No. 1

THE CHEMISTRY OF TECHNETIUM

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The discovery of technetium was reported by Perrier and Segre in 1937 and 1939. They first produced Element 43 by the bombardment of molybdenum metal in a cyclotron and gave the new element the name technetium from the Greek word for artificial. This was the first element to be synthesized. To date, isotopes with mass numbers from 92 to 107 have been discovered and among these is the metastable isotope ^{99m}Tc.

The choice of ^{99m}Tc for use in nuclear medicine imaging procedures rests upon its favorable nuclear properties and ready availability. As the decay product of ^{99}Mo, ^{99m}Tc is eluted with saline from a generator consisting of ^{99}Mo adsorbed on an alumina column. In the nuclear transformation, TcO_4^- (Tc VII), the most stable chemical state of technetium in aqueous solution is formed. However pertechnetate will not bind to chelating agents nor coprecipitate with particles and therefore less stable positively charged reduced states of technetium must be produced.

Reduced states of technetium can be achieved by treatment of pertechnetate with a variety of reducing substances. The most frequently used are: stannous ion, ferric chloride and ascorbic acid, ferrous ion, sodium borohydride and concentrated HCl. Pertechnetate can also be reduced electrolytically, although with the zirconium and tin electrodes, metallic reducing agents are present. In the reduced state Tc (III), Tc (IV) and Tc (V) predominate and form a variety of labeled chelates, colloids and particles. Lower oxidation states have been produced using stronger reducing agents. Tc (I) complexes with phenanthroline have been reported. Experiments with millimolar amounts of technetium have indicated that ^{99}Tc is reduced by stannous ion to the V state and then slowly to the Tc IV at pH 7 in citrate buffer. In HCl, ^{99}Tc is also reduced by stannous ion to the IV state. With a DTPA buffer at pH 4, the ^{99}Tc (III) state prevails. Certain Tc IV complexes have been shown to be substitution inert, thus lessening the need for large equilibrium constants to assure in-vivo integrity.

Most reports of technetium chemistry have dealt with the formation of oxides, sulfides and halides; few Tc chelates have been systematically studied.

In addition to the chemistry of technetium itself, the chelate chemistry of the metallic reducing agents must be considered. Regarding the most popular reducing agent, stannous chloride, the equilibrium constants for only a few chelates are known.

The first prerequisite to assure further development of ^{99m}Tc radiopharmaceuticals is the resolution of the many uncertainties concerning the chemistry of ^{99m}Tc. Further elucidation of structures, oxidation states, equilibrium constants and kinetics associated with technetium chelates is needed.

No. 2

THE RELATION OF STRUCTURE TO CHEMICAL REACTIVITY AND BIODISTRIBUTION OF TECHNETIUM-99m DIHYDROTHIOCTIC ACID (99m-Tc-DHTA) AND OTHER TECHNETIUM-MERCAPTO-CHELATES

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The chemical structure of most technetium-99m (Tc-99m) radiopharmaceuticals is unknown. Determination of the structures of these complexes is complicated by the fact that the concentration of the Tc-99m in these radiopharmaceuticals is below that which is detectable by standard chemical techniques. We have performed a series of mixed ligand experiments using 5 different 1,2 or 1,3-dimercapto ligands and Tc-99m to demonstrate that two such bidentate ligands are attached to Tc-99m in these complexes, including the investigational radiopharmaceutical Tc-99m-Dihydrothioctic Acid (Tc-99m-DHTA). We have also shown that these complexes, when prepared by the stannous chloride reduction of pertechnetate, are inert to exchange at neutral pH, but exchange readily at pH < 3.5. The 1,2-dimercapto ligands, as expected, form more stable complexes than the 1,3-dimercapto ligands and will displace the 1,3 dimercapto ligands at acid pH. This reaction proceeds through the intermediacy of mixed ligand complexes, containing one 1,2-dimercapto ligand and one 1,3-dimercapto ligand. When the technetium complexes are prepared using sodium borohydride as a reducing agent in place of stannous ion the same complexes are formed (confirmed by chromatography, electrophoresis and animal biodistribution studies), suggesting that tin is not part of the Tc-99m-dimercapto ligand complex. The charge on the Tc-99m-1,2 DMP (DMP = dimercaptopropane) complex was shown to be -2 by a standard ion exchange distribution method. A total of 9 complexes have been prepared (including 4 mixed ligand complexes), with charges ranging from -2 to -4. Biodistribution studies were performed in mice and a general trend of increasing urinary excretion with increasing water:octanol partition coefficients was noted. The only exception to this trend was Tc-DHTA which is excreted via the hepatobilliary system.

No. 3

THE CHEMISTRY OF STANNOUS DIMERCAPTOSUCCINIC ACID CHELATES

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This study explores the structure and chemistry of stannous mercapto acid chelates. The structure and chemistry of these chelates may determine the ultimate distribution of the Tc-99m labeled radiopharmaceutical.

Dimercaptosuccinic acid (DMSA) forms two distinct complexes with Sn^{+2}, one at low pH, e.g. 1-4 and another at neutral or alkaline pH, e.g. 7 and above. When labeled with Tc-99m, the complex formed at acidic pH concentrates in the kidneys as expected. The complex formed at alkaline pH however, is rapidly excreted. The stoichiometry of the two DMSA/Sn^{+2} complexes was determined by difference u.v. spectroscopy. At pH 4, the ratio of DMSA to Sn^{+2} is 2:1 while at pH 8, it is 1:1.

Qualitative and quantitative analysis of DMSA and stannous solutions can be done by differential pulse polarography. DMSA and Sn^{+2} give distinctive halfwave potentials ($E_{\frac{1}{2}}$) in 0.1 N $LiClO_4$ - 0.1 M citrate phosphate buffer at pH 3.0. DMSA has a $E_{\frac{1}{2}}$ of -0.31 V and Sn^{+2} has two halfwave potentials, one at -0.20 V and another at -0.44 V. When DMSA and Sn^{+2} are combined, the Sn^{+2} potentials are shifted to -0.44 V and -0.51 V while the DMSA potential remains the same. In the differential pulse mode, peaks are obtained at the $E_{\frac{1}{2}}$ and peak heights are directly proportional to the concentration of the species in solution. Other stannous mercapto acid chelates were also studied.

A stannous DMSA complex containing Sn-119m was prepared at pH 3. This complex was labeled with Tc-99m and injected into rats. Very little tin concentrated in the kidneys. Most of the labeled tin remained in the blood while the technetium was rapidly concentrated in the kidney. The technetium chelate does not have the same biological distinction as the stannous chelate.

No. 4

THE CHEMISTRY OF ^{99m}Tc LABELING KITS

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Numerous problems have recently been reported with the use of kit-produced ^{99m}Tc radiopharmaceuticals. These include the presence of undesired forms of technetium, such as free pertechnetate and reduced hydrolyzed technetium. The present research was carried out to better understand the chemistry involved in these kit systems which use stannous ion as the reductant. The interrelationship between technetium, tin(II), and the ligands used to complex reduced technetium has been explored. Our results demonstrate several possible reasons for poor performance of stannous kits. The problems may occur at various stages of formulation as well as at reconstitution. It has been found that the hydrolysis and oxidation of tin(II) and reduced technetium, combined with an unfavorable ligand-to-tin ratio, produce undesirable side reactions. Results show that the situation becomes worse in case of relatively weak complexing agents, and that sometimes complications arise because of slow Sn(II)-ligand-Tc reaction kinetics. These factors have also been shown to affect the reduction potential of the system, resulting in variable oxidation states of technetium and thereby a non-uniform performance of the final radiopharmaceutical preparation. As a result of the side reactions of tin(II), the tin actually available in the useful chemical form at reconstitution is drastically reduced in certain kit systems. In such situations the carrier content of ^{99m}Tc solutions becomes critical. The popular trend of using more and more tin(II) in the kits has been shown to have deleterious effects on the performance of ^{99m}Tc radiopharmaceuticals. A titration procedure using carrier technetium has been developed whereby the quantity of "usable" vs. "non-usable" tin can be determined in various kit systems. Carefully controlled conditions during formulation (preparation of stannous reagents, lyophilization), as well as a judicious choice of the ligand-to-tin(II) ratio may eliminate many of the existing problems. In general, the ratio of complexing agent to tin(II) should be high and the amount of tin used should be kept to a minimum. Based on our findings, the optimum amounts of tin and ligand, etc., should be evaluated for each system because of the differences in the chemistry of various kit systems.

No. 5

ION EXCHANGE STUDIES OF REDUCED TECHNETIUM SPECIES AND SOME TECHNETIUM-99m RADIOPHARMACEUTICALS

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An ion exchange distribution method was used to determine the charge on reactive intermediate technetium species produced when pertechnetate ion is reduced by stannous ion. The charge on some technetium-99m radiopharmaceuticals was also determined. To study the charge on an unknown ion or complex, its equilibrium distribution between the resin and solution is measured as a function of the concentration of an electrolyte of known charge. The slope of the distribution curve gives the charge on the unknown ion. This method has the advantage of being applicable to solutions containing very low concentrations or carrier-free amounts of unknown ion as is the case for Tc-99m compounds.

