.09	1.15±0.13	0.52±0.11
Lungs	1.24±0.42	1.89±0.36	1.63±0.19	1.77±0.23	2.58±0.85	3.90±1.01
Heart	0.89±0.23	0.64±0.09	0.99±0.13	0.66±0.09	0.35±0.03	0.26±0.02
Kidneys	1.82±0.50	3.45±0.64	2.80±0.24	4.38±0.57	0.98±0.19	1.83±0.40
Blood	0.06±0.01	0.79±0.06	0.09±0.01	1.00±0.05	0.12±0.01	1.87±0.16
Thyroid	4.73±1.00	0.10±0.02	6.06±1.25	0.12±0.03	2.76±0.17	0.06±0.01

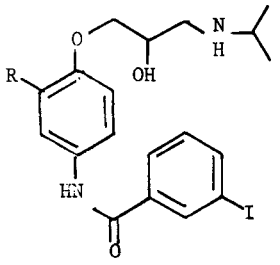
*average value and standard deviation for four rats

Table 2. Percent Injected Radioactivity Per Gram of Tissue in Rats Fifteen Minutes after Intravenous Administration of Thallium-201, and the Ortho- and Meta- Iodobenzoyl Derivatives of Acebutolol

	Tl	O-IBA	M-IBA
Liver	0.72±0.15*	1.57±0.22+	1.84±0.19*
Spleen	0.86±0.14	0.60±0.09	0.65±0.10
Lungs	2.03±0.30	1.07±0.08	1.76±0.40
Kidneys	5.83±1.05	0.95±0.03	3.42±0.48
Blood	0.07±0.01	0.24±0.03	0.07±0.01
Thyroid	10.91±2.42	5.65±0.53	4.25±1.13
Atria	2.16±0.68	0.42±0.06	0.77±0.15
Ventricles	3.09±0.67	0.43±0.08	1.06±0.23

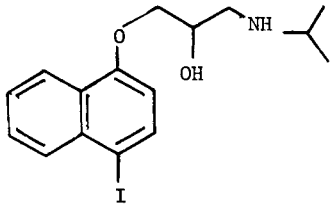
*average value and standard deviation for six rats

+average value and standard deviation for three rats

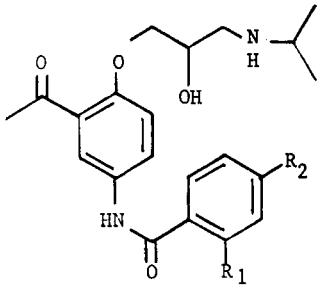


I R = H

II R = $CH_3C(=O)-$



III



IV R_1 = I, R_2 = H

V R_1 = H, R_2 = I

Paper No.33

BETA ADRENERGIC BLOCKING AGENTS FOR MYOCARDIAL IMAGING

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Beginning with practolol (1) a great number of cardioselective β-adrenergic blocking agents have been synthesized, some of which have been introduced into the therapy of cardiovascular and hypertensive diseases (2-4). From the pharmacologic point of view, a cardioselective β_1-blocker inhibits the response of β_1-adrenoceptors at much lower doses than required for the β_2-receptors (5). The cardioselectivity may result from higher affinity for β_1-receptors than β_2-receptors and/or from distributional effects. The distributional effects appear particularly important for imaging since a high affinity, nonselective β-blocker such as propranolol exhibits high lung concentrations relative to heart after intravenous injection (6). Although practolol exhibits an *in vitro* affinity for β-receptors three orders of magnitude lower than propranolol (7), lung concentrations are 15-fold lower probably due to increased polarity (8).

To resolve the different effects of affinity and distribution, we have synthesized three derivatives of practolol and one of the nonselective, high affinity drug alprenolol for *in vitro* assessment, radiolabeling and distributional studies. Two of the compounds (3 and 4) were prepared based on the conclusions of Basil (3) and Smith (4) that greater affinity and cardioselectivity can be imparted to the practolol-type β-blockers by lengthening the acyl chain or placing an electron withdrawing moiety in the ortho-position. To provide an easy site for iodination, tyramine was selected as the amino constituent.

The phenol substrates (purchased or synthesized by well known methods) were converted to epoxide intermediates as described by Shtacher (9). The epoxide intermediates were purified by crystallization from ethyl acetate (2) or column chromatography on silica gel in 10% MeOH in CH_2Cl_2. The reaction of epoxides with the tyramine free base in boiling MeOH rendered products which required tedious purfication by preparative and semi-preparative HPLC. Purified free bases of 1-[(4-hydroxy)phenethylamino]-3-aryloxy-propan-2-ols were converted to hemioxalates or oxalates.

The 1-[(4-hydroxy)phenethylamino]-3-aryloxy-propan-2-ols were radioiodinated using the chloramine-T method (10) and carrier free sodium iodide-125 (Industrial Nuclear, St. Louis, Missouri). A phenol to iodide ratio of 30 was used to assure monoiodination. Two TCL systems were used to assure that the HPLC purified compound (μ C_{18}, 1% AcOH/MeOH) contains only the desired species. Specific activities of the order of 90 - 110 Ci/mmole were obtained.

Turkey erythrocyte membranes (TEM) were prepared by the method of Bilezikian and Aurbach (11). Lung preparations were obtained from 200 to 250 g male Sprague-Dawley rats. The TEM preparations exhibited affinities in the order of isoproterenol > epinephrine = norepi-nephrine and the rat lung preparation gave isoproterenol > epinephrine > norepinephrine thus confirming the β_1 and β_2 nature of the respective preparations.

Tritiated dihydroalprenolol (^{3}H-DHA, New England Nuclear, Boston, Mass.) was used in binding assay described by Lefkowitz and Williams (12). The distribution studies of radioiodinated compounds 1 - 4 were performed in mature male Sprague-Dawley rats weighing approximately 300 g.

Distribution of the radioiodinated derivative of 2 based upon the cardioselective β-blocker practolol, shows that the large heart to blood ratios (H/B) needed for myocardial imaging were obtained in this class of drugs in rats. The heart to lung ratios (H/L) are also favorable for myocardial imaging. By contrast, 1 which is based upon the nonselective alprenolol, gives H/B and H/L values incompatible with imaging. In the studies of Basil (3) and Smith (4), cardioselectivity and β-affinity were improved by lengthening the acyl chain of the practolol structure and by the introduction of chlorine in the ortho position. Yet, the compounds 3 and 4 although considerably better than 1, were not as good as compound 2 with the respect to either H/N or H/L. It is interesting to note that the H/L values do not correlate with the ratios of *in vitro* K_d values for TEM and lung; increased β-affinity does not correlate with H/B and H/L values. As the affinity to the lung decreased, the H/B and H/L ratios increased. Distribution studies in guinea pig and rabbit with compd. 2 did not produce comparable results suggesting a species difference. This was confirmed by *in vitro* displacement studies which showed that most of the binding of compd. 2 was nonspecific.

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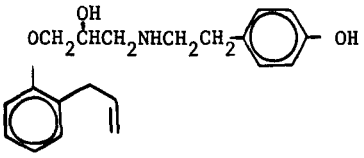
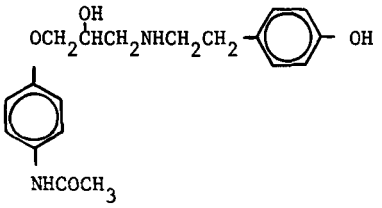


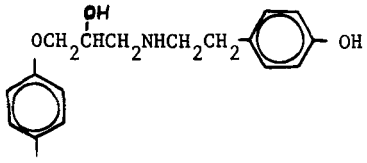
Compd. 1

TEM	K_d (M)	0.03
Lung	K_d (M)	0.25
H/B[a]		0.91
H/L[a]		0.1



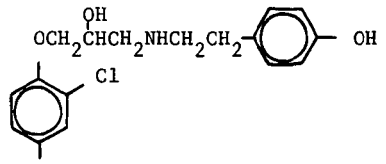
Compd. 2

TEM	K_d (M)	15.0
Lung	K_d (M)	78.0
H/B[a]		18.7
H/L[a]		2.0



Compd. 3

TEM	K_d (M)	0.5
Lung	K_d (M)	30.0
H/B[a]		6.2
H/L[a]		0.63



Compd. 4

TEM	K_d (M)	2.0
Lung	K_d (M)	26.0
H/B[a]		4.0
H/L[a]		0.64

[a] Two hours, iodinated with ^{125}I, tissue concentrations expressed as percentage of the injected dose per gram tissue wet weight.

Paper No.34

COMPARATIVE STUDIES OF ^{123}I-FATTY ACIDS IN MYOCARDIAL METABOLISM; DEVELOPMENT OF ω-^{123}I-HEPTADECANOIC ACID TO CLINICAL APPLICATION FOR IN VIVO DIAGNOSIS.

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The suitability of labelled fatty acids for heart imaging and measuring myocardial extraction rates has recently been demonstrated (1-4). Various α- and ω-halogenated fatty acids labelled with ^{34m}Cl, ^{77}Br and ^{123}I have been examined and compared with [1]-^{11}C-palmitic acid with respect to their myocardial uptake (5), and it was found that the ω-halofatty acids exhibit a similar uptake behaviour as [1]-^{11}C-palmitic acid while the α-halogenated fatty acids are less efficiently extracted. The results have led to the in-vivo application of ω-^{123}I-fatty acids for diagnosis of heart muscle insufficiencies (6).

For a better understanding of the biochemical reactions in the myocardial tissue we have also analyzed the heart muscle of mice at maximum accumulation of ω-^{123}I- and α-^{123}I-fatty acids. The radioiodine activity distribution among glycerides, free fatty acids and free iodide was determined, and two striking differences were observed: i) the amount of free α-iodofatty acids was ten times higher than that of the ω-iodofatty acid, ii) a considerable concentration of iodide (about 20%/g) was observed in the case of the ω-iodofatty acids, whereas the α-iodofatty acid gave rise to only about 5%/g. Since the blood contained less than 7%/g iodide at the same time, the high iodide concentration in the myocardial tissue seemed to be the result of a fast metabolism of ω-iodofatty acids. The observed results provide evidence that independent of the halogen all ω-halogenated fatty acids are metabolised in the same way: a fast catabolism via ß-oxidation with dehalogenation taking place only at the end, i.e. after degradation to a C_2- or C_3 unit. This is of particular interest for the application of the ω-^{123}I-heptadecanoic acid for studying metabolism in vivo.

The observations have led to clinical application of the ω-iodofatty acid as a radiopharmaceutical for diagnosis of myocardial insufficiencies. Results of in-vivo applications in normal patients, in patients suffering from coronary heart disease and patients with old infarctions have already demonstrated the excellent suitability of ω-^{123}I-heptadecanoic acid for myocardial imaging and for non-invasive assessment of energy metabolism of normal and pathological myocardium.

Infarcted zones are clearly visualized as defects of activity accumulation; also ischemic regions are detectable. The rate of release of the activity from the myocardium is determined from the time activity curves corrected for free iodide and serves as a parameter for the regional metabolic turnover of fatty acids. The average clearance half-time in normals is 25 ± 5 min; the values for infarcted zones range from 30 to 50 min.

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Paper No.35

CHEMISTRY OF TECHNETIUM RADIOPHARMACEUTICALS DERIVED FROM BIFUNCTIONAL CHELATING AGENTS

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A large body of information exists describing the in-vivo distribution, metabolism, and elimination of drugs and endogenous biochemicals. This information has provided the basis for designing radiopharmaceuticals containing the native labels ^{11}C and ^{13}N and for other radiopharmaceuticals containing foreign labels such as ^{131}I and ^{125}I. In contrast, this information source has yet to be effectively utilized for development of radiopharmaceuticals containing Tc99m chelates. These chelates differ from their ^{11}C, ^{13}N, and ^{131}I counterparts in that there is a paucity of information about their chemical and physical properties and even less information about their in-vivo structure-activity relationships (SARs). A new thrust to the research in chelate-type radiopharmaceuticals was provided by the work of Sundberg et al (1) which directed the chelate classification of radiopharmaceuticals away from the radiolabeling of existing chelating groups into the synthesis of bifunctional chelating agents. These bifunctional radiopharmaceuticals contain one functional group which binds the radioactive metal while the other functional group is either the partial or sole determinant of in-vivo distribution. Sundberg's work with protein analogs was expanded by other investigators to include technetium labeled analogs of small drugs and endogenous biochemicals, notably palmitate (2) and tolbutamide (3). These Tc-99m labeled analogs were incapable of serving as foreign labeled analogs of their respective parent compounds because of either in-vivo dissociation of the technetium chelate bonds or unacceptable structural differences between the parent compound and the labeled analog.

Our research goal has been to develop Tc-99m bifunctional radiopharmaceuticals to the point where their in-vivo distributions can be predicted from known SARs. Such a goal, while ambitious in its scope, must be considered the minimally acceptable outcome if technetium radiopharmaceutical development is to be a prospective rather than a retrospective science. As an initial step toward this goal we evaluated the chelating group iminodiacetate (IDA) and its N-substituted derivatives for use as bifunctional radiopharmaceuticals. IDA suggested itself for this role because of its relatively small size, its large K_f values with transition metals and the ease with which it undergoes N-substitution. Further, the Tc-99m chelates of various IDA compounds were shown to fulfill the prerequisite requirements: their in-vivo distribution varied with the chemical structure of the N-substituted group (4), they were inert under physiological conditions with respect to hydrolysis and ligand exchange reaction (5), and the Tc-chelates existed as single radiochemicals. Table 1 contains HPLC retention times for Tc-HIDA and a series of structurally similar IDA derivatives. When the pKa of the imino nitrogen was 5, there was minimal chelation with Tc, around a pKa of 6 a single radiochemical resulted, and between pKa values of 8.3 and 8.7 there were multiple radiochemicals each of which possessed a different in-vivo distribution. It would appear, therefore, that N-carbamoylmethylIDA is better suited for radiopharmaceutical incorporation than is IDA itself .

Initial structure-activity studies showed that the addition of Tc to the chelating group significantly altered its in-vivo distribution. Table 2 contains in-vivo distribution data for ^{14}C-HIDA, ^{99m}Tc-HIDA, ^{14}C-cholic acid, ^{14}C-CIDA (an analog of cholic acid) and ^{99m}Tc-CIDA. The observed changes in the in-vivo distribution indicated the importance of information on the radiochemical structure of these analogs. Subsequent data demonstrated that Tc-HIDA exists as a bis structure with two molecules of HIDA for each atom of Tc^{+3}, and that there is neither Sn^{+2} nor Sn^{+4} in the final radiopharmaceutical (6). These data would predict an overall charge on the radiopharmaceutical of -1, a value which was confirmed by the work of Burns et al (7).

With the advent of these stable, radiochemically pure Tc-99m chelates of known structure, it is now possible to begin the most difficult portion of the investigation, the description of those SARs which prescribe the in-vivo distribution of Tc-99m chelates. Radiopharmaceuticals to be studied further include the radiochemically pure Tc-99m-I, an analog of pregneneolone and Tc-99m labeled, III, IV, and V. ^{99m}Tc-V has an asymmetrical structure most consistent with that of a fatty acid containing two saturated ten carbon chains symmetrically placed around a Tc atom with a methyl group on one terminal end, and a carboxylic acid on the other. Work to date with Tc-99m labeled bifunctional radiopharmaceuticals has demonstrated that they can be made equivalent to their radioiodinated counterparts. Both groups can be synthesized radiochemically pure and inert, but detailed knowledge of SARs is needed if the foreign labeled radiopharmaceuticals are to successfully trace drugs and endogenous biochemicals. When these SARs are described, Tc-99m radiopharmaceutical development will become a truly prospective science.

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TABLE 1: RADIOCHEMICAL PURITY OF ^{99m}Tc-IMINODIACETATES □

Ligand	pKa Values	HPLC Retention Time (Min) •
$C_6H_5CH_2CH_2N(CH_2COOH)_2$	8.7	2 (26%), 5 (52%), 22 (22%)
$C_{10}H_7OCH_2CHOHCH_2N(CH_2COOH)_2$	8.6	2 (23%), 5.5 (45%), 16 (32%)
$Br\text{-}C_6H_5CH_2N(CH_2COOH)_2$	8.5	2 (20%), 5 (45%), 25 (35%)
$C_6H_5COCH_2N(CH_2COOH)_2$	8.3	5 (42%), 8 (30%), 22 (28%)
$(CH_3)_2C_6H_5NHCOCH_2N(CH_2COOH)_2$*	6.2	20 (99%)
$C_6H_5CH_2CH_2NHCOCH_2N(CH_2COOH)_2$	6.1	23 (99%)
$C_6H_5CON(CH_2COOH)_2$	5.1	2.5 (10%)
$C_6H_5N(CH_2COOH)_2$	5.0	2.5 (10%)

□ Radiolabeled at a pH of 5.5

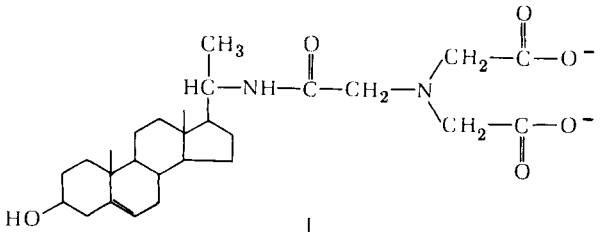
• μ Bondapak C-18 column; .025 M phosphate buffer : acetonitrile (90:10); 2 ml/min

* HIDA

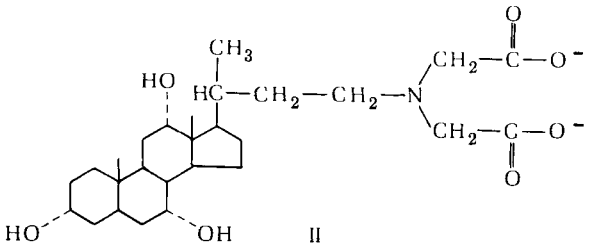
TABLE 2: IN-VIVO DISTRIBUTION DATA IN MICE•

ORGAN	^{14}C-HIDA*	^{99m}Tc-HIDA	^{14}C-Cholate	^{14}C-CIDA*	^{99m}Tc-CIDA
Blood	0.2	0.8	.05	4.7	3.7
Liver & Gallbladder	0.4	8.0	5.8	9.1	50.0
Intestines	1.4	68.3	75.5	67.3	30.3
Urine	74.0	-----	.05	1.7	6.0

• Percent Injected Dose at 1 hour post-injection
* Compound Number II

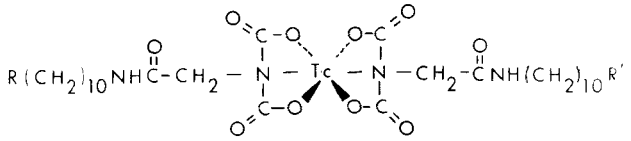


I



II

I N- (5, pregen-3β ol-2oyl-carbamoylmethyliminodiacetate
II 3α: 7α: 12α Trihydroxy-24 norcholanyl-23 iminodiacetate (CIDA)



Tc-III : R = R' = COOH

Tc-IV : R = R' = H

Tc-V : R = H, R' = COOH

Paper No.36

TECHNETIUM IN 99m-Tc RADIOPHARMACEUTICALS I. TETRAVALENT MONONUCLEAR TECHNETIUM PENICILLAMINE COMPLEX.

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Some evidence for a relationship between the chemical state of 99m-Tc in 99m-Tc radiopharmaceuticals and their in vivo behavior has been recently published by our group. In the course of study of 99m-Tc-penicillamine (99m-Tc-Pen), a cholescintigraphic agent, it has been found that the hepatobiliary excretion is mainly restricted to two complexes. Although these two complexes were excreted in the gallbladder, the dynamic study of the in vivo distribution was completely different. As previously reported (1,2) it has been postulated that in these complexes, Pen is coordinated with tetravalent mononuclear and dinuclear technetium to form Complex I and Complex II as follows:

$TcO(Pen)_2^{2-}$ $(Pen\ TcO\langle^{O}_{O}\rangle TcOPen)^n$ or $[\ (Pen)_2TcO\text{-}O\text{-}TcO(Pen)_2\ ]^n$

Complex I Complex II

This paper deals with the studies of the characterization of the mononuclear Tc-Pen complex, Complex I, by spectrophotometry and Sephadex column chromatography.

The Sephadex column chromatography (G-15, 17 cm x 2.0 cm; eluant, 0.15 M NaCl; 108 ml/min) of various labeling solutions has shown that the elution times of the various Tc species are as follows:

TcO_4^- (95-120) Complex I (34-45) Complex II (17-22) other polymerized Tc-Pen complexes (8-15) [elution time in minutes]

This elution order reflects the possible relationship of the molecular size of Complex I and Complex II. In fact, no other fraction with a slower elution time than Complex I was detected with the exception of TcO_4^-; this analysis appears to offer evidence for the small molecular size proposed for the mononuclear Tc-Pen species. This Complex I fraction, isolated by Sephadex column chromatography from a labeling reaction using 99-Tc, showed an absorption maximum at 420 nm. An identical absorption spectrum was obtained when $TcCl_6^{2-}$ was reacted with Pen. It is well known that Tc as the hexachloride salt is in the tetravalent state (3). So, for further characterization of this complex, the effects of pH and concentration of Pen on this reaction were considered.

In the presence of a large excess of Pen over $TcCl_6^{2-}$, the formation of Complex I was observed at pH range of 4 to 6 (Fig. 1). This feature can theoretically be explained by considering a competitive reaction between the formation of a mononuclear tetravalent Tc complex, $TcO(Pen)_2^{2-}$ and the hydrolysis of a tetravalent Tc species, TcO^{2+}.

A molar ratio of Tc:Pen of 1:2 could be estimated by the effect of Pen concentration on the reaction of $TcCl_6^{2-}$ (Fig. 2) and the data were analyzed

based on the competitive reaction mentioned above, as follows:

$$TcO^{2+} + 2\ Pen \longrightarrow TcO(Pen)_2^{2-}$$

$$TcO^{2+} + 2\ H_2O \longrightarrow TcO(OH)_2 + 2H^+$$

Further support for the structure of $TcO(Pen)_2^{2-}$ is given by the formation of a zero charge complex with penicillamine ethyl ester.

A stable character of Complex I was observed either at carrier free concentrations or at 99-Tc concentrations; a tetravalent d^3 state of "mononuclear complex" is regarded as the criterion for its stability.

Another characteristic phenomenon was noticed in the formation of Complex I following the labeling reaction using $SnCl_2$ reduction. The preparation under similar pH range as stated above, was very sensitive to the stannous ion concentration. Complex I was detected only at 1.5 to 2.0 $SnCl_2/TcO_4^-$ molar ratios.

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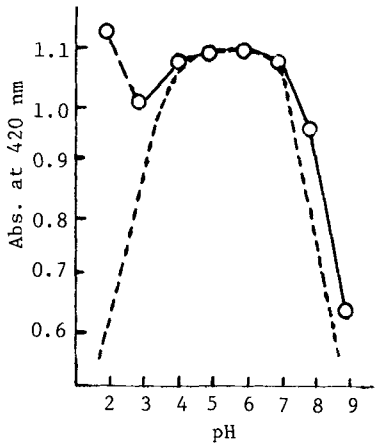

Fig. 1. pH Effect

——O—— Experimental data

------- Estimated data from $K[H^+]^2[Pen^{2-}]$;

$K = K_f \times K_{OH}$ (Ref. (1))

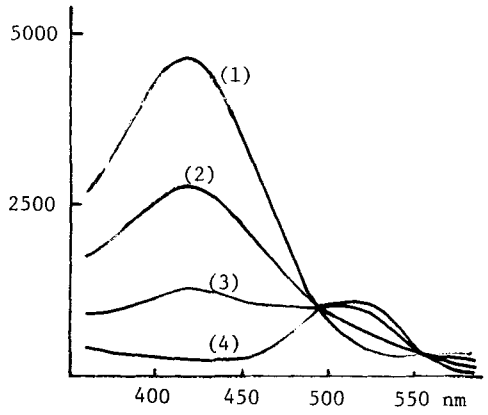

Fig. 2. Pen Concentration Effect at pH 6.5

$Pen/TcCl_6^{2-}$: (1) 200
(2) 20
(3) 2
(4) 0.2

Paper No. 37

THE IDENTIFICATION AND CHARACTERIZATION OF SIX COORDINATE HEXAKISTHIOCYANATOTECHNETATE(IV) AND SEVEN COORDINATE HEPTAKISTHIOCYANATOTECHNETATE (III).

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There has been considerable speculation as to the nature of the two technetium complexes that are obtained by the reduction of TcO_4^- by thiocyanate (1). We have reinvestigated this system and find the previous formulations to be erroneous.

The purple octahedral technetium species $Tc(IV)(NCS)_6^{2-}$ was isolated as a tetraphenylarsonium salt. It is paramagnetic, μ = 4.10 BM, consistent with a $^4A_{2g}$ ground state; it has a charge transfer absorption band at 500 nm. It does not undergo reduction in acetonitrile with tetra-n-butylammonium perchlorate as a supporting electrolyte. However, cyclic voltammetric studies in this system with added thiocyanate show a reversible one-electron cathodic process at +0.18 V w.r.t. S.C.E. This wave is independent of the thiocyanate ion concentration at constant ionic strength.

The reduced species is very conveniently prepared by the reduction of $Tc(IV)(NCS)_6^{2-}$ in methanol, containing added thiocyanate ion, by hydrazine. The resulting seven-coordinate complex, $Tc(III)(NCS)_7^{2-}$, can be isolated as well formed lemon yellow crystals using tetra-n-butylammonium as a counter ion. The cyclic voltammetry of this complex shows a reversible one-electron oxidation wave at +0.18 V w.r.t. S.C.E. (CH_3CN-$(C_4H_9)_4NClO_4$). The yellow Tc(III) complex is paramagnetic, has a charge transfer absorption band at 410 nm, and is very sensitive to oxidation, being converted rapidly into $Tc(IV)(NCS)_6^{2-}$.

The nature of both compounds and their electrochemical behavior will be discussed.

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Paper No.38

MIXED-LIGAND COMPLEXES OF TECHNETIUM (V)

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$^{99}TcO_4^-$ is reduced to Tc(V) by $SnCl_2$ in alkaline solutions of polyhydric compounds containing vicinal OH groups. The complexes of various polyol compounds are all pink-violet in color (λ_{max} near 520nm, ϵ_M~100) (1).

$^{99}TcO_4^-$ is reduced by $SnCl_2$ to Tc(IV) in the presence of cysteine (2), and to either Tc(IV) or Tc(111) in the presence of dimercaptosuccinic acid, depending on conditions (3). $^{99}TcO_4^-$ is reduced by excess $SnCl_2$ to Tc(111) in the presence of diethylenetriaminepenta-acetic acid (DTPA) (4). A Tc(IV)-DTPA complex has also been prepared (5). There is no indication of the formation of Tc(V) compounds containing either sulfhydryl compounds or DTPA.