The net charge on the intermediate technetium species was determined by equilibrating reduced technetium between cation resin, stannous form, (AG 50W - X8, 50-100 Mesh) and a solution of stannous perchlorate as the divalent bulk electrolyte. The slope of the reduced technetium distribution curve indicated a charge of +2 at pH $\lesssim$ 2. The species carrying this charge is either the dihydroxy technetate ($Tc(OH)_2^{2+}$) ion or the oxotechnetate (TcO^{2+}) ion. These hydrolyze to technetium dioxide dihydrate ($TcO_2\ 2H_2O$) at higher pH values. To confirm the validity of this technique, similar experiments were carried out on known tracer species, Na-22 and Zr-95, with the $Sn(ClO_4)_2$ - AG50W - X8 system. The charge on Na^+ was confirmed as +1 and the Zr data showed a charge of +2 corresponding to $Zr(OH)_2^{2+}$ or ZrO^{2+}.

The net charge on some ^{99m}Tc radiopharmaceuticals was determined by individually equilibrating ^{99m}Tc pertechnetate, ^{99m}Tc-hydroxy-ethylidene diphosphonate, ^{99m}Tc-glucoheptonate and ^{99m}Tc-pyrophosphate between an anion resin, perchlorate form, (AG1-X8, 50-100 Mesh) and a solution of sodium perchlorate as the monovalent bulk electrolyte. The measured slope for $^{99m}TcO_4^-$ ion is 0.98 corresponding to the known charge of -1. The ^{99m}Tc-HEDP contained two components: one had a charge of -0.5 while the other had a charge of -2.0. Technetium-glucoheptonate exhibited a charge of -1.0. The inability to determine the charge on any complex of technetium species in sodium pyrophosphate medium was attributed to oxidation of reduced technetium by the method employed. It is suggested that Tc(IV) forms chelates with HEDP and probably with pyrophosphate and a complex with glucoheptonate.

No. 6

COMPLEXES OF REDUCED Tc-99 WITH POLYHYDRIC COMPOUNDS

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Pertechnetate-99 reacts in alkaline solution in the presence of various polyhydric alcohols and polyhydroxy acids, forming a series of pink compounds whose molar extinction coefficients are approximately 100. The compounds include members of the alcohol series from ethylene glycol to perseitol (the seven-carbon member or heptitol), and members of the hydroxy-acid series from glyceric acid to glucoheptonic acid. A more detailed study of the gluconic acid system by spectrophotometry, iodometry and polarography was made in the presence of excess stannous chloride. In 0.1M gluconate, 5×10^{-4}M pertechnetate was reduced (at pH 12) to Tc(V). The complex is decolorized in acid solution, but on raising the pH the original spectrum re-appears, virtually unchanged. When the same experiment is performed with ethylene glycol in place of gluconate, the acidification of the alkaline solution causes an immediate precipitation of TcO_2, and pertechnetate is found in the filtrate. The ratio TcO_2/TcO_4^- is 2:1, pointing to the following disproportionation reaction

$$3Tc(V) \rightleftarrows Tc(VII) + 2Tc(IV) \qquad (1)$$

The greater stability of the gluconate complex in acid is attributed to a Tc-O-Tc dimer which complexes with four alcohol groups on the gluconate backbone.

These results are compared with earlier experiments on the elution of Tc-99m polyhydroxy complexes from sephadex columns, and basic similarities are noted.

No. 7

LABELLING MECHANISM OF HUMAN SERUM ALBUMIN (HSA) WITH Tc-99m

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The labelling of HSA with ^{99m}Tc has been studied by several investigators. In many cases, the published work includes a discussion on the possible mechanisms of the reactions involved in that preparation, the final valency state of the technetium and the possible structure of the labelled species. Not very much is known about these problems, and many contradictory observations and interpretations have been published. In an attempt to clarify the present panorama, especially regarding the reaction mechanisms of the reactions connected with the preparation of ^{99m}Tc-HSA, we list and discuss in this paper the evidence that has been obtained so far by the different investigators and the principal problems which have already been solved as well as the most important ones which so far have remained unsolved.

No. 8

CYSTEINE AND ALBUMIN COMPLEXES OF Tc-99 AND Tc-99m

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Cysteine reacts readily with $^{99}Tc(V)$ (present as the gluconate complex), forming a 1:1 complex absorbing at 420 nm, with a molar extinction coefficient of 2300, a 2:1 complex absorbing at the same wavelength with a coefficient equal to 3700, and possibly some others. Both complexes show maximum stability at pH 4. The formation constant of the 2:1 complex appears to be of the same order as that of Ni(II) — about 10^{20} — based on competitive reactions.

An earlier report from this laboratory showed that albumin is labelled by technetium-99m at the one sulfhydryl group in the protein. Solutions of human serum albumin with $^{99}Tc(V)$-gluconate yielded no characteristic spectrum down to 300 nm in the pH range 2-5. However, a mixed complex was formed at pH 5 from equal moles of albumin sulfhydryl, $^{99}Tc(V)$ and cysteine. This complex has an absorption band at 410 nm, with a molar extinction coefficient of 2060, and a second band at 360 nm with a coefficient of 1350, when measured against an equal concentration of albumin at pH 5. Difference spectra supported the conclusion that the band at 410 nm represents the cysteine contribution to the complex, and that the band at 360 nm is associated with the albumin sulfhydryl group.

Ordinarily human serum albumin cannot be labelled with technetium-99m at pH 5 or 6. An acid or an alkaline solution is required. It has been labelled at pH 5 by: reducing pertechnetate with $SnCl_2$ in mannitol, adjusting to pH 5, adding albumin at pH 5, and then forming a mixed complex with dilute cysteine. Analysis by sephadex gel chromatography showed that up to 30% of the technetium was associated with the protein. Other sulfhydryl compounds also worked. This will be discussed in connection with the physical chemistry.

No. 9

KINETIC AND THERMODYNAMIC DESCRIPTION OF EXCHANGE REACTION BETWEEN Tc-HIDA AND EDTA

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The radiochemical structure and kinetic stability of Tc-HIDA, an N-substituted iminodiacetic acid, were examined by following the ligand exchange reaction between Tc-HIDA and EDTA. The exchange reaction was monitored as a function of time, temperature, and pH using sequential paper electrophoresis at 300 volts in a 0.05 M phosphate buffer, pH 6.8 for 3 hours. Tc-HIDA was shown to be anionic existing in two discrete radiochemical forms at pH's around 3.0 with only one of these structures in evidence at pH's greater than 4.5. Tc-HIDA underwent acid catalyzed unimolecular dissociation in the presence of EDTA with half-lives of 2.5, 11 and 72 hours at pH's of 2.9, 4.4, and 6.4, respectively. The reaction rate was virtually independent of EDTA concentration over a fivefold range. The overall formation constant for Tc-HIDA was calculated to be 4×10^{16} liters/mole when compared to a K_f for Tc-EDTA of 10^{19}.

Tc-HIDA was also shown to exist as a bis compound with two molecules of the N-substituted iminodiacetic acid for each atom of technetium. This was inferred since only when Tc-HIDA was assumed to exist in a bis form was its K_f independent of pH. This bis structure for Tc-HIDA was confirmed by synthesizing Tc-99-C-14 HIDA using Tc-99 and C-14 HIDA of known specific activity. Similarity of Tc-HIDA with and without carrier was confirmed by tissue distribution studies in mice and by paper electrophoresis. The dual labeled HIDA was separated from both HIDA and Sn-HIDA using descending paper chromatography in 95% acetonitrile. The molar ratio of HIDA to technetium was determined by this technique to be 1.8 ± 0.3. This work suggests that Tc-HIDA exists as an anionic bis compound and that the bond between technetium and N-substituted iminodiacetic acids should remain intact for relatively long periods of time at physiologic pH.

No. 10

CHEMICAL KINETIC STUDIES OF THE LABELLING OF EDTA AND DTPA WITH TECHNETIUM-99m

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Experiments have been performed to determine the optimal conditions to get a Tc-99m EDTA preparation with a high labelling yield. Thus various concentrations of tin were used with constant EDTA concentrations at various pH values. Gelchromatography Column Scanning (GCS) technique was used for the determination of reduced hydrolyzed Tc-99m, free pertechnetate, and Tc-99m EDTA complex. The optimal conditions for Tc-99m EDTA preparation were found at concentration 10 mM of $EDTAH_4$ and 0.5 mM of $SnCl_2$ in 10 ml Tc-99m eluate as a final volume and pH 2.8-2.9. The rate constant for forming the Tc-99m DTPA complex is considerably higher than that for Tc-99m EDTA. Thus one has to wait about 30-60 minutes before one gets high labelling yield of Tc-99m EDTA but only a few minutes with Tc-99m DTPA at room temperature.

Kinetic studies were performed at different temperatures by adding 10 ml Tc-99m eluate to a kit containing 29.2 mg of $EDTAH_4$ and 0.17 mg $SnCl_2 \cdot 2H_2O$, and shaking well several times. The content of the kit was filtered through a 0.22 µm membrane filter and samples were taken at different times, and analyzed with the GCS-method. Comparative studies were performed by adding 10 ml Tc-99m eluate to a kit containing 39.3 mg $DTPAH_5$ and 2.3 mg $SnCl_2 \cdot 2H_2O$. The reaction kinetics were studied according to the scheme

$$TcO_4^- \rightleftarrows \text{Tc red. hydr.} \rightleftarrows \text{Tc complex}$$

and the various rate constants k_i were determined by fitting the experimental results of the GCS analysis to these reactions.

In vitro study of the degree of binding the Tc-99m EDTA with red-blood cells and plasma proteins has also been performed using the GCS-method. The degree of plasma protein binding with Tc-99m EDTA as resulted from this study was much higher than for Tc-99m DTPA.