We have found that cysteine and DTPA, among other reagents, form mixed-ligand complexes with the various Tc(V)-polyol compounds previously described. A Tc(V) cysteine 1:1 complex (6) was formed by adding cysteine to a Tc(V)-gluconate complex. When made in this way, the yellow complex is indefinitely stable in solution (λ_{max} 420nm, ϵ_M2300). Attempts to prepare it in the absence of gluconate or another polyol yielded TcO_2, or solutions whose spectra changed in time.

The addition of DTPA to an alkaline solution (pH > 9) of a gluconate, mannitol, ethylene glycol or Dextran complex of Tc(V) produces no change in the absorption spectrum. Acidification (pH < 6) changes the color to blue (λ_{max}~620nm, ϵ_M~60). The change is reversible.

The Tc(V) polyol complexes of longer-chain compounds like mannitol are themselves stable in acid, whereas that of ethylene glycol disproportionates in acid, forming TcO_2 and TcO_4^- (1). The difference is attributed to the formation of a linear Tc-O-Tc dimer stabilized by at least four OH groups in the same molecule. The number of polyol molecules per Tc atom is not known because a large excess of ligands is needed to maintain the complex. It is now possible to conclude that (in acid solution) there are at least two mannitol molecules per Tc-O-Tc unit. The attached Figure shows the spectra of a 8.6 x 10^{-4}M solution of ^{99}Tc in 2% mannitol containing various concentrations of added DTPA. The curves suggest, but do not prove, that there is a competition between DTPA and mannitol. Lowering the mannitol concentration (to 1% and then to 0.2%) progressively increases the yield of the mixed complex at a fixed DTPA:Tc ratio and at a fixed Tc concentration. This means that at least one mannitol molecule is displaced by DTPA, and at least one remains in the mixed complex.

The mixed complex containing ethylene glycol is (relatively) stable in acid solution if the DTPA concentration is larger than that of Tc. A mol ratio study at pH 4 in 1% ethylene glycol shows that there is less DTPA than Tc in the mixed complex. Disproportionation of Tc(V) (slowed down, but still present) did not permit measurements to be made below a DTPA:Tc ratio of about 0.6 but it appears likely that in the complex the ratio of DTPA to Tc is 1:2.

An attempt to prove by gel chromatography that a mixed complex still contains the polyol ligand was only partially successful. A Dextran-^{99}Tc (V) complex (Dextran molecular weight 40,000) was prepared in alkali, tagged with ^{99}Tc, DTPA to 1% (ψ) was added, the solution was acidified, and chromatographed on a Sephadex column. ^{99}Tc-polyol complexes absorb on Sephadex (7) and we have found that ^{99}Tc-polyol complexes undergo some disproportionation on Sephadex. In this experiment approximately 75% of the technetium (eluted with normal saline) was found in the void volume. It can be concluded that the polyol moiety is still part of the new Tc complex.

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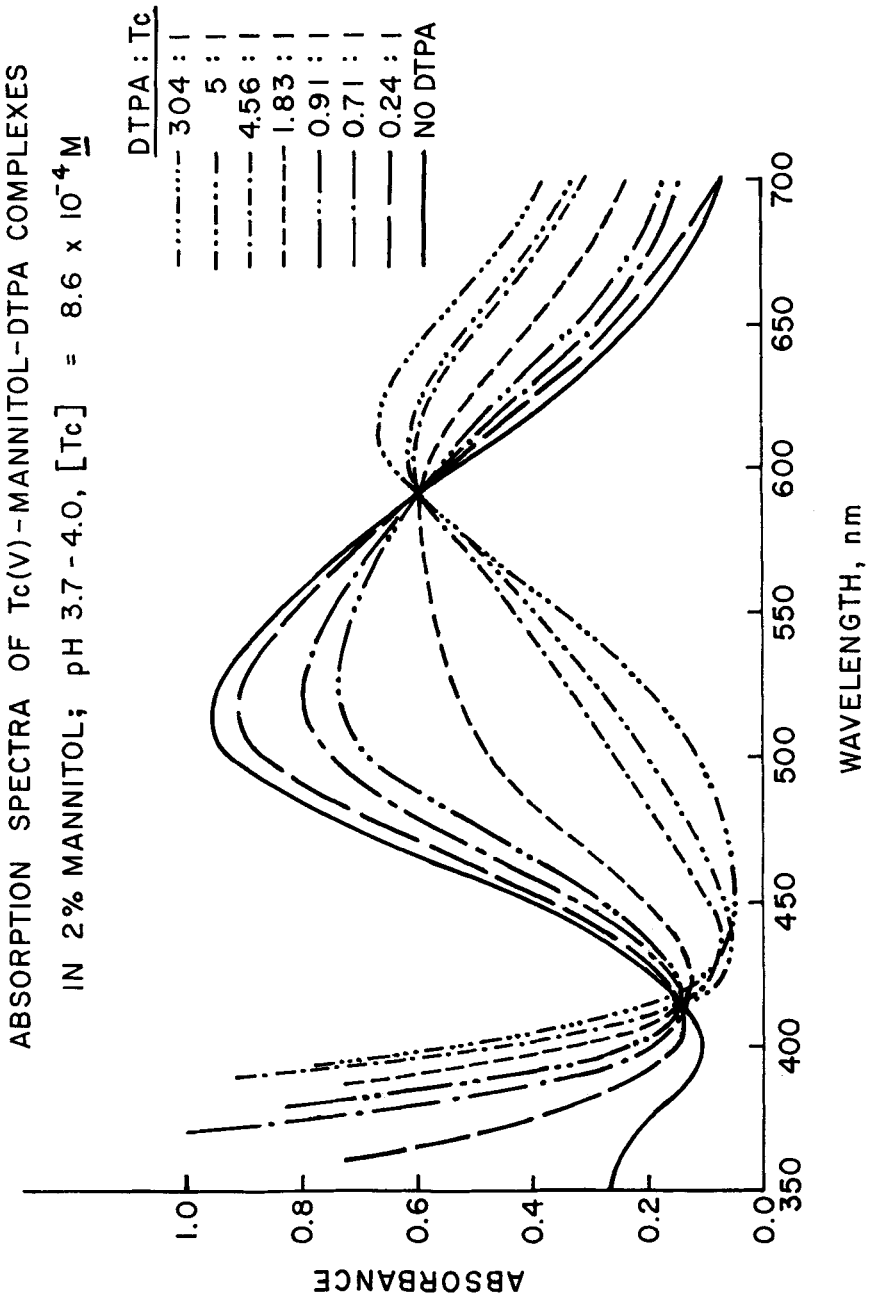
ABSORPTION SPECTRA OF Tc(V)-MANNITOL-DTPA COMPLEXES
IN 2% MANNITOL; pH 3.7 - 4.0, [Tc] = 8.6 x 10⁻⁴ M
DTPA : Tc
304 : 1
5 : 1
4.56 : 1
1.83 : 1
0.91 : 1
0.71 : 1
0.24 : 1
NO DTPA
ABSORBANCE
0.0
0.2
0.4
0.6
0.8
1.0
350
400
450
500
550
600
650
700
WAVELENGTH, nm

Paper No.39

^{99m}Tc-Cu-EDTA, PREPARATION AND BIOLOGICAL STUDIES

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Fe(II) and Sn(II) have been used in the preparation of ^{99m}Tc-EDTA (1,2). The use of the sparingly soluble cuprous chloride as a reducing agent in the preparation of ^{99m}Tc-DTPA has been previously described (3). The present study entails the use of induced Cu(I) as a tool for labelling EDTA with technetium; the formation rate of the complex ^{99m}Tc-Cu-EDTA with a high yield was fast and achieved within a few min., in contrast to that formed with Sn(II) (2). The rapid reduction of technetium with Cu(I) illustrates the formation of Tc(IV) which is the valence state present in Tc-EDTA (4,5).

The experimental work involved the study of the effect of different concentrations of $CuCl_2$, ascorbic acid and disodium-EDTA on the labelling yield of the complex. The Gelchromatography Column Scanning (GCS) technique was used for the determination of reduced hydrolyzed-Tc-99m, free pertechnetate, and ^{99m}Tc-Cu-EDTA complex (6,7). The optimal conditions for a ^{99m}Tc-Cu-EDTA preparation with a high radiochemical yield were found at concentrations of not less than 1 mM of $CuCl_2$, 110 mM of ascorbic acid, and 10 mM of EDTA in the final preparation. The labelling yield of the complex is significantly decreased with concentrations of $CuCl_2$ below 1 mM.

The labelling efficiency of the ^{99m}Tc-Cu-EDTA complex at various pH values was investigated, and the stability of the complex was studied by analyzing the samples at different time intervals by the GCS-method. The GCS profiles of samples taken from a mixture of technetium pertechnetate, cupric chloride and ascorbic acid indicate the formation of a complex at pH below 1, however the complex yield was increased with the rise in pH.

In vitro studies of the degree of binding the ^{99m}Tc-Cu-EDTA to red blood cells and plasma proteins have also been performed using the GCS-technique. The binding of ^{99m}Tc-Cu-EDTA to plasma protein was found to be much lower than that for ^{99m}Tc-Sn-EDTA. *In vivo* studies showed that the kidney uptake of activity reached a maximum within 30 min. after i.v. administration of the complex into the tail vein of rats.

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Paper No.40

RADIOCHEMISTRY OF NUCLEOGENIC N-13 IN AQUEOUS SOLUTIONS

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We have developed a cyclotron target for production of ^{13}N by the $^{16}O(p,\alpha)$ reaction on recirculating aqueous solutions in a stainless steel target. The total target system includes facilities to produce $^{13}NO_3^-$, $^{13}NO_2^-$, ^{13}NN and $^{13}NH_3$. The target has been irradiated with currents of up to 40 μA with a nuclear yield of 29±1 mCi/μA for bombardment with 19 MeV protons for 20 min. Further engineering details of this target system as used at the Crocker Nuclear Laboratory have been described elsewhere (1,2). The purpose of this report is to describe the radiochemical form of the initial ^{13}N labeled species resulting from the nucleogenesis of ^{13}N in aqueous solutions.

The radiochemical yields from the ^{16}O (p,α) reaction in water are probably a complex function of hot atom chemistry and radiation chemistry. The ^{13}N nucleus recoils from the compound nucleus, ^{17}F, with a broad kinetic energy distribution from 0.5 to 6.5 MeV, the exact limits depending on the proton irradiation energy. The recoiling ^{13}N will be highly positively charged (initially $\geq +4$) but will rapidly loose energy and become neutralized. The $N^+ + H_2O \rightarrow \overline{N} + H_2O^+$ reaction cross section maximum as predicted by the adiabatic principle occurs at about 7 keV, therefore we believe that the ^{13}N reaches the chemical reactivity range, below about 0.1 keV, as a neutral atom.

Parameters that effect the radiation chemistry of matter include both the quantity of radiation incident upon it and the mass available to absorb that radiation. Our irradiations of H_2O have involved beam currents varying from 0.5 to 30 μA for times of 3 to 20 min, resulting in total integrated current on target from 88 to 36,000 μcoul. Irradiations were done at measured beam energies of 16.5 and 20.8 MeV, which are degraded in energy by two foils and strike the water at about 14 and 19 MeV, respectively. The target irradiation cavity is entirely SS and is 3.53-3.61 mm deep, the range of a 17 MeV proton in water. All of the 14 MeV beam is absorbed by the target solution, whereas only two-thirds of the 19 MeV beam is absorbed. The high energy beam thus deposits less energy into a target of this thickness than the lower energy beam, yet the integrated ^{16}O (p,α) cross section is almost twice as high for the higher energy beam.

The total volume of water held by our target is 60 cm^3; however, the target solution is continuously circulating at 150 cm^3/min. The actual irradiation cavity volume is 2.35 cm^3 with a front surface area of $\simeq$ 6 cm^2 exposed to the defocused proton beam. The result of all of these factors is that the water in the irradiation cavity absorbs about 0.02 eV/molecule during each pass in front of a 20 μA proton beam. For a 20 min irradiation at this beam intensity, an average of about 1 eV is absorbed per water molecule, a relatively low amount for water radiation chemistry.

Figure 1 shows the radiochemical $^{13}NO_3^-$ yield determined by both anion and cation exchange HPLC for water samples receiving radiation doses from 0.003 to 1.5 eV/molecule under the complete range of irradiation conditions (Ep, μA, time) described earlier. At radiation doses $\geq$ 0.3 eV/molecule we found $\geq$ 97% NO_3^-. At lower doses the yield was lower and more variable. The poor reproducibility at very low absorbed doses may result from considerable

variability in defocusing the cyclotron beam. The more defocused beams distribute the absorbed energy over more molecules during each passage in front of the target; however, the overall dose averaged over many circulations would not be affected by beam focusing.

We conclude that at doses of > 0.3 eV/molecule we can reliably make $^{13}NO_3^-$ of sufficient radiochemical purity for biochemical kinetics measurements (3) and for use as a synthetic precursor or for conversion to $^{13}NO_2^-$ (2). On the other hand, it has not been possible to achieve reliably high yields of $^{13}NH_3$ under low dose irradiation conditions, and hence, we have adopted Devarda's method (2) for making $^{13}NH_3$ via the reduction/distillation of nitrate.

The present results suggest a role for both neutral hot atom and radiation chemical mechanisms in the formation of observed ^{13}N products. High yields of $^{13}NO_3^-$ were found at very low total absorbed doses during some irradiations; whereas, $^{13}NH_3$ yields never exceeded 60% and were generally < 25%. These observations led us to reject a mechanism limited to $^{13}NH_3$ as the primary product via sequential abstraction of hydrogen atoms from water by nucleogenic ^{13}N free radicals. We believe that the radiochemistry of the newly formed ^{13}N is dominated by the highly excited microcosm at the end of the track where the ^{13}N reaches near thermal energy, a track only a few microns long and a few millimicrons in diameter into which 0.5 to 6.5 MeV of recoil energy is instantaneously deposited. This environment contains many different ions, radicals and hydrated electrons with both oxidizing and reducing properties, leading to ^{13}N in many valence states. The lifetime of species found in this small space are sufficiently short that each ^{13}N birth can be considered as an independent event, isolated from other ^{13}N atoms and from protons that stop without undergoing nuclear reaction. When nearly one eV is absorbed per water molecule, sufficient oxidant may be formed (probably H_2O_2) to modify the initial product spectrum, converting it entirely to $^{13}NO_3^-$.

We acknowledge the assistance of Eugene Russell, David Vera, William Brady and Ken Yoneda with the experiments and thank Dr. Richard McDonald for the beam energy measurements and Professors Neal Peek, John Jungerman and Robert Stadalnik for helpful discussions. This work was supported by NSF (DEB 77-01199), DOE (EY-76-C-03-0472) and Crocker Nuclear Laboratory.

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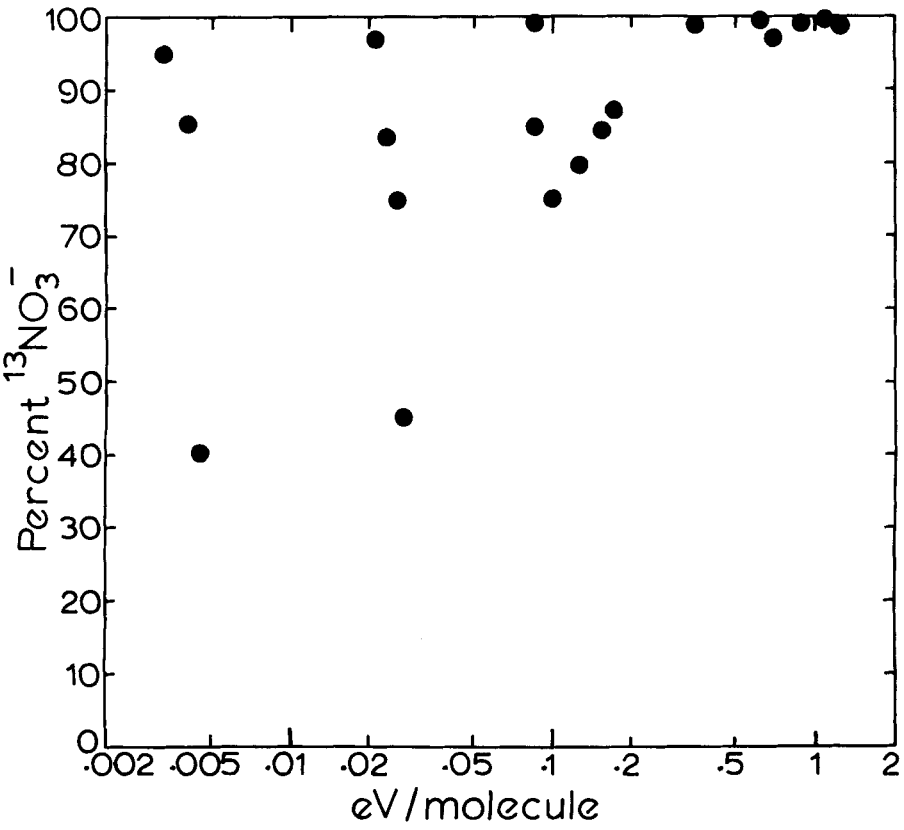
Figure 1


100
90
80
70
60
50
40
30
20
10
0
Percent $^{13}NO_3^-$
.002
.005
.01
.02
.05
.1
.2
.5
1
2
eV/molecule

Paper No.41

HIGH YIELD SYNTHESIS OF THE SEQUENCE $^{13}N_2$, $^{13}N_2O$, $^{11}C_2H_2$, $H_2{}^{15}O$, $H_2{}^{14}O$ AND $^{17}F^-$ FOR rCBF STUDIES

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Physiological flow can be quantified by measuring the clearance of inert, diffusible tracers. The low solubility of xenon and poor scatter rejection at 81 keV conspire to make ^{133}Xe ill-suited for regional blood flow (rCBF) studies. A positron emitting gas of moderate solubility would permit sharp definition of the studied brain volume through coincidence detection. For this reason, synthetic techniques were developed to provide a sequence of increasingly soluble agents $^{13}N_2$, (^{133}Xe), $^{13}N_2O$, $^{11}C_2H_2$, $H_2{}^{15}O$ and $H_2{}^{14}O$ with cerebral kinetics clearly differentiated from the non-diffusible ion $^{17}F^-$.

^{13}N-Nitrogen. Carbon-13 powder, enriched to 98%, was irradiated with an 11 MeV proton beam from the UW EN tandem accelerator, inducing a saturation ^{13}N-yield of 100 mCi/μA consistent with reference 1. The 1 mm^2 beam spot was centered on the thick ^{13}C target, with heat removed both by conduction and a nitrogen stream passing over the target face. Trapping of $^{11}CO_2$ from this cooling gas allowed parasitic ^{11}C-labelling experiments to proceed in parallel with ^{13}N-production. Following bombardment, the 20 mg of ^{13}C was transferred to a combustion apparatus in which the target assembly doubled as a furnace to reduce radiation exposure. The target material was burned in a controlled oxygen jet, with the evolved activity monitored in a flow-through detector (2) to be quantitatively collected in a 60 ml syringe. Processing time averages 10 minutes, decay-corrected yields exceed 90% and negligible (stable) carbon monoxide concentrations result over a wide range of combustion conditions.

^{13}N-Nitrous Oxide. Ten MeV proton irradiation of a recirculating water target induced 4 mCi/μA of ^{13}N-nitrate at saturation. Carrier ammonium nitrate was added and evaporated to dryness. Pyrolysis in concentrated sulfuric acid (3) at 220°C released ^{13}N-nitrous oxide with less than 3% ^{13}N-nitrogen contamination, as evident by radio gas chromatography. Decay-corrected yields exceed 80% of the initial activity, with processing times averaging 18 minutes. The ^{13}N-nitrous oxide is easily dissolved in cold saline for intravenous injection.

^{11}C-Acetylene. Proton irradiation of nitrogen results in ^{11}C as carbon dioxide which is trapped in aqueous sodium hydroxide containing 1 mmole sodium carbonate carrier. The carbonate is precipitated as barium carbonate, filtered and dried. Excess magnesium is added and a thermite reaction carried out in hydrogen at 700°C, yielding barium carbide (4). The ground carbide is flushed with helium, wetted with ethanol and treated with boiling water to release ^{11}C-acetylene in 80% yield with radiochemical purity in excess of 98%. Processing times average 40 minutes, with 10 mCi/μA of $^{11}C_2H_2$ available for inhalation or injection.

Labelled Water. Deuteron irradiation of a high pressure nitrogen target induces ^{15}O-activity as oxygen, which is swept through a teflon capillary to the user site 500 meters distant. Carrier oxygen and excess hydrogen

are added to the incoming nitrogen gas stream in a 1:3:10 ratio. The trinary gas mixture passes over a palladium-on-alumina catalyst, contained in a water-cooled quartz tube (2). Calorimetry on the reaction power levels (typically 100 watts) indicate quantitative conversion of the oxygen to water. Under tuned transport conditions and 8 MeV deuteron energy, 15 mCi/μA of ^{15}O-labelled water saturates in a volume that is readily controlled by the user. Production of ^{14}O-labelled water requires proton irradiation of the nitrogen target. A soda-lime trap is placed on-line to remove the prodigious ^{11}C-carbon dioxide that would otherwise dissolve in the generated water, masking the 1/2 mCi/μA ^{14}O saturation activity present at 11 MeV.

^{17}F-Fluoride. Nine MeV deuteron irradiation of a recirculating water target induces 100 mCi/μA of $^{17}F^-$activity at saturation. This extraordinary yield is a direct result of the large proton transfer cross section to the weakly-bound, first excited state of ^{17}F. Low level ^{19}O-contamination is quickly removed by decay. The aqueous $^{17}F^-$ is directly suitable for injection in studies requiring an ionic positron emitter constrained by the blood-brain barrier. The 66-second half-life dominates the recirculation, suggesting steady state imaging applications (5). In our preliminary rCBF investigations using coincidence arrays, the rapid passage of $^{17}F^-$ through the cerebral vasculature acts as the observed (shunt spike) input function common to the other diffusible tracers in the sequence.

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Paper No.42

A NOVEL SYNTHESIS OF ^{13}N-L-ASPARAGINE

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The enzymatic synthesis of ^{13}N-L-asparagine from aspartic acid, ^{13}N-ammonia and asparagine synthestase has been reported (1,2). The use of ^{13}N-L-asparagine has provided superior myocardial images in dogs (1,2) and in other animal species (2).

The preparation of ^{13}N-L-asparagine described in these investigations suffers from some disadvantages; the enzyme used is not commercially available and must be isolated and purified via an involved procedure; since the enzyme is unstable, it may not be immobilized on a solid support to improve product safety; finally the yield is low (10%). These drawbacks have limited the use of this radiopharmaceutical in the past.

We have developed a novel method of preparing the compound by a non-enzymatic procedure which is both rapid and efficient. The β-carboxylic group of the protected L-aspartic acid; α-N-t-Boc-t-Bu-Aspartate is activated with N-Hydroxysuccinimide to yield α-N-t-Boc-α-t-Bu-β-N-hydroxysuccinimidyl aspartic ester. Without purification the latter is refluxed for 10 min with ^{13}N-ammonia. A hydrolysis for 1 min in 1 N HCl yields up to 30-40% of ^{13}N-L-asparagine and about 40% of ^{13}N-ammonia. The ammonia appears in the product mixture as unreacted starting material and as a result of the partial hydrolysis of ^{13}N-L-asparagine to ammonia and aspartic acid. The labeled asparagine is purified by cation exchange.

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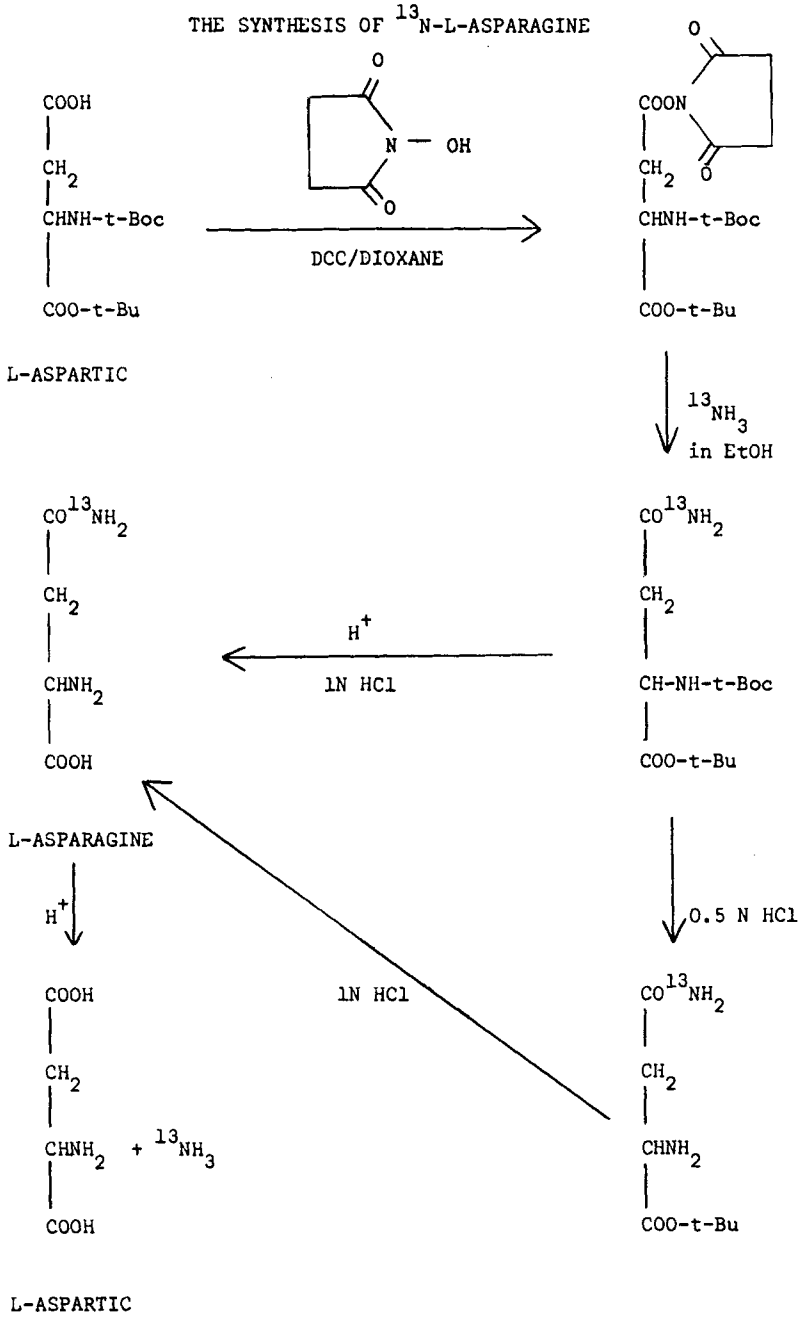
THE SYNTHESIS OF ^{13}N-L-ASPARAGINE
DCC/DIOXANE
L-ASPARTIC
$^{13}NH_3$ in EtOH
H^+
1N HCl
L-ASPARAGINE
H^+
1N HCl
0.5 N HCl
L-ASPARTIC

Paper No.43

SYNTHESIS OF (^{13}N)-LABELED AROMATIC L-AMINO ACIDS BY ENZYMATIC TRANSAMINATION OF (^{13}N)-L-GLUTAMIC ACID

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Aromatic amino acids are of significant medical interest because of their role in the pathogenisis of certain inborn errors of metabolism and as catecholamine precursors. Abnormal catecholamine metabolism is associated with such pathological conditions as Parkinson's disease and various mental disorders. The labeling of L-tyrosine and L-phenylalanine with a short-lived positron emitter may prove useful for in-vivo physiological studies of these disorders.