We also found significant differences in the clearance of the complexes through the kidneys of rabbits.

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No. 11

RECENT DEVELOPMENTS OF THE GELCHROMATOGRAPHY COLUMN SCANNING METHOD. A RAPID ANALYTICAL TOOL IN RADIOPHARMACEUTICAL CHEMISTRY

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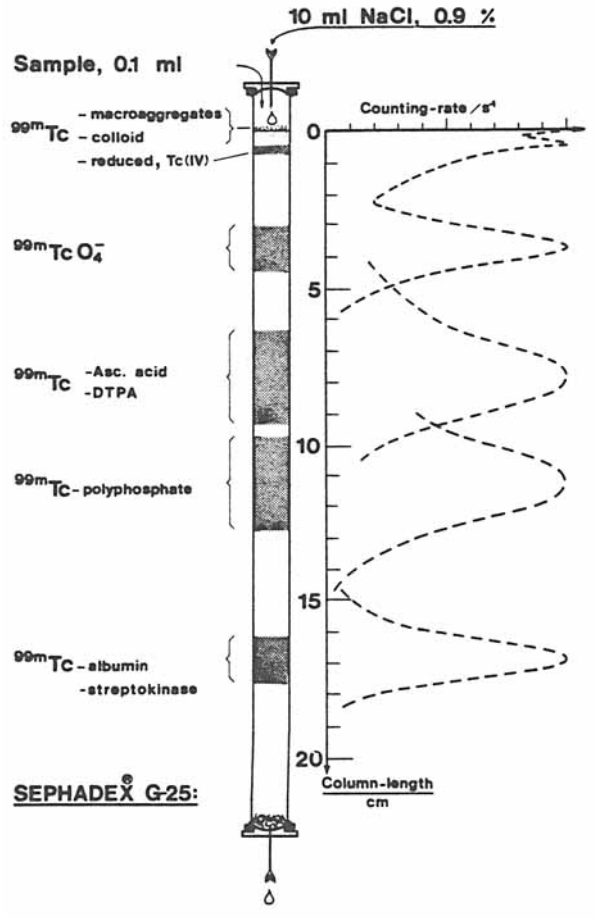
Gel filtration using Sephadex[1] has been used extensively for studying the chemical state of ^{99m}Tc in labelled compounds and radiopharmaceuticals[2-6]. Hydrolyzed reduced technetium is however absorbed on the top of the sephadex column and is not eluted. Therefore the method of Gelchromatography Column scanning (GCS) was introduced in which the column was eluted with a volume less than the void volume and then scanned[7-9]. The scanning profile thus obtained exhibit individual peaks for hydrolyzed reduced ^{99m}Tc, ^{99m}Tc pertechnetate and various ^{99m}Tc-complexes.



The GCS-method has been extensively used for studying the labelling with ^{99m}Tc or ^{123}I of various radiopharmaceuticals such as Tc-Ascorbic acid[7-10], Tc-DTPA[7,8,11], Tc-Streptokinase[12,13], Tc-labelled macroaggregates[14], and Tc-plasmin[15], and ^{123}I-labelled streptokinase and plasmin. Sephadex gels (dextran) allow fractionation of molecules of molecular weights up to about 0.5 x 10^6. Sepharose gels (agarose) may be used to separate molecules and particles up to molecular weights of several millions. Sepharose gels have therefore been used for studying Tc-labelled colloids. By using the GCS-method the fraction of free pertechnetate and particle weight distribution spectrum can be obtained in one single measurement[16].

The great advantage of column chromatography is the possibility to perform the developing in a closed system with the same or similar buffer medium as in the radiopharmaceutical, and in inert atmosphere if desired[8]. This is especially important for studying ^{99m}Tc-phosphates and weak complexes[17].

Thus the effect of using phosphate buffer for ^{99m}Tc-labelled phosphates and protein solutions for ^{99m}Tc-labelled proteins will be demonstrated.

The GCS-method is very simple and rapid compared to other chromatographic techniques. It is therefore well suited for studying the labelling kinetics. The variation of the GCS-profile with time and temperature gives information about the mechanism and thermodynamics of the labelling procedure. The same principle is very useful in determination of the degree of denaturation of the preparation in other media, e.g. blood plasma. Examples of kinetic studies will be shown for Tc-streptokinase and Tc-plasmin.

The GCS-method has been shown to be a very useful analytical tool both during the development of new radiopharmaceuticals and for routine quality control.

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No. 11a

LABELLING OF PLASMIN WITH Tc-99m

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The stability of plasmin in neutral and alkaline solution is very poor and therefore plasmin is not well suited for standard iodination procedures. We therefore suggested that it would be well suited to labelling with ^{99m}Tc by using the stannous method at low pH which was previously used by us for labelling streptokinase.

The aim of this investigation was to study in detail the labelling of plasmin (NOVO Industri A/S Denmark) with ^{99m}Tc in order to prepare a radioactive indicator for rapid scintigraphic detection of thrombi.

Gel chromatography column scanning (GCS) was used to analyze the fraction of ^{99m}Tc-plasmin, ^{99m}Tc-complex (of lysin and other constituents of low molecular weight), ^{99m}Tc-pertechnetate and reduced hydrolyzed ^{99m}Tc under various condition of labelling. The influence of concentrations of $SnCl_2$, NaCl, plasmin and lysin was studied as well as incubation time pH and temperature.

The best method found for preparing ^{99m}Tc-plasmin involved the reduction of 2.5 ml ^{99m}Tc-pertechnetate solution with 0.5 ml of 4 mM $SnCl_2$, 2 M NaCl and 70 mM HCl. This mixture was then added to 5 mg of plasmin to give a final pH of about 2.

After 60 min of equilibration the labelling efficiency was 80-90% as determined by gel chromatography column scanning.

The enzymatic activity after labelling was determined by the casein method according to NOVO. A solution of casein is decomposed by the enzyme for 20 minutes, pH 7.5, 35.5°C. The reaction is stopped by precipitating the protein with perchloric acid and the amount of substrate decomposed is determined by measuring the optical density at 275 nm. The enzymatic activity of ^{99m}Tc-plasmin was reduced by less than 15% by the labelling process.

At present a clinical pilot study is carried out at the University hospital in Lund using ^{99m}Tc-plasmin for detection of deep venous thrombosis.

No. 12

POLAROGRAPHY OF Tc(IV) AND Re(IV) COMPLEXES

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The nature of the reduced state of technetium in radiopharmaceuticals is the subject of some debate. A clear understanding of the accessibility of oxidation states (+3, +4, +5, +6) in aqueous solution as a fraction of ligand donor atom sets is hampered by (i) the absence of a large body of systematic chemistry of technetium and (ii) the ease of hydrolysis of the simple complexes of technetium(+4) to $TcO_2 \cdot 2H_2O$ in aqueous solution. Polarographic studies in acetonitrile of characterized technetium(+4) complexes, and their rhenium analogs will be described. These studies have provided information about the inherent ease of oxidation and reduction of these species as a function of the ligand donor sets in the absence of complications due to hydrolysis. Using a rotating platinum electrode, halo and pseudo halide(+4) complexes MX_6^{2-} as tetra alkyl ammonium salts undergo reversible one electron oxidations to the +5 state. $MX_6^{2-} \rightarrow MX_6^{-1} + e^-$. These oxidations with the exception of $X = SCN^-$ are very difficult for both Tc and Re. The rhenium complexes are easier to oxidize than those of technetium, e.g. $E_{\frac{1}{2}}$ (Re, $X = Cl^-$) = 1.18 volts vs. SCE, but $E_{\frac{1}{2}}$ (Tc, $X = Cl^-$) = 1.94 volts vs. SCE. Using the dropping mercury electrode a one electron reduction is noted for the tetra alkyl ammonium salts of rhenium.

No. 13

ANOTHER TECHNETIUM SULPHIDE COLLOID WITH COPPER AS A CARRIER

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The usefulness of Technetium sulphide preparation for liver scanning purposes is greatly dependent on the colloid characters of the product, since the presence of millicurie quantities of pertechnetate in the generator eluates involve such a minute amount of Tc^{99m} that chemically TcS_7 formed in the reaction mixture does not form a precipitate or even an opalescence. Therefore the use of a carrier becomes an important factor to secure the colloidal properties of the preparation. Different formulations for the preparation of Technetium-99m sulphide colloid had been adopted, whereas in all of them technetium heptasulphide is formed, however, the choice of best conditions to secure the heptasulphide and the stability of the colloid are the most important. In general the preparation of the colloid includes the selection of a proper source of sulphide ions, the incorporation of a suitable metal ion as a carrier for (Tc_2S_7), the use of a certain hydrophilic stabilizer for colloid and the adjustment of the final pH by a suitable buffering system to safeguard stability of the colloidal preparation. Hence a new formulation for Technetium sulphide colloid using Cu II as a carrier had been tried which gives promising results where the CuS formed in the reaction mixture acts as collector for the heptasulphide of technetium. Investigation of the radiochemical purity has shown that a high radiochemical yield was obtained which reveals a good indication for the stability of Tc_2S_7 in the preparation.