(^{13}N)-L-Tyrosine and (^{13}N)-L-phenylalanine were enzymatically labeled by the sequential synthesis of (^{13}N)-L-glutamic acid with Sepharose-immobilized glutamate dehydrogenase followed by transamination of p-hydroxyphenyl pyruvate and phenylpyruvate, respectively, in reactions catalyzed by a commercial source of pig heart glutamate oxalacetate transaminase (GOT). When the newly synthesized (^{13}N)-L-glutamic acid was incubated with GOT and the appropriate α-keto acid, the products were analyzed by high pressure liquid chromatography on a Whatman SAX column eluted with 5 mM potassium phosphate-HCl buffer of pH 3.5. Transfer of up to 40% of amino (^{13}N) from glutamic acid to L-tyrosine and 10% to L-phenylalanine were obtained. The formation of (^{13}N)-L-tyrosine was further confirmed by thin layer chromatography. The enzyme(s) catalyzing the transaminations of the keto acid analogues of the aromatic amino acids are constant contaminants of the commercial GOT preparations. Spectrophotometric and chromatographic assays have demonstrated that the α-keto acids of aspartate, methionine, leucine and dihydroxyphenylalanine but not valine or tryptophan can be transaminated by the enzyme preparations.

Paper No.44

PREPARATION OF ^{13}N-LABELED STREPTOZOTOCIN AND NITROSOCARBARYL

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We have recently described a relatively simple procedure for the preparation of millicurie quantities of the cancer chemotherapeutic agent bis(2-chloroethyl)nitrosourea (I) (BCNU), labelled in the nitroso group with ^{13}N (1). We now report the synthesis of the antitumor antibiotic IIa and the nitrosocarbamate IIIa. The latter is a potent mutagen that has been suggested to be formed from the reaction of nitrite, in saliva or the stomach of man, and the carbamate insecticide III (2).

A mixture of 250 mg (1.02×10^{-3} mole) of 2-deoxy-2(3'-methylureido)-D-glycopyranose urea (II) and 600 mg of copper dust was allowed to react with 16 ml of a carrier solution for 5 min. at room temperature. This solution was prepared by the addition of 2 ml of 8 M HNO_3 to 160 ml of glacial acetic acid, yielding a NO_3^- concentration of approximately 1×10^{-4} mole/ml. The dark blue reaction mixture was then passed through a column (2 cm x 8 cm) of a cation exchange resin (AG 50W-X12) to remove the copper salts. [A polystyrene preparation bearing triethylenetetramine functional groups (3) was also shown to remove copper salts from the reaction mixture.] High pressure liquid chromatography (HPLC) (Partisil S.A.X. packing, and elution with phosphate buffer, pH 4.5) and TLC (Silica Gel) with CH_3OH-$CHCl_3$ (1:3) (R_f=0.5) showed that the column effluent contained one compound which co-chromatographed with authentic IIa. The effluent was freeze-dried and the residue yielded 220 mg of a light yellow crystalline product, (yield 81.5%), mp 113-115° (lit. 115°) which exhibited identical IR and NMR spectra with authentic IIa.

^{13}N-labeled IIa was obtained by reacting 10 mg of II, 250 mg of copper dust and 0.5 ml of carrier solution (80 mCi of $^{13}NO_3^-$) as above. The product was obtained from the reaction by passing through the cation exchange column and adjusting the pH to 4.5 with 10N NaOH. Product was shown to be chemically pure by HPLC and TLC. Two mCi of ^{13}N labelled IIa was obtained in a volume of 1.5 ml of solution and in a total synthesis time of 30 min. The radiochemical yield was calculated to be approximately 20%.

A mixture of 0.5 g (2.4×10^{-3} mole) of 1-naphthyl N-methylcarbamate (III) and 1.0 g of copper dust and 24 ml of carrier solution was allowed to react for 10 min. Subsequently, 50 ml of water was added and the mixture extracted twice with 50 ml of CH_2Cl_2. The CH_2Cl_2 extracts were washed with H_2O, dried over Na_2SO_4 and evaporated under reduced pressure.

The residue was recrystallized from ethanol-H_2O (1:1) to yield 0.4 g (1.9×10^{-3} mole) (70% yield) of IIIa. The identity of IIIa was confirmed by IR, NMR spectra and melting point.

The ^{13}N-labeled nitrosocarbaryl (IIIa) was synthesized by utilizing the above procedure, with a radiochemical yield ranging from 15 to 20% yield. In a typical experiment, starting with 120 mCi of $^{13}NO_3^-$, 2.8 mCi of IIIa was produced in a synthesis time of 30-35 min. The specific activity of the product was 350 μCi/mg.

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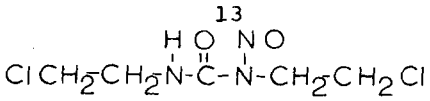
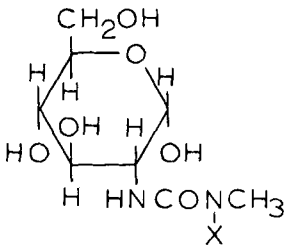
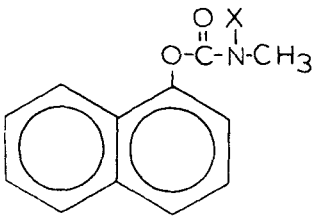


I = BCNU





II=X=H=Streptozotocin precursor

IIa=X=NO=Streptozotocin

III=X=H=Carbaryl

IIIa=X=NO=Nitrosocarbaryl

Paper No.45

AN EXAMPLE OF A COMPLETELY AUTOMATIC METHOD TO SYNTHESIZE A RADIOPHARMACEUTICAL MOLECULE : ^{11}C-IMIPRAMINE.

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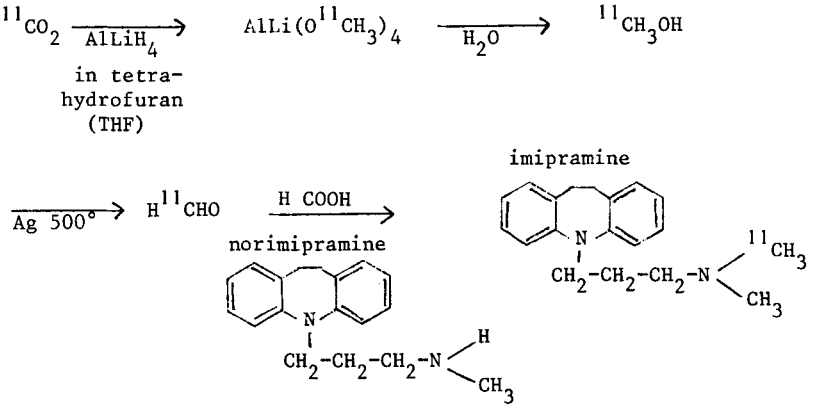
^{11}C-imipramine, an anti-depressant which easily crosses the blood-brain barrier, is currently used in our laboratory for brain examinations in patients suffering from various neurological diseases.

The preparation frequency and the radioactivity level involved (0.5 to 1 Ci) are such that an entirely automatic synthesis method had to be developed. The principle has already been described in reference to ^{11}C-chlorpromazine synthesis (1) :



The changes needed to automate the method concern the nature and size of the apparatus and the purification method.

The reaction vessels are 1 ml conical tubes joined together by flexible teflon capillaries fitted to hypodermic needles. These tubes are closed with plastic septa and the passage of fluids is controlled by electrovalves (Durrum). The mobile reaction vessels are handled by remote control and may be transferred from a hot bath (100°C) to a cold bath (-20°C). The purification is carried out by HPLC (Waters) on a Partisil column magnum 9 (Whatman).

The ^{11}C carbon dioxide produced with the cyclotron by $^{14}N(p,\alpha)^{11}C$ reaction is trapped in liquid oxygen, reduced in the first tube to methanol and dehydrogenated to formaldehyde by passage over the silver catalyst. The reaction with norimipramine takes place in the second tube, after which the reaction mixture is injected automatically onto the chromatographic column in a 100 µl volume and the tube rinsed with solvent fed in from outside by a syringe. The imipramine fraction is collected, the solvent evaporated under nitrogen and the product taken up in physiological serum buffered to pH 4. After sterilisation through millipore the solution is ready for use. This sequence of operations lasts 45 minutes.

Tests, carried out during the development stage with non-radioactive carbon dioxide (1 µM), give a final yield of about 30 % (dehydrogenation of methanol to formaldehyde : 50 %, transfer onto the chromatographic column : 75%). The imipramine obtained is chromatographically pure, free from solvent (dimethylformamide) and excess norimipramine.

This method may also be applied to other molecules prepared routinely in the laboratory [chlorpromazine (1), chlorimipramine (2), nicotine (3)], while owing to the smaller amounts of reagent neccessary ($AlLiH_4$) specific radioactivity better than that obtained by semi-automatic methods may be expected.

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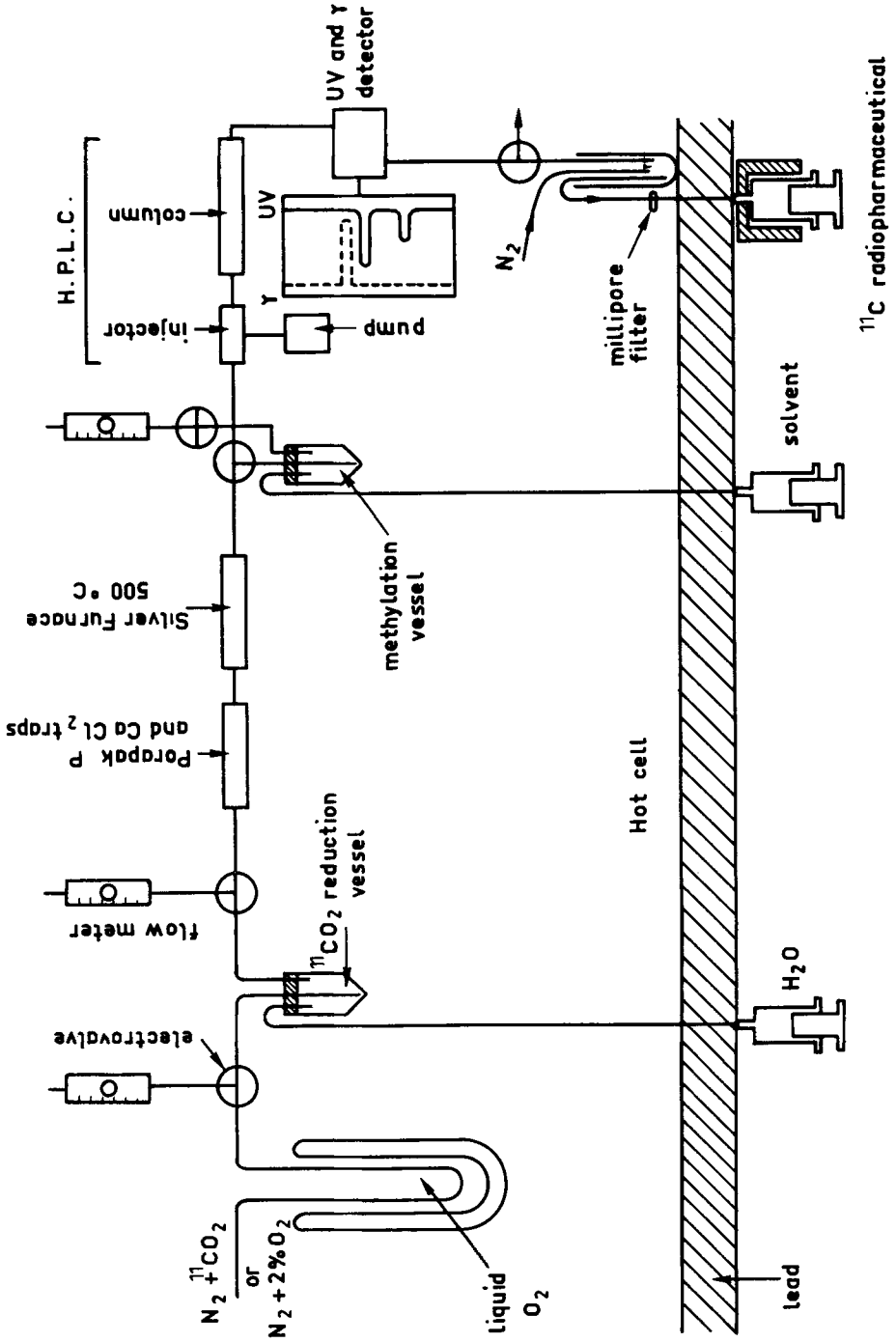
UV and γ detector
H.P.L.C.
column
injector
UV
γ
pump
N_2
millipore filter
^{11}C radiopharmaceutical
solvent
methylation vessel
Silver Furnace 500 °C
Porapak P and Ca Cl$_2$ traps
flow meter
$^{11}CO_2$ reduction vessel
Hot cell
H_2O
electrovalve
$N_2 + ^{11}CO_2$ or $N_2 + 2\% O_2$
Liquid O_2
lead

Paper No.46

NUCLEAR DATA FOR THE PRODUCTION OF MEDICALLY USEFUL RADIOISOTOPES

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An important basic parameter in the choice of a radioisotope for radiopharmaceutical use is a detailed knowledge of the relevant nuclear data, including both decay properties and production reaction cross-sections. The data are needed for diverse considerations like targeting, level of radionuclidic impurities, radiochemical separation schemes, radiation chemistry and biology, radiation dosimetry etc. In this paper the present status of nuclear data will be reviewed. Special attention will be paid to the production cross-section data of the present-day as well as some potential medically important radioisotopes and the various radionuclidic impurities associated with them. For various radioisotopes production can be effected via several nuclear reactions. Wherever possible, an intercomparison of the merits and disadvantages of the various production methods will be presented. Special consideration will be given to some classical as well as some new "organic" (^{18}F, ^{34m}Cl, $^{76,77}Br$, ^{123}I, ^{30}P) and "inorganic" (^{28}Mg, ^{48}Cr, ^{97}Ru, ^{201}Tl etc.) radioisotopes, and the areas needing more radiochemical work will be outlined.

Paper No.47

YIELDS OF RADIOXENONS 122 TO 129m FROM 43 TO 160 MeV PROTONS ON ^{133}Cs

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Recent experiments to determine the yield of ^{123}I from the spallation reaction $^{133}Cs(p,2p9n)^{123}Xe$ have shown a relatively small dependence on the proton energy over the energy range 210 to 590 MeV (1,2). The yields of the radiocontaminants ^{125}I from $^{125}Xe \rightarrow ^{125}I$ and ^{121}Te from $^{121}Xe \rightarrow ^{121}I \rightarrow ^{121}Te$ also appear to have only a slight energy dependence. This result may be an advantage for ^{123}I production by use of the waste beam at meson factory accelerators, where proton beam currents of tens to hundreds of microamperes are available at beam dumps with a wide range of energies depending upon the prime experiment's requirement.

The present study considers another option available to the radioisotope producer, specifically, the possibility of adjusting the proton energy of the waste beam, by means of beam energy degraders, to achieve an optimum energy for the yield of a particular radioxenon. That is,optimization of the yield, by adjustment of the proton energy, can be achieved not just for $^{123}Xe \rightarrow ^{123}I$ but for each of the neutron deficient radioxenons.

Nature has been benevolent in making caesium monoisotopic and in separating the radioisotopes ^{123}Xe, ^{125}Xe, ^{127}Xe, and ^{129m}Xe by the stable isotopes ^{124}Xe, ^{126}Xe, ^{128}Xe and ^{130}Xe. As a consequence, there should exist an incident proton energy at which the yield of a particular radioxenon is a maximum. The present study reports the yields of each of the neutron deficient radioxenons for protons in the energy interval 43 to 160 MeV. The irradiations were done at the Indiana University Cyclotron Facility (IUCF).

The target used for this study was a 5cm diameter by 1cm thick stainless steel container of liquid caesium to which there was attatched a vertical reflux column. The caesium was electrically heated to about 80°C so that radioxenons rapidly reached the liquid surface and were evolved from the target. The liquid caesium target and radioxenon collection system were similar to that used in a study (3) of ^{127}Xe and ^{129m}Xe production with

protons in the energy interval 28 to 41 MeV.

The proton bombardments were done with low beam currents (<1 μa) and for only a few minutes. Radioxenons were carried away from the target by a low flow of helium gas (<50 cc/min) as they evolved from the caesium. They were adsorbed on charcoal at liquid nitrogen temperature and Ge(Li) spectra of gamma radiation from the charcoal trap were taken repeatedly for a 30 minute period following bombardment. The trap was sealed off and then removed from the target system for later determination of the yield of the longer lived radioxenons.

Another objective of this report is to point out the advantages of a liquid metal caesium generator target over the fused caesium salt target. In addition to the obvious advantage of the avoidance of processing a radioactive target, there is an improved yield when a generator type target is used (4), because with the fused caesium salt the decay ($^{123}Xe \rightarrow {}^{123}I$) in the target reduces the yield, whereas with the generator target the ^{123}Xe is removed as it is produced. To be presented are the results of ^{123}I production runs at IUCF with the generator type target and also a description of ^{123}I production facilities at TRIUMF, in Vancouver,B.C., Canada, where a liquid caesium target is planned.

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Paper No.48

COMPUTED YIELDS OF ^{11}C, ^{13}N, ^{15}O, and ^{18}F FOR PROTON BOMBARDMENT OF ENRICHED STABLE ISOTOPE TARGETS

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The emergence of positron tomography as an important technique for the study of physiology and the diagnosis of disease (1) has caused us to emphasize the development of positron radiopharmaceuticals and their evaluation with our ORTEC ECAT scanner (2,3,4). Since radionuclide production in support of our work must be accomplished using the 22 MeV internal beam of a proton accelerator, the ORNL 86-Inch Cyclotron (5), we have been motivated to emphasize research on high-yield, proton reactions on solid or liquid target materials for the production of ^{11}C, ^{13}N, and ^{18}F. As a result of this approach, we have observed that important advantages can be gained by using p,n reactions on targets which are highly enriched in the stable isotopes ^{11}B, ^{13}C, ^{15}N, and ^{18}O.

Excitation functions were constructed by combining and adjusting data from three references (6,7,8) for $^{11}B(p,n)^{11}C$, two references (9,10) for $^{13}C(p,n)^{13}N$, one reference (11) for $^{15}N(p,n)^{15}O$, and three references (12, 13,14) for $^{18}O(p,n)^{18}F$. The resulting composite curves were then put in digital form with an Elographics Model E241 Graphic Data Digitizer and entered in the data file of our Accelerator Target Simulation (ATS) computer code. ATS is an extensive Fortran program which calculates and graphically depicts performance parameters of accelerator target systems. A matrix representation is used to model complicated target geometries, and output includes two-dimensional and three-dimensional plots of yield, energy attenuation, heat generation, and temperature. A modification of the Stopping Power and Ranges (SPAR) computer code (15) is used by ATS to calculate energy attenuation of charged particle beams. ATS calculations for thick target saturation yields of ^{11}C, ^{13}N, ^{15}O, and ^{18}F are shown in the figures (solid lines) for the p,n reactions on ^{11}B, ^{13}C, ^{15}N, and ^{18}O. Yields of other proton and deuteron reactions are also shown for comparison purposes.

The enriched stable isotope target materials are readily available (16,17) in high enrichments (99%) at reasonable costs ($75/g to $125/g) and in various chemical forms. Using a ^{11}B-boron oxide internal beam target bombarded by 175 μA of 22 MeV protons, we have recovered up to 5 Ci of ^{11}C-oxides in 40 min. Under the same conditions, we have recovered up to 3 Ci of gaseous ^{13}N activity from a ^{13}C powder target. We are also developing a system for the large-scale production and recovery of ^{18}F from a target utilizing ^{18}O-water in a recirculating mode.

We conclude that the p,n reactions on enriched stable-isotope targets offer the potential of producing high yields of ^{11}C, ^{13}N, ^{15}O, and ^{18}F at low energies with one charged particle beam. Target size and cost can be minimized by exploiting the recovery of gaseous activity from bombarded solids or liquids. (Oak Ridge Associated Universities operates under Contract Number EY-76-C-05-0033 with the U.S. Department of Energy.)

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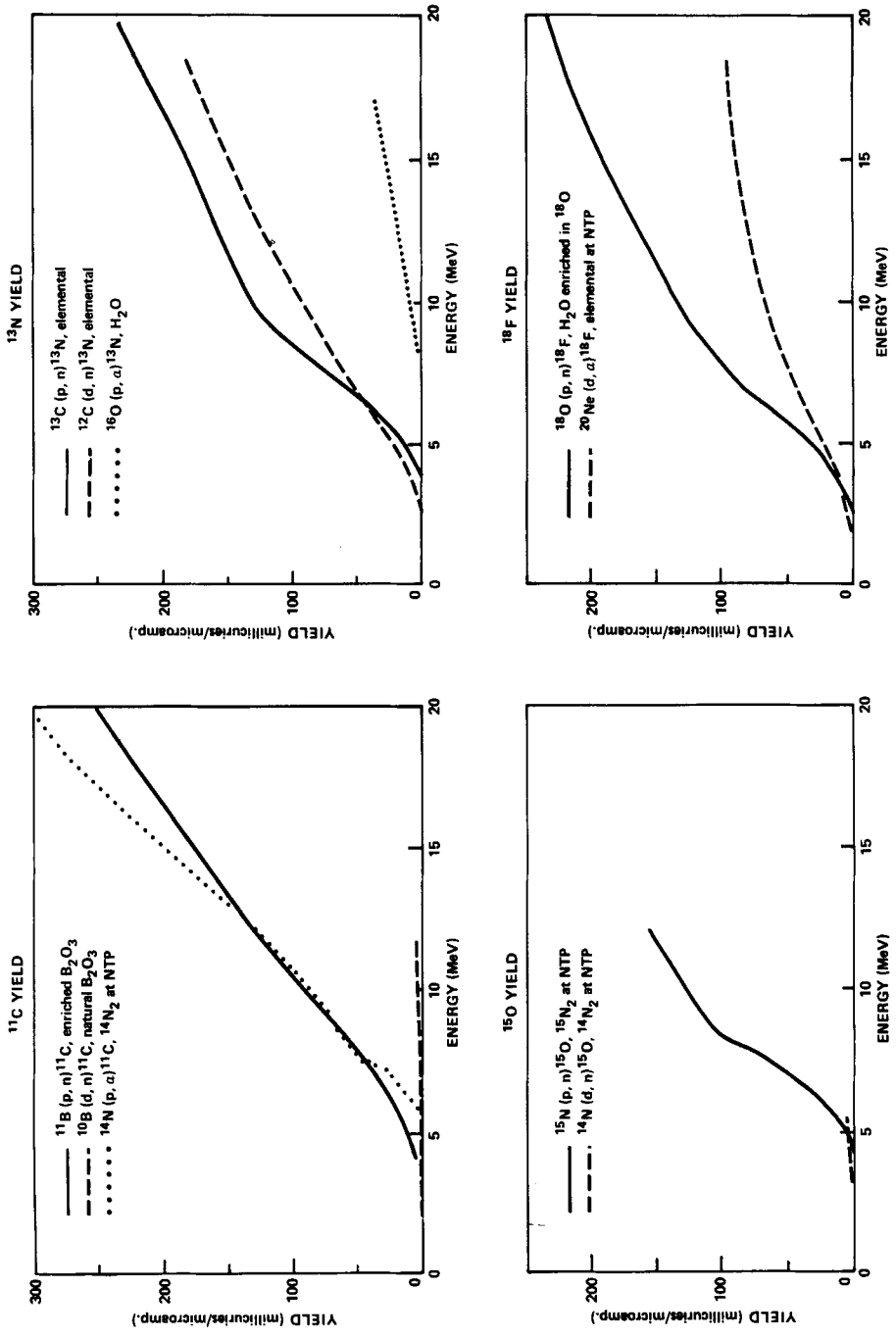
^{13}N YIELD
^{13}C (p, n)^{13}N, elemental
^{12}C (d, n)^{13}N, elemental
^{16}O (p, α)^{13}N, H_2O
YIELD (millicuries/microamp.)
ENERGY (MeV)
^{18}F YIELD
^{18}O (p, n)^{18}F, H_2O enriched in ^{18}O
^{20}Ne (d, α)^{18}F, elemental at NTP
^{11}C YIELD
^{11}B (p, n)^{11}C, enriched B_2O_3
^{10}B (d, n)^{11}C, natural B_2O_3
^{14}N (p, α)^{11}C, $^{14}N_2$ at NTP
^{15}O YIELD
^{15}N (p, n)^{15}O, $^{15}N_2$ at NTP
^{14}N (d, n)^{15}O, $^{14}N_2$ at NTP

Paper No.49

CYCLOTRON PRODUCTION OF SHORT-LIVED ^{30}P

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Phosphorous is an important element in living systems. For tracer studies in life sciences, out of its two fairly long-lived isotopes $^{32}P(T_{1/2} = 14.3d)$ and $^{33}P(T_{1/2} = 25.3d)$, so far the former has been most commonly employed. Both of these isotopes, however, are pure β^- emitters and are not suitable for in-vivo studies. The only other isotope of phosphorous worth considering is ^{30}P, although its half-life ($T_{1/2} = 2.5min$) is rather short. Its β^+ emission, however, may also prove to be advantageous with the increasing use of positron tomographs.

We have carried out development work on the production of ^{30}P and have measured the relevant nuclear data of the following four nuclear reactions:
$^{27}Al(\alpha,n)^{30}P$; $^{31}P(p,pn)^{30}P$; $^{31}P(n,2n)^{30}P$ and $^{32}S(n,t)^{30}P$.