No. 14

"BIFUNCTIONAL" CHELATING AGENTS FOR THE BINDING OF METAL IONS TO BIOLOGICAL MOLECULES: PRINCIPLES AND PRACTICAL ASPECTS

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A large number of metal chelates with ethylenediaminetetraacetic acid (EDTA) and its close analogs are stable under physiological conditions. The ability to attach such powerful chelating agents to biomolecules provides a fundamentally important new approach to the preparation of radiodiagnostic agents. The features of this approach include the separation of synthetic chemistry from radiolabeling, which may be achieved rapidly before use. Chelates of ^{111}In with para-substituted derivatives of 1-phenyl-EDTA have been found to possess great thermodynamic stability and to be almost kinetically inert with respect to dissociation. However, the rates of formation of such chelates are quite rapid and the chelation proceeds to completion - even at low pH in the presence of competing metal-binding groups. The compound 1-(p-aminophenyl)-EDTA is quite versatile chemically, in that the aromatic amino group may be acylated, alkylated, diazotized, or otherwise modified to yield products possessing different properties of chemical reactivity and selectivity. In principle, this allows control over the sites at which a bifunctional chelating agent can react with a target molecule. The thermodynamic principles and multidentate ligand kinetics which form the foundation of this technique will be presented, and recent experimental results will be described.

No. 15

APPLICATION OF THE MANNICH REACTION FOR INTRODUCTION OF CHELATING GROUPS INTO A BIOLOGICALLY ACTIVE CARRIER

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The process of screening a large number of biologically active compounds as a potential carrier of gamma emitting radioisotopes requires a fast method of derivatization.

Usually the introduction of the chelating group is achieved by the replacement of an active halogen with an amine, amide or ester formation, or by opening of an epoxy ring. All of these approaches require several additional reactions prior to the final synthesis.

Tha Mannich reaction customarily consists of the condensation of a primary or secondary amine, paraformaldehyde and a compound capable of supplying one or more active hydrogens. Therefore this reaction can be carried out directly by using the biologically active compound without chemical alteration. The Mannich reaction does not alter one of the functional groups but rather replaces an active hydrogen. The products of the condensation, known as "Mannich bases", are in general stable and easy to isolate.

Our group has studied the possibility of connecting the iminodiacetic acid moiety, a well known chelating group, with compounds of known biological activity. The following compounds were selected for condensation with iminodiacetic acid: i) uracil, ii) hexestrol, iii) dihydrotestosterone.

i) A number of effective inhibitors of cell growth like 5-fluorouracil, 5-trifluoromethyl-uracil or 5-metcaptomethyl uracil has been known for some time. Uracil reacted with paraformaldehyde and the disodium salt of imminodiacetic acid in boiling aqueous methanol to give 5-N',N'-dicarboxymethylaminomethyl-uracil. Preliminary investigation of this compound as the ^{57}Co chelate in tumor bearing rats indicates that the compound localizes in tumors and clears the blood rapidly giving a 5:1 tumor-to-blood ratio at 24 hr.

ii) The extremely high affinity of hexestrol to estradiol receptors makes this compound an ideal starting material for condensation with imminodiacetic acid. Unfortunately the number of active hydrogens in the molecule leads to a large number of cross linked and/or polysubstituted products. The isolation of the desired product has been achieved by high pressure high-performance liquid chromatography. Proper manipulation of the ratio of starting materials leads to 2'-dicarboxymethylaminomethyl hexestrol, whose distribution and accumulation is being investigated at the present.

iii) Dihydrotestosterone (5-α-androstan-17β-ol-3-one) is the active metabolite of testosterone localizing in the prostate. Only two active hydrogens are present in the molecule but nevertheless the condensation reaction produces a number of cross-linked and/or polysubstituted products. The purification and identification of the desired product is in progress.

The use of the Mannich reaction to prepare derivatives should result in a unique class of compounds which contain a biologically active biochemical or drug with all of the native functional groups intact.

No. 16

THE SYNTHESIS OF CHELATING AGENTS FOR THE PREPARATION OF DRUG DERIVATIVES

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The ready availability and ideal nuclear properties of ^{99m}Tc has led to its widespread use for imaging purposes. In general, the localization of the present radiopharmaceuticals is based on the ability of an organ to remove foreign substances from the blood. Further application of this ideal nuclide seems related to the development of methods to label biologically active molecules or drugs in such a way as not to interfere with their desired in vivo behavior. However directly binding the ^{99m}Tc to these molecules may change the expected distribution (except in the case of large molecular weight proteins and cells) by altering the functional groups. Therefore the synthesis of derivatives containing a chelating group to bind the technetium is suggested as a possible solution to the problems associated with direct labeling. This could result in a molecule with similar biological properties to the parent molecule.

The synthesis of two chelating groups which easily can be reacted with various biochemicals or drugs has been completed.

The first chelating group contains a primary amine (the reactive group) connected to a DTPA like molecule. This compound N'-(2-aminoethyl) diethylenetriamine-N,N,N",N" tetraacetic acid has been prepared in the following way: Bis(2-chloroethyl) amine is reacted with potassium phthalimide to produce the diphthaloyl derivative of diethylenetriamine. This product contains diethylenetriamine with the primary amines blocked and the secondary amine free. This secondary amine is then reacted with N,N dibenzyl chloroethyl amine. The phthaloyl blocking groups are then removed with hydrazine and the primary amines are carboxymethylated with chloroacetic acid. The removal of the N,N dibenzyl group frees the primary amine for reaction with the biochemicals or drugs. This primary amine will replace halogen substituents, form a Schiff base with ketones, and open epoxide rings to form amines. To date, this compound has been reacted with 1,2 epoxy-3-o-allyl phenoxy propane to produce a derivative of the β adrenergic drug alprenolol.

The second chelating group is similar to the first except that the reactive group is the amine group in aniline. This amine can be reacted directly or converted to a diazonium ion and then reacted with an activated benzene ring.

This compound has been prepared by starting with the diphthaloyl derivative of diethylenetriamine used in the first synthesis. With the primary amines blocked, the secondary amine is reacted with p-nitro benzyl bromide. The blocking groups are then removed with hydrazine and the primary amines are carboxymethylated. The nitro group is then reduced by mild hydrogenation to give the amine. The diazonium salt produced from this compound has been reacted with hexestrol and estradiol to give substitution in the phenolic ring.

These derivatives will be tested in vitro with the appropriate receptor system to determine if they retain biological activity. Hopefully, the preparation of drug derivatives containing a strong chelating group will produce a compound which can trace accurately biochemical pathways with readily available gamma emitting radionuclides.

No. 17

A NEW TYPE OF BIFUNCTIONAL CHELATE FOR PROTEIN LABELING: APPLICATION TO ^{99m}Tc-FIBRINOGEN

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Bifunctional chelating agents have been developed for binding metallic radioisotopes to biologic compounds. These agents are based on an EDTA-like structure to provide the metal chelating function, with a second moiety such as diazo to provide the coupling function. Coupling to labile biologic compounds such as easily denatured proteins must be effected rapidly under mild conditions. The succinimidyl ester function readily satisfies this requirement, and if this group can be incorporated along with appropriate ligands into a molecule, the resulting structure may be suitable as a bifunctional chelate. To evaluate this idea, we prepared a simple compound of this type, N-succinimidyl-3-(3,4-dihydroxyphenyl) propionate. The applicability of this compound to a model system, ^{99m}Tc-fibrinogen, was assessed by comparing ^{99m}Tc-fibrinogen labeled directly (FLD) to ^{99m}Tc-fibrinogen labeled with the chelate (FLC).

Coupling to fibrinogen was accomplished by simply adding the chelate in 5 µl dimethylformamide to fibrinogen in pH 8.0 borate buffer, at molar ratios of 2:1-10:1, and incubating for 15 minutes. Labeling efficiency of both FLD and FLC was 80-85%, and isotopic clottability of both was 75-80% in vitro. Chromatography of both FLD and FLC on Sephadex G-50 revealed a single sharp peak corresponding to authentic fibrinogen, with nearly complete recovery of activity. In animal studies the relative amount of circulating activity remaining fibrinogen-bound was consistently higher with FLC than with FLD, and electrophoresis indicated less transfer of activity from FLC to other plasma proteins. Exchange studies of FLD and FLC with EDTA were followed by electrophoresis and Biogel P-100 column chromatography.

Bifunctional chelates containing the succinimidyl ester function may have potential for radiopharmaceutical preparation if ligands such as diamino or iminodiacetic acid can be employed, and if labeling can be effected prior to coupling.

No. 18

SOME CHEMISTRY OF BLEOMYCINS PERTINENT TO THEIR DIAGNOSTIC AND CHEMOTHERAPEUTIC USE

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The interaction between the bleomycin group of antineoplastic agents and various metals (Fe, Co, Ni, Cu, Ga, In, Pb) has been investigated using a variety of techniques including gel chromatography, thin layer chromatography, electrophoresis, visible and U.V. spectroscopy, and mass spectroscopy. The results of these investigations have shown that copper is bound to the bleomycin by four nitrogen ligands in a highly distorted environment. It has been possible to assign a tentative binding site for copper to bleomycins and probably with some slight variation for other metals as well. The gel chromatographic data show that there are differences in elution characteristics between BLM A_2 and B_2 which are not removed when a metal is bound. There are also differences between the metal complexes of bleomycins with ^{111}In, ^{57}Co, and ^{61}Cu which are attributed to the different co-ordination characteristics of these metals. Stability studies have shown that ^{111}In BLM is not stable to substitution by cupric ion under approximate physiological conditions whereas ^{57}Co BLM is stable. It is suggested that the known differences in in vivo distribution of the metal complexes is due to the different co-ordination characteristics of the metals imposing different conformations on the complex. The instability of ^{111}In BLM to exchange by cupric ion is proposed as a mechanism for the rapid appearance of ^{111}In labelled transferrin after the injection of ^{111}In. Preliminary experiments have indicated that there is a relationship between the non-cerruloplasmin-bound serum copper levels and the release of ^{111}In from the bleomycin complex, and also the advent of toxic effects when bleomycins are used in chemotherapy. These phenomena may be altered by prior treatment with drugs such as penicillamine that reduce non-cp-bound serum copper levels. Studies are in progress along these lines to try and improve the usefulness of bleomycins and their metal complexes in the diagnostic and chemotherapeutic fields.