The excitation function of the (α,n) reaction was measured over E_α = 8 to 28 MeV and that of the (p,pn) reaction over E_p = 16 to 36 MeV. The neutron induced reactions were investigated using a fast neutron spectrum produced in the break-up of 53 MeV deuterons on Be. From considerations of product yields, radionuclidic purity, carrier-free form of ^{30}P, and ease in target construction etc., the reaction $^{27}Al(\alpha,n)^{30}P$ appears to be most promising. This reaction is especially suited for production of ^{30}P using high-intensity compact cyclotrons. Theoretically, ^{30}P thick target yields of 4.7 mCi/μA min can be achieved at E_α=28 → 8 MeV.

A method for the rapid isolation of carrier-free ^{30}P in the form of $^{30}PH_3$ has been developed. The irradiated Al is dissolved in 6M HCl in a reducing atmosphere, the formed $^{30}PH_3$ is swept off by a stream of helium gas and is absorbed in alcohol, saline solution (containing a few drops of H_2O_2) or dilute bromine water. The procedure takes about 5 min to complete and gives ^{30}P of very high radionuclidic purity (> 99.99%). Radiochemical yields between 30 and 70% are achieved and under these conditions > 70% of the activity in solution appears in the phosphate form. mCi amounts of pure ^{30}P can easily be produced for potential medical applications.

Paper No.50

CYCLOTRON PRODUCTION OF SHORT-LIVED Na-21

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Sodium is one element which can be used as tracer for the detection of functional stenosis of kidney. From the 16 known radioactive isotopes of sodium only Na-22 and Na-24 have been used recently in nuclear medicine. Use of these nuclides becomes troublesome because of the large radiation dose they deliver.

Na-21 with a short half-life of 22,6 seconds, is a typical cyclotron nuclide and positron emitter which allows the use of a positron imaging device.

From the various reaction mechanisms leading to Na-21 we have examined only two methods which were practical. The first - deuteron bombardment of gaseous neon $^{20}Ne(d,n)\,^{21}Na$ and the second - ^{3}He particle irradiation of neon gas ^{20}Ne $(^{3}He,2n)$ $^{21}Mg \xrightarrow{0,12\ s}\, ^{21}Na$ or direct $^{20}Ne(^{3}He,pn)^{21}Na$.

Both reactions have been examined and the $^{20}Ne(d,n)^{21}Na$ reaction has been found more useful for routine production.

Degradation of incident deuteron energy below 3,4 MeV, which is treshold of the $^{20}Ne(d,\alpha)^{18}F$, avoided the co-production of F-18. The method used required scavenging of Na-21 from the target and fast transport of Na-21 in a tubing system over a relatively long distance.

Principally, there are two ways to transport Na-21 from the target during bombardment to the dispensing site in the shortest time.

1) Situate the special apparatus for washing of Na-21 from the gas phase to the liquid phase immediately behind the target and transport the Na-21 in isotonic saline solution over 15 m distance.

2) Situate the trap at the application site and transport the Na-21 over the 15 m distance with target gas.

The second method using Ne gas as transport medium, has been found more simple and efficient. Optimal irradiation conditions and experimental parameters such as target fabrication and recovery procedures of Na-21 are discussed. Under optimal conditions, the activity in the Neon gas required a flow time of about 10-11 seconds to reach the dispensing reservoir which was situated in the diagnostic area.

The optimal experimental conditions, using a 10 μA deuteron beam current continously delivered 3 mCi/ml of Na-21 in injectable isotonic saline to the clinical area.

The radionuclidic purity and half-life of the Na-21 was determined. The extraordinary behaviour of Na-21 in different trapping solutions and the fact that the Na-21 could be so easily removed from the target by a sweeping gas and then conveyed on a relatively long distance, led us to the study of a transport mechanism and the chemical state of Na-21 after the bombardment.

Paper No.51

GENERATOR FOR IONIC GALLIUM-68.

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Several generator systems for gallium-68 have already been proposed. They are based either on adsorption chromatography (1,2) or ligand-ligand extraction (3-5). A commercially available generator elutes gallium as an EDTA complex, which must undergo additional separations when different gallium compounds are needed. A generator delivering ionic gallium would greatly simplify the production of gallium-68 labeled compounds.

Distribution coefficients for germanium and gallium were determined by batch equilibration. The systems tested were hydrous zirconium oxide (HZO) with nitric acid as eluant, titanium dioxide with either dilute sodium hydroxide or dilute sodium phosphate buffers as eluants, and silica gel with nitric acid as eluant.

Germanium adsorbs strongly on HZO from nitric acid, while the K_D for gallium drops rapidly with increasing nitric acid concentration. However at nitric acid normalities higher than one, HZO starts to decompose. Thus, no acceptable generator system could be developed with the HZO/HNO_3 system.

The best separation system is a titanium dioxide/dilute sodium hydroxide system. However, titanium dioxide slowly dissolves in dilute alkali, causing undesirable levels of titanates to appear in the gallium-68 fractions. Unless this difficulty can be overcome this system cannot form the basis for a generator. In the acidic pH range, gallium adsorbs better than germanium, as shown in Fig. 1.

Silica gel/HNO_3 allows a separation of germanium and gallium as shown by the K_D values in Table I. The adsorption of germanium onto the SiO_2 is a slow process which is, however, of no consequence once a generator has been built up. Although HNO_3 is an undesirable agent in pharmaceutical preparations, it can readily be removed by evaporation of the gallium fraction before the desired complexing agent is added. This system therefore is the most promising among those tested as a potential generator system.

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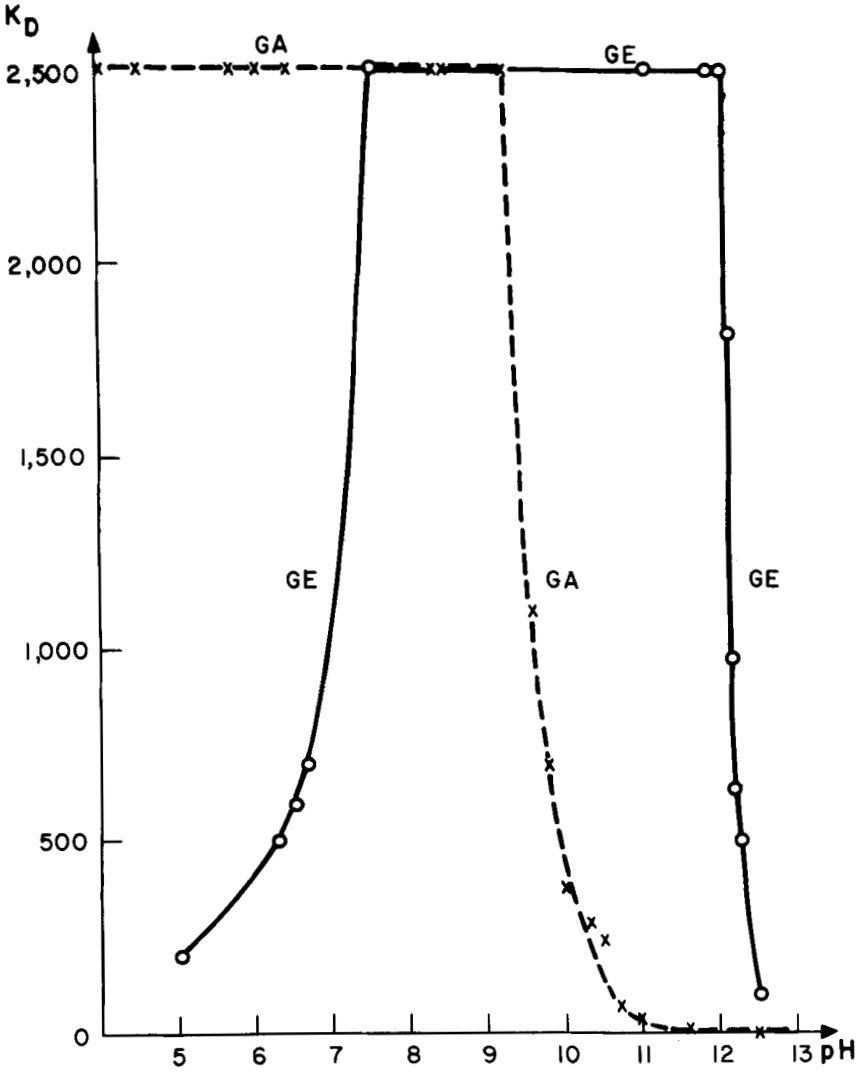
Table I
Distribution Coefficients (K_D) for Germanium and Gallium between SiO_2 and Nitric Acid

N_{HNO_3}	K_D	
	Ga	Ge
0.12	<1	54
0.50	<1	66
1.0	<1	98
2.0	<1	160



K_D - VALUES FOR Ge AND Ga ON TiO_2 IN F (pH)

Paper No.52

APPLICATIONS OF GALLIUM-68 PRODUCED BY THE GALLIUM-68 OXINE GENERATOR

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Recently (1) we described a new gallium-68 solvent extraction generator producing the readily convertible weak chelate gallium-68-8-hydroxyquinoline (gallium-68-oxine). We have used the eluate from this generator to produce gallium-68 radiopharmaceuticals and labeled blood components. The bone agent gallium-68-EDTMP (2), produced by chelate substitution of EDTMP for oxine in aqueous solution, has provided excellent bone scans of rabbits and has produced bone/blood ratios in rats of 7 and 16 after 1 and 2 hours, respectively. Gallium-68 ferric hydroxide colloid has been prepared by displacement of gallium-68 from the oxine complex by Fe^{+3}, followed by coprecipitation of gallium-68 with ferric hydroxide from a pH 7.5 phosphate buffer solution. The gallium-68 colloid has been used to image the canine liver and spleen via multi-slice positron emission transaxial tomography. Using Krejcarek and Tucker's procedure (3) we have attached the polyamine carboxylate chelating agent DTPA to albumin which creates a hexadentate chelating site for the gallium-68. Comparative rabbit blood clearance studies of gallium-68-DTPA-albumin labeled protein complex (4), versus iodine-125-labeled albumin suggest that the gallium-68 preparation clears more slowly than the iodine-125-labeled protein and therefore may be a better blood pool scanning agent. Gallium-68-oxine has also been used to label DTPA-HSA microspheres, produced in a similar fashion, for use as a lung scanning agent. The gallium-68 microspheres should provide a stable in vivo label, since after 4 hours in a physiological solution of transferrin 95% of the activity remained attached to the DTPA-HSA microspheres. Labeling of canine red cells (5) with gallium-68-oxine has been studied, demonstrating an efficiency of $\sim$80%. The labeling was found to reach a maximum in less than 10 minutes for concentrated red cell suspensions and showed a marked dependence on red cell concentration. Canine platelets have also been labeled with gallium-68-oxine (5) and used to successfully detect pulmonary emboli in dogs by positron emission transaxial tomography.

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Paper No. 53

BEHAVIOR OF GALLIUM-67 IN THE BLOOD: THE ROLE OF TRANSFERRIN

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While Gallium-67 citrate has been shown to localize in a wide variety of soft tissue tumors and abscesses, the exact mechanism of these processes is still unknown. Previous studies on the nature of gallium binding in blood have yielded widely divergent results, indicating that gallium in blood behaves mostly as a free form or is loosely associated with albumin, transferrin or haptoglobin. Hartman and Hayes (1) first demonstrated that gallium in blood is bound to plasma proteins and later suggested that α or β globulins, particularly transferrin, are active components. Gunasekhara et al. (2) reported that gallium in blood remains mainly associated with serum proteins of which 70% appears to be loosely associated with albumin, transferrin or haptoglobin. With regard to the nature of gallium binding to transferrin, Toshihiko Hara (3) reported that gallium in blood behaves mostly as a free form and is partially associated with transferrin, whereas Clausen et al. (4) showed that gallium in blood is firmly bound to transferrin, which has 14 binding sites for gallium.

We have reinvestigated the nature of gallium binding in blood to more clearly identify the plasma components binding gallium in vivo. Blood samples were obtained at various times following intravenous injection of Ga-67 (high citrate) in rabbits. To determine the total protein binding, ultrafiltration, dialysis and ammonium sulfate precipitation were performed on the plasma samples. Relative distribution of gallium among the various plasma protein fractions was determined by agarose electrophoresis at 2 mA constant current in pH 8.6 barbital buffer. The electrophoresis was run at different time periods of 15, 30, 45 and 60 min (Fig.1). Upon electrophoresis for 15 min, 70% of Ga-67 activity was associated with the β protein fraction, while only 14% appears at the α protein band, 9% at the albumin band, and 6% beyond. After 30 min electrophoresis, these figures were 47%, 19%, 24% and 10% respectively. After 45 min electrophoresis β_1 fraction (transferrin) retained only 30% of the activity, while the activity beyond the albumin band increased to 38% (Fig. 2A-D). Plasma samples dialyzed prior to electrophoresis showed a similar pattern of gallium distribution.

Similarly, when transferrin was incubated with Ga-67 citrate in a plasma ultrafiltrate medium in vitro, a 15 min agarose electrophoresis showed an initial 70% binding, which decreased to 48% at 30 min (Fig. 3A-B). To a portion of the incubated gallium-transferrin mixture 5 mA current was passed for 5 min prior to electrophoresis to test the effect of electric field on gallium binding. A 15 min electrophoresis showed an initial binding of only 39%, which decreased to 27% at 30 min (Fig. 4A-B). Albumin incubated with Ga-67 citrate failed to show any binding after a 30 min electrophoresis (Fig. 5A-B). Electrophoresis of gallium citrate alone was run for comparison (Fig. 6A-B). Ultrafiltration of the incubation mixture of transferrin with gallium showed that 97% of the gallium is bound to transferrin. Ultrafiltration of the rabbit plasma samples showed that 98% of the injected gallium is protein-bound.

These results suggest that, in vivo, gallium present in blood is bound exclusively to transferrin, and the activity which appears in other protein fractions after electrophoresis is the dissociated gallium from transferrin. Most of the previous work to identify the plasma components that bind

gallium was based on electrophoresis technique. As a result, the data showing most of the gallium activity in blood as free or loosely associated with albumin or haptoglobin may be due only to experimental limitation of electrophoresis technique and not to binding of gallium by the other proteins. These results may have significance in the study of the mechanism of gallium localization and in the application of electrophoresis to determining protein binding of other radiopharmaceuticals.

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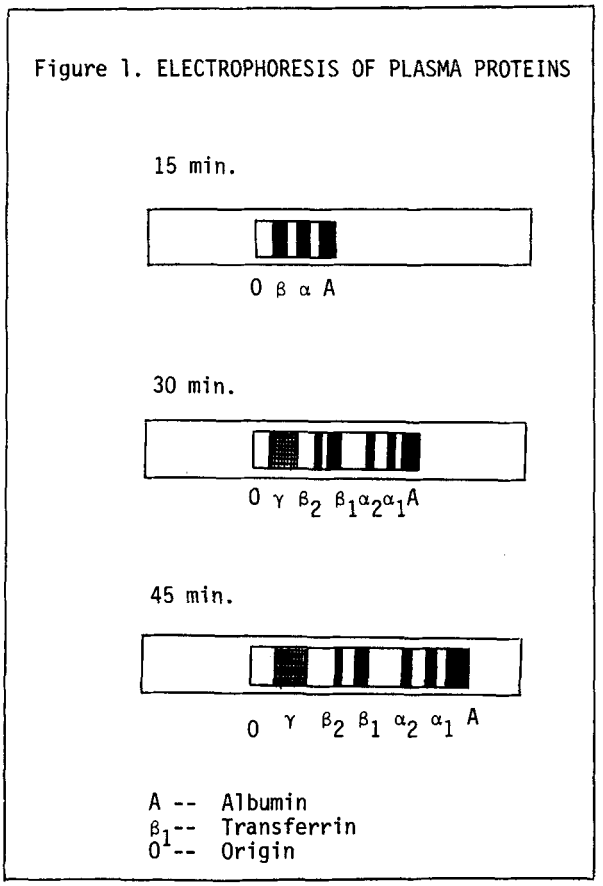
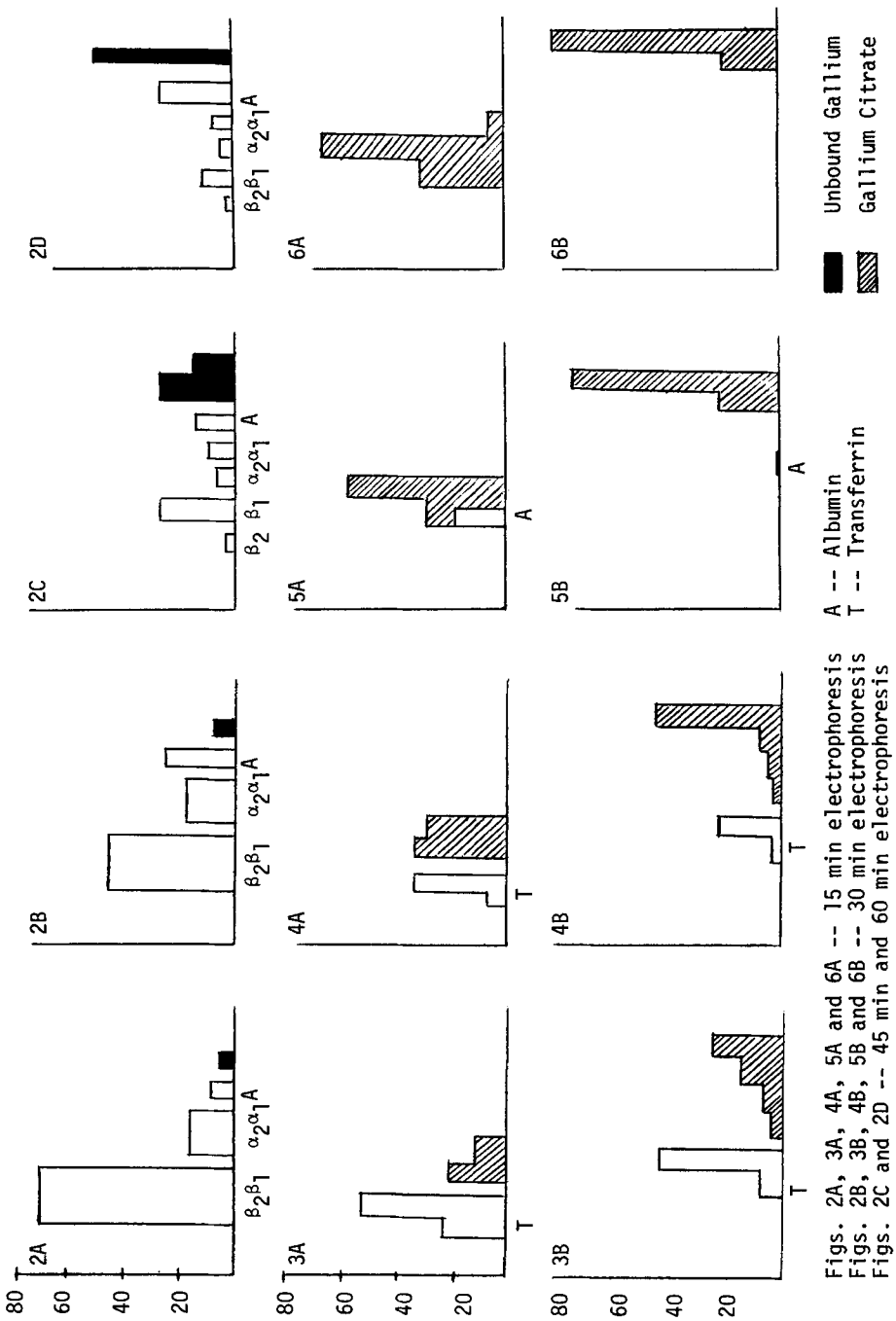
Figure 1. ELECTROPHORESIS OF PLASMA PROTEINS





Figs. 2A, 3A, 4A, 5A and 6A -- 15 min electrophoresis
Figs. 2B, 3B, 4B, 5B and 6B -- 30 min electrophoresis
Figs. 2C and 2D -- 45 min and 60 min electrophoresis

Paper No.54

IN VITRO AND IN VIVO STUDIES OF THE COMPLEX-FREE BEHAVIOUR OF RADIOGALLIUM AND RADIOINDIUM

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As members of the same group of the Periodic Table, gallium and indium resemble each other chemically. This study compared both elements in complex-free condition in saline and serum environments. The results of paper chromatography performed at different pH values showed the effects of the higher hydrolysis constants of gallium; at neutral pH both gallium and indium hydroxides were the predominant species, at pH 8-11 gallium is hydrolyzed to gallate while indium remains as the hydroxide. Electrophoretic studies at buffer pH 8-9 of both elements incubated in serum and transferrin solutions showed that under identical conditions, indium binds to transferrin more avidly than gallium; whereas essentially all the radioindium appeared at the transferrin peak position, radiogallium appeared at the cathode (as hydroxide) and at the anode (as gallate) in addition to the transferrin peak. Using an equilibrium dialysis method, we have measured the first formation constant of gallium and indium transferrin to be 10^{23} and 10^{31} respectively, in agreement with trends apparent in the electrophoretic study. Finally both tracers were administered to dogs and blood levels determined over a one hour period. When injected at acid and neutral pH, both tracers showed similar clearance from blood, when administered at pH 10-11 however, radioindium was cleared more rapidly. As established earlier at this pH only indium exists as colloidal hydroxide, therefore the rapid blood clearance for indium is expected as the tracer is removed by organs of the reticuloendothelial system.

Paper No. 55

CELL DAMAGE ASSOCIATED WITH ^{111}In-OXINE LABELLING OF A HUMAN TUMOUR CELL LINE (HeLa S3)

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^{111}In-oxine has been shown to be of use as a marker for polymorphonuclear leukocytes (1) and lymphocytes (2) in a variety of clinical studies. For studies such as these it is important that the labelled cells should remain viable and functionally normal. However, radiation and chemical damage to the cells might be expected to occur because of the predicted radiation dose from the low-energy Auger electrons emitted by the ^{111}In (D.J. Silvester, personal communication) and the known and suspected toxicity of at least some of the contaminants found in ^{111}In-oxine preparations.

In the present studies, the effects of ^{111}In-oxine, some of the possible contaminants (stable In-oxine, Cd-oxine and oxine) and some of the related metal salts ($InCl_3$ and $CdCl_2$) on various markers of cell damage in a human tumour cell line (HeLa S3) have been investigated.

Mitotic-cell death

Cell damage resulting in mitotic-cell death was determined by assaying the colony-forming ability of 2×10^5 cells ml^{-1} in isotonic saline treated with 1-50 μCi ml^{-1} nominally carrier-free ^{111}In-oxine in ethanol (prepared by the method of Thakur *et al*(1)) for 15 mins. at 20°C. The excess ^{111}In was then complexed to transferrin by the addition of 15% serum. The cells were diluted, plated into dishes of tissue culture medium and incubated at 37°C for 10 days. The cells remaining viable after this treatment attached to the plastic dishes and divided to produce visible colonies which were stained and counted. The dose-response curves obtained, (Table 1) showed considerable variation in colony-forming ability with different batches of ^{111}In-oxine. In order to ascertain whether this was due to chemical toxicity from the variable amounts of stable impurities in the ^{111}In-oxine preparations, cells were treated with some of the known stable contaminants of ^{111}In-oxine preparations (see earlier) under identical conditions to the above and their colony-forming ability measured. Although stable In-oxine is very toxic, the concentrations of the known stable components of a 50 μCi ml^{-1} nominally carrier-free ^{111}In-oxine preparation was too low to cause significant cell killing and therefore the reduction in colony-forming ability and the variation between different batches of ^{111}In-oxine is probably due to radiation and/or unidentified chemical contaminants. All these results are dependent on cell concentration and if the cell number is reduced to 2×10^4 ml^{-1} increased toxicity is observed.

Inhibition of DNA Synthesis

Oxine is the main contaminant of nominally carrier-free ^{111}In-oxine preparations and is present at approximately 2.5×10^{-5} M in a 50 μCi ml^{-1} ^{111}In-oxine preparation. Using a recently published method (3), the effect of oxine on DNA

synthesis of HeLa cells was measured. It was found that cells treated with 10^{-5}M oxine showed a 70% depression in DNA synthesis relative to control cells 60 mins. after the end of oxine treatment. This indicates that oxine may be causing DNA damage to HeLa cells and the fact that oxine is known to be mutagenic to bacteria (4), would indicate that the oxine concentrations in ^{111}In-oxine preparations should be kept as low as possible.

DNA strand breakage

In order to determine DNA strand breakage, cells were grown in ^{3}H-thymidine for 24 hours and then treated with nominally carrier free ^{111}In-oxine (1-50 μCi), stable In-oxine (10^{-7} - 10^{-6}M) and oxine (10^{-6} - 10^{-5}M) for 15 mins. as above. The cells were then incubated in the dilute alkali, to unwind the DNA strands where breaks had occurred, neutralised and the single and double-strand fragments were separated by hydroxyapatite column chromatography (5). The amount of single-strand material eluted from the column was proportional to the number of breaks produced. Of the compounds tested, only stable In-oxine (10^{-6}M) produced breaks. Therefore the effect of oxine on DNA synthesis is not due to the production of DNA strand breaks.

The demonstration that nominally carrier-free ^{111}In-oxine, stable In-oxine and oxine cause cell damage leads to the following suggestions of ways to minimise the potential harmful effects involved in the clinical use of ^{111}In-oxine cell-labelling.

(a) The use of high specific activity material.
(b) Decrease the ^{111}In-oxine : cell ratio by increasing the cell numbers.
(c) Eliminate excess oxine and stable metal impurities from ^{111}In-oxine preparations.

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Table 1

Colony-forming ability of 2×10^5 cells ml^{-1} HeLa S3 treated with nominally carrier-free ^{111}In-oxine

Batch	^{111}In-oxine µCi ml	µCi Bound per 2×10^5 Cells	% Binding	Colony-forming ability, % Control
1	1.17	0.09	8	100
	2.0	0.196	10	100
	10.0	2.86	29	73
	22.5	6.42	29	2.8
2	0.56	0.076	14	91
	1.1	0.17	15	73
	4.9	1.98	40	11
	10.5	5.46	52	0.1
	22.4	13.72	61	0.01
3	13.3	0.24	2	61.5
	28.5	0.86	3	11.0
	59.7	4.94	8	0.06
4	5.1	0.54	11	99
	10.6	1.38	13	96
	22.4	2.18	10	57
5	8.4	0.30	4	98
	17.0	0.80	5	89
	30.4	1.37	5	15
6	1.05	0.09	9	100
	5.1	0.71	14	100
	10.2	1.65	16	100
	22.4	6.08	27	38
	27.1	4.8	18	4