No. 19

SIMULTANEOUS PRODUCTION OF Ga-67, Cu-67 AND Cu-64 AND LABELLING OF BLEOMYCIN WITH Cu-67 AND Cu-64

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An electroplated-zinc target, capable of withstanding at least 600 µA of 16 MeV deuterons, is being used to produce gallium-67. Simultaneously appreciable activities of copper-64 and copper-67 are formed. Using 16 MeV deuterons on natural zinc the production rates are 143 µCi Cu-64/µAh and 0.12 µCi Cu-67/µAh, respectively.

A 600 µA 16 MeV deuteron beam produces per hour 570 mCi Ga-67 and yields — after an eight day cooling period — 10 µCi Cu-67 containing 2.5 µCi of Cu-64. By using electrolytic plating and deplating one can easily recover enriched zinc from a bombarded zinc target[1]. Since Cu-67 is presumably produced primarily by (d,2p) reaction on Zn-67, the use of enriched zinc-67 is being contemplated. For preliminary experiments with bleomycin carrier-free copper-64 is used.

The routinely used procedure for Ga-67 production[2] separates zinc and copper as a first fraction from gallium-67. This copper fraction is subsequently subjected to an anion-exchange separation in dilute hydrochloric acid which yields a pure copper product.

Divalent copper is complexed by bleomycin upon mixing of the aqueous solutions. The degree of complexation is determined by TLC in a 10% ammonium-acetate in water: methanol (1:1) solution on a silica gel plate. Less than 0.1% of the copper remains uncomplexed if the capacity of the bleomycin is not exceeded.

This capacity was determined to be 2.8% weight/weight. A program is in progress to compare copper bleomycin with Co-57 bleomycin. The Cu-67 has good decay characteristics for use with a gamma-camera and its convenient half-life would make it the compound of choice if its production rate from enriched zinc-67 is sufficiently high and if the stability of the bleomycin complex proves satsifactory in vivo.

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No. 19a

PRODUCTION OF Ni-57, Co-55 AND Zn-62 AND THEIR COMPLEXATION WITH BLEOMYCIN

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For a program aimed at a more practical radioactive bleomycin a number of isotopes, belonging to the first transition series, were produced. According to Renault et al.[1] the stability sequence of the bleomycin complexes follows the Irving-Williams stability series. Hence the nickel, copper and zinc complexes should be more stable than the complex with the long-lived cobalt-57. The short half-lived cobalt Co-55, produced simultaneously with the nickel-57 was also made available to the hospitals, involved in the program. Nickel-57 and cobalt-55 were produced by 25 MeV helium-3 bombardment on natural iron. Zinc-62 was produced by alpha bombardment on natural nickel.

The production rates for the various nickel and cobalt isotopes produced by 25 MeV He-3 in the natural iron target were determined and are summarized in Table 1. The excitation function for the $^{60}Ni(\alpha,2n)^{62}Zn$ reaction was determined. The calculated thick-target yield for a 30 MeV alpha beam on natural nickel is 93 µCi/µAh. Separation procedures for the various isotopes were developed. Nickel-57 and cobalt-55 are separated from each other as well as from the iron matrix by a liquid-liquid extraction, followed by anion-exchange chromatography in hydrochloric acid. Zinc-62 is purified from the nickel matrix by cation-exchange in an HCl 0.2 N + 95% ethanol solution.

Nickel-57, cobalt-55 and zinc-62 are chelated by mixing with an aqueous solution of bleomycin. Quality control is by atomic absorption measurement of the total amount of stable carrier in the radioactive solutions and by TLC on silica gel in 10% ammonium acetate in H_2O:methanol (1:1) to determine the amount of residual free metal ion.

In all cases complexation was better than 98%. The capacity of bleomycin for nickel, cobalt and zinc was determined as 2.7%, 1.9% and 2.9% weight/weight respectively.

Table 1

Isotope	Production rate (µCi/µAh)
Ni-56	< 0.03
Ni-57	32 ± 2
Co-55	21 ± 1
Co-56	0.2 ± 0.05
Co-57	2.3 ± 0.2
Co-58	0.7 ± 0.1

References

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No. 20

LABELLING BLOOD COMPONENTS WITH 8-HYDROXYQUINOLINE CHELATES: SIMPLIFIED PROCEDURE AND MECHANISM OF LABELLING

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Indium-111 and Gallium-68 8-hydroxyquinoline (oxine) chelates have been shown to label erythrocytes, leukocytes and platelets in high yields with retention of their viability. For labelling, these blood components have to be isolated from plasma to eliminate plasma transferrin in presence of which the radioactivity does not incorporate with the cells. We have shown the feasibility of depleting transferrin by passing plasma through a (CNBr) Sepharose-4B column activated with 5 mg transferrin antibody and glycine. The results have been confirmed by transferrin radioimmuno assay and by the lack of the formation of indium-111 transferrin. This may allow the labelling to be carried out in plasma. When labelled leukocytes and platelets are incubated in normal saline an increased amount of oxine is released without indium-111 activity. Labelled leukocytes and platelets were lysed and passed through 0.22 μm filter. More than 90% of the activity was filterable. This was dialysed for 18 hours against normal saline. Only 18% of the activity was dialysed. The solution was also subjected to G-10 and G-200 Sephadex filtration. The results confirmed that approximately 20% of the activity was associated with low molecular weight (M.W.) component and indicated that 65% was bound to M.W. > 700 (G.10 exclusion limit) and 15% was associated with M.W. > 2×10^5 (G-200 exclusion limit). Labelled leukocytes were homogenised and the homogenate was fractionated with density gradient ultracentrifugation. Greater than 80% activity was in the soluble fraction, of which only about 15% was extractable in chloroform. The balance of activity ($\sim$20%) was associated with the insoluble fractions. These results indicate that the oxine chelate diffuses through the membrane and transfers approximately 80% of the activity to intracellular components during time of incubation. The activity which is dialysable, extractable in $CHCl_3$ and which is retained on the G-10 Sephadex Column may still be as oxine chelate at the end of the incubation period.

No. 21

HOMOGENEOUS AND HETEROGENEOUS PHASE REACTIONS IN THE LABELING OF INDIUM-EDTA COMPLEXES

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As part of a study to develop a reliable and rapid method to label microspheres with ^{113m}In, we studied the use of the EDTA-exochelator proposed originally by Sundberg, et al. [Nature 250 (1974) 587]. Human albumin microspheres could be readily coupled with phenyl-azo-EDTA, but incubation of the azo-phenyl-EDTA-microsphere with indium resulted in a preparation where up to 50% of the ^{113m}In could be easily washed off with buffer and 0.05 N HCl, and which primarily localized in the liver and spleen. When, however, indium-111 was reacted with the diazo-phenyl-EDTA in solution, and this complex then coupled to the microspheres, excellent binding resulted. Similarly, complexing of ^{113m}In to azo-phenyl-EDTA albumin in solution followed by aggregation, also yielded radiolabeled particles which were resistant to washings and localized over 90% in the lung, immediately upon injection to rats. We believe that these results can best be explained by considering a set of competing reactions.

$$In(Ac)_3 + 3OH^- \rightarrow In(OH)_3 + 3Ac^- \quad \text{and}$$

$$IN(Ac)_3 + \text{HSA-Microsph.-EDTA} \rightarrow \text{In-EDTA-HSA-Microsph.} + 3Ac^-$$

In solution, complexing to the EDTA moiety is very fast and the resulting complex is strong. When the EDTA is on the microsphere, the frequency of collisions is significantly lower in heterogeneous phase than when these collisions are dependent on diffusion kinetics in solution. Under heterogeneous phase reaction conditions, the homogeneous phase formation of the hydroxide is favored. The latter will deposit itself, but probably not bind to, the microspheres. Application of these principles has led to the development of indium labeled aggregates of potential interest in lung scanning or other capillary bed physiological studies.

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No. 22

EVALUATING THE STABILITY OF GALLIUM RADIOPHARMACEUTICALS

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A method employing paper chromatography has been used to assess the stability of several Ga-67 and Ga-68 labeled radiopharmaceuticals in basic, neutral, and acidic solutions. Ascending paper chromatography with a pyridine, ethanol, water solvent was shown to be capable of resolving the decomposition products of gallium chelates, namely gallium hydroxide and gallate, from that of the chelates themselves. The stability of gallium-labeled citrate, pyrophosphate, EDTA, and ethylene-diamine tetramethylene phosphonate (EDTMP) were each investigated by paper chromatographic analysis in which the solvent was adjusted to seven pH values in the range 2.5 to 9.6. The acidity of the solvent was therefore relied upon to alter the chemical environment of the compound under study. In the case of Ga-EDTA and Ga-EDTMP, no decomposition products were observed throughout the entire pH range. Gallium citrate was found to be stable in the pH range from about 2 to 7. As the pH is raised above 7, the degree of Ga-citrate dissociation increases with the formation of increasing concentrations of gallium hydroxide and gallate. Gallium pyrophosphate was found to be stable only in acid solutions of pH less than about 5. This latter compound was found in our laboratory to be unsuitable as a bone imaging agent due to poor bone localization accompanied by excessive liver accumulation. This unfavorable *in vivo* result may be expected since the complex has now been shown to be unstable at the pH of blood.

No. 22a

THE EFFECT OF PREPARATION QUALITY ON BIODISTRIBUTION FOR ^{67}Ga CITRATE

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Differences in tumor and abscess localization observed in clinical studies with ^{67}Ga citrate have recently been attributed to preparation quality. Using paper chromatographic techniques we have analyzed commercial preparations and have confirmed that quality is variable with up to 80% of the activity present in some preparations as gallate ion rather than as the desired citrate chelate. To determine whether these differences in quality effect biological distribution, ^{67}Ga citrate of "excellent" and "poor" quality were prepared by adjusting the pH to 6 and 9, respectively. Biodistributions in subcutaneous tumor bearing rats were established for both preparations and for a typical commercial preparation. The serum binding properties were determined by equilibrium dialysis with pooled human serum. No significant differences among the three preparations were observed in either study. Because of the labile nature of gallium complexes, it is possible that the preparations achieve uniform quality in this citrate concentration range (0.5 mg/ml) upon being buffered in a serum environment.

In agreement with a published report, we have observed differences in preparation quality with citrate concentration. Paper chromatographic studies have shown that the formation of gallate ion may be suppressed by increasing the citrate concentration over a wide range. The concentration of citrate was also found to effect the serum protein binding properties of gallium citrate preparations. Using equilibrium dialysis and gel filtration chromatography, we have found that serum binding is significantly reduced at a citrate concentration of 20 mg/ml over that of 2.0 mg/ml. The significance of these observations will be discussed.

No. 23

SYNTHESIS OF A NON-LABILE ASTATINE-PROTEIN CONJUGATE

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Proteins labeled with ^{211}At could be of great utility in several areas of therapeutic medicine. For example, malignant cells could be selectively destroyed by labeling tumor specific antibodies with ^{211}At. The specificity of the immune response could then be used to localize the tagged antibodies in close proximity to the tumors so that α particles emitted in the decay of ^{211}At would have a high probability of destroying the tumor. Similarly the rejection of transplanted organs by the recipient's antigen recognition cells might be significantly reduced by selectively eliminating the antigen recognition cells specific for the transplanted organ.

^{211}At is the isotope of choice for these applications since being an α emitter, it decays with intensely ionizing radiation of short range. This should maximize the destruction of the target cells and should minimize the destruction of neighboring healthy cells. Furthermore, astatine is a halogen so its chemical properties should be similar to those of iodine. However, utilization of the standard protein iodination techniques has not resulted in an astatine-protein bond which is stable under in vivo conditions. The lack of a method for forming non labile astatine-protein bonds has prevented the successful application of astatinated proteins to the aforementioned medical problems. Therefore, the development of a better method for labeling proteins with ^{211}At is of obvious importance.

We have developed a two step process for forming non labile At-protein bonds. First, a stable, astatine-carboxylic acid compound was prepared and second, this compound was added to a protein via a condensation reaction between the carboxylic acid group and the complementary function on the protein. The probability of forming a biologically stable ^{211}At-labeled protein should be increased by first incorporating the At$^-$ into a stable intermediate compound. An astatinated, aromatic intermediate was chosen because aryl halides have a lower reactivity towards halogen displacement than alkyl halides.

The ^{211}At was prepared by bombarding ^{209}Bi with 29-30 MeV α particles via the ^{209}Bi(α,2n)^{211}At reaction. The targets were heated in a furnace and as the astatine was distilled off, it was collected on a cold finger coated with a frozen layer of 0.1 M NaOH/0.02 M $Na_2S_2O_3$. Then, p-astatobenzoic acid was prepared via a diazonium salt intermediate using the Sandmeyer reaction. The p-astatobenzoic acid was then conjugated to bovine serum albumin using a variation of the mixed anhydride reaction. A Sephadex G-25 column was used to separate the ^{211}At-labeled BSA from unreacted p-astatobenzoic acid and lower molecular weight impurities. Typically, 12% of the initial ^{211}At activity was eluted with the protein.

The tissue distribution patterns of the p-astatobenzoic acid-BSA conjugate were determined and compared to those of both At$^-$ and p-astatobenzoic acid. In all mice injected with either At$^-$ or p-astatobenzoic acid, the highest concentrations of activity were in the stomach and intestines. In the mice injected with astatinated BSA, the highest concentration was in the liver. The probability that the observed difference in the distributions for the BSA compound and that of At$^-$ (or p-astatobenzoic acid) is statistically significant is greater than 99%. These results suggest that the astatinated protein is non-labile in vivo over a period of at least 20 hours.

The immunogenic properties of the astatinated BSA were determined from both immunodiffusion and haemagglutination measurements. The results indicate that the majority of the immunospecific properties of unlabeled BSA have been retained by the ^{211}At-BSA conjugate.

No. 24

SYNTHESIS OF RADIOLABELED PLATINUM COMPLEXES

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Platinum complexes continue to be of great interest in cancer chemotherapy, and we have also suggested that they have potential in defining mechanisms of tumor localization.

Platinum possesses several radionuclides, of which Pt-191, Pt-193m, and Pt-195m are the only usable pure γ-emitters. Their $t_{\frac{1}{2}}$ are 3.0, 4.3, and 4.0 days, respectively and they emit 106.7, 17.4, and 93.9 photons per 100 disintegrations. We believe that Pt-195m is the best of these, all its photons are below 130 keV, and its $t_{\frac{1}{2}}$ of 4.0 days allows for ample time for synthesis and purification. It is produced in the $^{194}Pt(n,\gamma)$ reaction, usually on 96.8% enriched Pt-194, with a production cross-section of 0.1 barn. Unfortunately, its burnup cross-section is 13,000 barns, thereby limiting the specific activity attainable to 1 mCi/mg Pt.

The irradiated target is dissolved in HNO_3/HCl, and the H_2PtCl_6 can be converted to Pt(II) by hydrazine reduction. This step is critical, and conditions have been optimized for work at a 1-10 mg scale, using either hydrazine hydrochloride on dry K_2PtCl_6, or free hydrazine hydrate on wet H_2PtCl_6. The synthesis of pure *cis*-ammino-complexes is favored by the use of iodine as a *trans*-directing ligand, followed by conversion to the chloride via the di-aquo intermediate. Thus, in a typical run, pure *cis*-dichlorodiammine platinum(II) can be attained in >98% purity and in 50% radiochemical yield, from 10 mCi of ^{195m}Pt as H_2PtCl_6, containing 10 mg of platinum. Other platinum complexes can be similarly prepared. Problems of further scaling down will be discussed.

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No. 25

CYCLOTRON PRODUCTION OF CARRIER-FREE COBALT-55. A POTENTIAL LABEL FOR BLEOMYCIN

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The development and increased availability of positron cameras in the medical community, and the known property of bleomycin to form metal chelates which later concentrate in tumors, has induced us to develop a production method for the 18.2 h positron emitter Co-55. Cobalt-57 (270 d) labeled bleomycin has been successfully applied for the scintigraphic detection of tumors and it is considered a far superior agent than the In-111 and Ga-67 labeled bleomycin. The better decay properties of Co-55 and the possibility of its use with an imaging device with a coincidence circuit, suggests then a significant improvement if Co-55 becomes routinely available as a potential label for bleomycin.

Production cross sections for the (p,xn) reactions on natural Fe were measured at CNL in the 10-65 MeV region. Cobalt-55 is produced by the $^{56}Fe(p,2n)^{55}Co$ nuclear reaction (Q = -16 MeV), utilizing an external 35 MeV proton beam impinging on a 17 MeV thick target. Beam intensities up to 30 μA were utilized to produce 75-80 mCi/h Co-55 on a He(front)-H_2O(back) cooled target.

After bombardment, the Fe foil is dissolved in aqua regia, the bulk of the Fe extracted repeatedly with diethyl ether and a final Fe-Co separation is accomplished by taking advantage of the difference in the complex forming behavior. Anionic chloride complexes of Fe^{+3} and Co^{+2} are formed in a 12 N HCl solution and separated with a Dowex 1 x 8 anion exchange column. The Co-55 is eluted with a 4 N HCl solution, evaporated to dryness and finally dissolved in a 0.5 N HCl solution.

The final solution contains >99.9% Co-55, with Co-56 (78.5 d) being the only detectable impurity. Solutions with up to 100 mCi/ml can be obtained by this method.

Similar bleomycin labeling yields were obtained when comparing the CNL preparations with commercially available Co-57. Imaging and biological studies with Co-55 labeled bleomycin are presently under consideration.

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No. 26

PRODUCTION OF ^{61}Cu BY ^{4}He AND ^{3}He-REACTIONS ON COBALT TARGET

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Among the eleven radioisotopes of copper, only ^{61}Cu (3.32 h), ^{64}Cu (12.8 h) and ^{67}Cu (61.7 h) could be useful for biological and medical applications from the aspects of half-life and energy. ^{61}Cu is thus the most suitable radioisotope of copper for nuclear medicine.

^{61}Cu is advantageous to ^{64}Cu and ^{67}Cu at scanning due to the presence of the desirable γ ray (0.284 MeV - 12%). Since it is a β^+ emitter, it is also suited to positron camera. The absorbed dose to the various organs and tissues was estimated to be the smallest for ^{61}Cu, larger for ^{64}Cu, increasing up to about 5 times for ^{67}Cu. In this study the cobalt targets were bombarded with α or ^{3}He particles to construct the excitation curves and thick target production yields for both ^{61}Cu and the by-product nuclides (see Figures 1 and 2). The maximum cross section of the $^{59}Co(\alpha,2n)^{61}Cu$ reaction was found to be 340 mb at 25 MeV. The ^{61}Cu thick-target yield was 6 mCi/μAh from a natural cobalt target of 210 mg/cm^2 thick bombarded with 40 MeV α particles.

In case of ^{3}He bombardment, the $(^{3}He,n)$ reaction on ^{59}Co has a low Q value of +6.61 MeV, but the maximum cross section is as low as 6 mb at 35 MeV. Accordingly the thick-target yield is also small, 110 μCi/μAh at 40 MeV. This is probably due to the reason that ^{3}He particles which overcome the coulomb barrier of 9.72 MeV in this reaction simultaneously induces the following competing reactions having larger cross sections than that of $(^{3}He,n)$ reaction, $^{59}Co(^{3}He,\alpha)^{58}Co$, $^{59}Co(^{3}He,\alpha n)^{57}Co$ and $^{59}Co(^{3}He,\alpha 2n)^{56}Co$. The maximum cross sections of these reactions in this order were found as large as 300, 75 and 63 mb respectively under 40 MeV of ^{3}He particles bombardment. Accordingly, from the standpoint of ^{61}Cu production, the maximum cross section of the α reaction is 61 times larger and thick target yield is 63 times larger than the ^{3}He reaction.

As the radiochemical separation method, the co-precipitation, solvent extraction and anion exchange resin method were examined to separate ^{61}Cu free from such by-product nuclides as ^{58}Co, ^{57}Co, ^{62}Ni and unreacted target cobalt. The anion exchange method on chlorocomplex ions of cobalt and copper provided a carrier-free separation of ^{61}Cu requiring 150 minutes. The radiochemical yield of the separation process for ^{61}Cu was 95% according to the tracer test performed with ^{64}Cu.

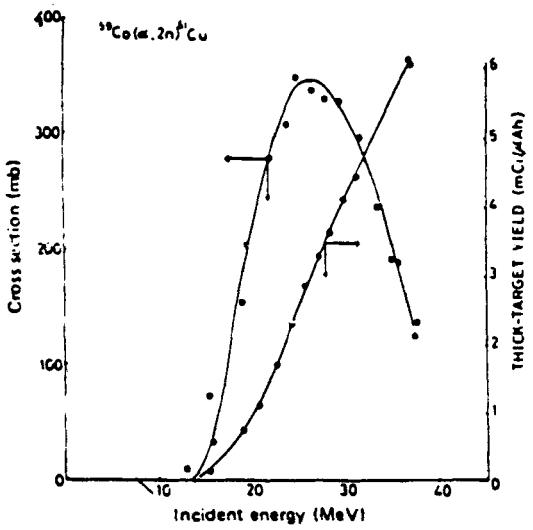

Fig. 1. Excitation curve and thick-target yield curve for ^{61}Cu from (Co + α) reaction.

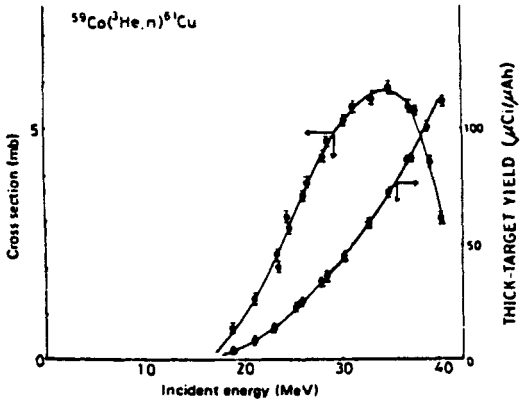

Fig. 2. Excitation curve and thick-target yield curve for ^{61}Cu from (Co + ^{3}He) reaction.

No. 27

COPPER-62 RADIOPHARMACEUTICALS

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Copper-62 is a generator produced, positron emitting radionuclide with a 9.8 min half-life. Its Zn-62 parent (T 1/2 = 9.3 hr) can be produced by the Cu-63(p, 2n)Zn-62 reaction when natural copper is irradiated with 22 MeV protons. A simple generator system, using an anion exchange column and elution with 0.1 N HCl containing 100 mg/ml NaCl, has been described.

Copper-62 labeled CuS was prepared by bubbling H_2S through the acid Cu-62 eluate. After removing excess H_2S by purging with N_2, the solution was neutralized and buffered at pH = 5.5. Copper oxide is spontaneously produced upon heating $Cu(OH)_2$ in alkaline solution. Copper-62 labeled CuO was prepared by adjusting the pH of the eluate to 11, heating in an autoclave for 10 min, and finally buffering at pH = 7.

Albumin aggregates encorporating Cu-62 labeled CuS were formed by adding heat denatured human serum albumin (pH = 11) to Cu-62-CuS, which was prepared as described above. The labeled MAA forms spontaneously when the denatured HSA precipitates at pH = 5.5. A final short duration heating at 100°C "fixes" the labeled MAA in the 20-60 μ size range. Human albumin microspheres which have been pretreated with Sn(II) were rapidly labeled with Cu-62 by adjusting the eluate to pH = 3, adding the "instant" HAM and heating the suspension at 121°C for 10 min.

Copper-62 labeled chelates were prepared by mixing 10 mg of the desired chelating agent with the acidic Cu-62 eluate and adjusting the pH to neutral. Cu-62-EDTA and -DTPA were rapidly and quantitatively formed, but liver uptake after IV injection in rats suggested lack of in vivo stability. The Cu-62 chelate of trans-1,2-diaminocyclohexanetetraacetic acid (DCTA) was found to be stable in vivo with rapid renal uptake and excretion. The Cu-62 chelate of 8-hydroxyquinoline was slowly taken up in the canine liver with subsequent concentration in the gall bladder. Copper-62-trifluoroacetylacetonate (TFA) and -hexafluoroacetylacetonate (HFA) are of special interest since they are highly volatile and can be used for inhalation studies.

No. 28

A NOVEL METHOD FOR THE CHEMICAL SEPARATION OF CARRIER-FREE THALLIUM-201 FROM ENRICHED THALLIUM-203 CYCLOTRON TARGETS

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Thallium-201 appears to be a very useful radionuclide for various medical applications including myocardial imaging and possibly tumor detection. This nuclide decays by electron capture with a half-life of 73 hours. It emits K x-rays of 69-83 keV in 98% abundance, and has photon energies of 135- and 167-keV in approximately 10% abundance, which are excellent for efficient collimation and detection. Thallium(I) is known to be a good potassium analog and to possess an affinity for potassium activating sites.

Carrier-free lead-201 ($T_{1/2}$ = 9.4 hr) is produced from Tl_2O_3 by the $^{203}Tl(p, 3n)$ nuclear reaction. The chemical separation of carrier-free lead-201, parent of thallium-201, involves the extraction of thallium(III) chloride into isopropyl ether, and passage of the aqueous lead-201 solution over Dowex 1 anion exchange resin to insure removal of trace amounts of Fe(III) and Tl(III). This procedure has proven to be simple, reliable and readily suited for hot cell manipulations.

The distribution ratio has been calculated at various ionic strengths and the effect of pH of the solvent on the oxidation state of the thallium-201 has been investigated. The reduction of thallic oxide to thallous oxide by the heat dissipated in the target caused by the proton beam has necessitated an investigation of various oxidizing agents for thallium.

Chemical analysis reveals that less than 1 microgram/ml of stable thallium appears in the product. Radiochemical purity of the product is checked by paper chromatography and radionuclidic purity is analyzed by multichannel pulse-height analysis, using a Ge(Li) detector. The radioisotopic purity is greater than 99% at end of processing. Recovery of the enriched thallium-203 is 95-97%. Further, a 'generator' system is being investigated for the continuous milking of Tl-201 from Pb-201.

No. 29

CYCLOTRON PRODUCTION OF THALLIUM - 201 FOR MYOCARDIAL IMAGING

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Thallium-201 in an aqueous solution suitable for human use is being produced in multimillicurie amounts by the Crocker Nuclear Laboratory (CNL). Thallium-201, as thallous chloride, is indicated for myocardial imaging in acute infarction and/or ischaemia. Thallium-201 is produced by the $^{203}Tl(p,3n)^{201}Pb \rightarrow {}^{201}Tl$ nuclear reaction. A proton beam of 30.4 MeV incident energy on a natural metallic Tl target ($900mg/cm^2$) is used. Proton beams of up to 30μA are available producing 19 - 20mCi/h of Tl-201. A specially designed water cooled target block is utilized to contain the Tl target material, which, due to the energy absorbed from the beam, is melted during the irradiation. After bombardment, the Tl is dissolved in 6 M H_2SO_4 and the parent ^{201}Pb(9.4h) is precipitated as $PbSO_4$ ($Ksp = 2.3 \times 10^{-8}$) by adding 10 mg of Pb^{+2} carrier. The acid dissolution of the Tl target yields about 20% Tl^{+3}, which may be adsorbed by the $PbSO_4$ precipitate. A fraction of the Tl^{+} formed may also be adsorbed and/or coprecipitated as $Tl_2Pb(SO_4)_2$. It is then necessary to further purify the parent Pb fraction from the Tl carrier. This can be done by redissolving the $PbSO_4$ followed by either A) Adding 30% H_2O_2 and boiling the solution to reduce Tl^{+3} prior to reprecipitating the $PbSO_4$. This method yields > 99.99% separation of Pb from the Tl carrier; B) Reprecipitating the $PbSO_4$ in the presence of C_2H_5O and then washing the solid with large volumes of ether. This method produced $\lesssim$ 0.02% Tl contamination in the final product. A product >98% Tl-201, with <2% Pb-203, Tl-200 and Tl-202 is obtained. The final Tl-201 administration dose (1 - 2mCi/patient) in the CNL product contains <5ppm of Tl carrier. From cross section data measured at CNL for the 10 - 60 MeV proton energy region, the alternative reaction $^{205}Tl(p,5n)^{201}Pb \rightarrow {}^{201}Tl$ (from either natural or Tl-205 enriched targets) has been found to be inadequate due to the larger radionuclidic contamination (especially Tl-200) in the final product. Production cross sections for Pb-200, Pb-202, Pb-203 and Pb-204 were also measured.

*On leave from Naval Research Laboratory, Washington D.C.

No. 30

A SYSTEM FOR ROUTINE PRODUCTION OF ^{99m}Tc BY THERMAL SEPARATION TECHNIQUE

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Technetium-99m is one of the most widely used radionuclides in diagnostic medicine, and it is a general opinion that ^{99m}Tc and the radiopharmaceuticals obtained from it will be used for a long time. ^{99m}Tc has convenient physical and biological parameters for clinical applications. ^{99m}Tc is formed by the beta decay from its ^{99}Mo parent. ^{99}Mo is produced by neutron irradiation of natural Mo or enriched ^{98}Mo, or as a fission product of ^{235}U. A third approach is cyclotron production of ^{99}Mo by proton bombardment of enriched ^{100}Mo over $^{100}Mo(p,pn)^{99}Mo$ reaction. The most common method of separating ^{99m}Tc from its ^{99}Mo parent is the elution of ^{99m}Tc with normal saline from an aluminium oxide column containing adsorbed ^{99}Mo. The second method is solvent extraction using methylethylketone. The sublimation method based on different volatility of MoO_3 and Tc_2O_7. Previously the development of a practical sublimation generator has been studied and some of them have been used routinely. As a reactor and cyclotron owner we have examined the possibility of producing our own ^{99m}Tc with the aim of being independent of supplier and transportations problems. We have studied the separation of ^{99m}Tc from MoO_3 by complete distillation of gram amounts of natural MoO_3 or enriched $^{98}MoO_3$ irradiated in reactor. The irradiation was performed either at Triga Heidelberg II reactor (5×10^{13} n/cm^2sec) or at GFK Karlsruhe FR 2 reactor ($8\times10^{13}n/cm^2sec$). The principle of the described method is based on the complete evaporation of MoO_3 and Tc_2O_7 oxides and its following fractionated condensation. To realize this idea, two different generator systems for repeated multiple separation were developed and studied. The first based on using a two-oven technique. A quartz apparatus is placed in two ovens which are situated side by side and heated to different temperatures. Sweeping gas carries irradiated $^{99}MoO_3$ from a middle part of the first oven heated to a higher temperature (1100^oC), to the beginning of the second oven (550^oC) where the $^{99}MoO_3$ condenses. $^{99m}Tc_2O_7$ remains in the gas phase and is carried through the second oven and condenses on a coldfinger. ^{99m}Tc can be washed out with a very small volume of water for injection or normal saline. To repeat the separation process the same procedure is followed with the two ovens exchanging roles. The second studied generator system is based on a different distribution of Mo and Tc oxides along a quartz column having a temperature gradient.

Two different types of generators have been designed and constructed with special equipment for the routine semiautomatic separation of ^{99m}Tc. Optimal conditions for gas flow and temperature have been studied along with the effects of using different tube geometries and quantities of MoO_3. The described method of ^{99m}Tc separation is more reliable and has given a higher production rate than previously published methods.

No. 31

PHOTON AND CHARGED-PARTICLE PRODUCTIONS OF CARRIER-FREE Tm-167 FOR MEDICAL USE

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Recent studies on radioisotope scanning agents have shown that rare-earth radionuclides of high atomic numbers concentrate favorably in tumor tissues and bones. Among them attention is paid to the 9.24 d Tm-167 as being one of the acceptable tumor localizing agent, in view of its nuclear characteristics, the half-life and the EC decay followed by a 208 keV γ emission. The present authors report their studies on the production methods of this nuclide free from carrier and long-lived isotopes of thulium by using bremsstrahlung from electron linac and protons and alpha-particles from cyclotron. The photoproduction of Tm-167 is a new method, which uses the process of Yb-168(γ,n)Yb-167 ($T_{1/2}$ = 17.7 m, EC) ⟶ Tm-167. A 10 hr irradiation with about 150 μA of 60 MeV electrons of a 100 mg $^{168}Yb_2O_3$ enriched to ∿20% produces 0.5∿1 mCi of Tm-167 and the same irradiation of one gram of Yb_2O_3 of the natural abundance produces 30∿50 μCi. This process produces no Tm-168 ($T_{1/2}$ = 85 d) and the (γ,p) reactions yield undetectable Tm-170 and Tm-171 ($T_{1/2}$ = 129 d and 1.91 y, respectively). The amounts of the target could be increased up to several tens of gram without any appreciable decreases of the Tm-167 yield per unit weight and used repeatedly without any appreciable losses of the target material, provided that the supply of the enriched material could be allowed. The cyclotron productions currently used were also reinvestigated by measuring the excitation functions of the (Ho-165+α) and (Er+p) reactions. Erbium oxides of the natural abundance and of an enriched in Er-167 were used for the latter reactions. The present results on the excitation functions were compared well with the calculated values based on the statistical model. Great discrepancies were found between the present and reported values. The alpha- and proton energy intervals for the Tm-167 productions are to be optimized from the present results to yield minimum contaminations with the long-lived Tm nuclides. Cost problems and chemical procedures will be discussed.

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No. 32

SAFETY HANDLING FOR CYCLOTRON PRODUCTION OF RADIOPHARMACEUTICALS

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The short-lived radiopharmaceutical produced by cyclotron gives patients the benefit of the radiation exposure dose. But the manufacturing staff may receive much higher exposure dose than the patients. Under the health-physical aspects, the following safety control is to provide. (1) The maximum permissible concentration for these short-lived radionuclides is not described in the Recommendation of ICRP Report of Committee II (1959) and according to the ICRP formula, their MPC (168 hours week) are calculated.

	^{11}C	^{13}N	^{15}O	^{43}K	^{52}Fe	
MPC air	6×10^{-7}	5×10^{-7}	4×10^{-7}	1×10^{-7}	3×10^{-8}	
MPC water	4×10^{-1}	7×10^{-1}	3	5×10^{-4}	1×10^{-5}	μCi/ml

(2) Our machine has the facility for focusing the accelerated ions (d: 16-35 MeV, 46 μA, p: 40-50,10, α: 32-70,20, ^{3}He: 24-80,20) down 2 beam tubes for the production of radionuclides in target room outside cyclotron chamber. This room is shielded with 2 meters thick concrete walls in all directions including floor and ceiling. Beside the wall, 4 hot cells with 5 or 10 cm thick lead wall and lead glass window are arranged. As a result of these shielding, the exposure dose in an adjacent room, for example, is 0.04-1.3 mrem of neutron and 10-50 μR/hr of γ ray in case of d: 16 Mev, 15 μA on aluminum target. (3) In order to reduce the exposure dose to the staff and to save the loss of cooling time, the next automatic and remote controlled systems of radiopharmaceutical production are designed. (a) ^{18}F-transport from $^{16}O(\alpha,pn)^{18}F$ water target into vials through fractional injector, (b) ^{13}N-ammonia preparative apparatus which consists of $^{16}O(p,\alpha)^{13}N$ water target, transport machine, reduction flask by Devarda's alloy and ammonia absorption vials, (c) on-line gas preparation system for $^{11}CO_2$, ^{11}CO, $^{13}N_2$ and $^{15}O_2$ will be shown by photo-slides.

No. 33

RADIOPHARMACEUTICALS LABELED WITH POSITRON-EMITTING ISOTOPES FOR USE IN EMISSION TOMOGRAPHY

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Many of the problems associated with the use of single photon emitting isotopes and the Anger scintillation camera can be overcome by using emission tomography with positron-emitting radiopharmaceuticals. Utilizing this technique, quantification of the radiopharmaceutical distribution can be obtained. One major limitation in the general applicability of positron emission tomography is the scarcity of suitable radiopharmaceuticals. Because of the high photon flux necessary for emission tomography and the high radiation dose associated with the positron mode of decay, these isotopes must be short-lived.

One source of such isotopes is the "in house" biomedical cyclotron which can be used to produce oxygen-15, nitrogen-13, carbon-11 and fluorine-18. A second source of such isotopes are generator systems and the leading candidates for broad application appear to be the strontium-82/rubidium-82 and germanium-68/gallium-68 systems. The half-life (75 seconds) of rubidium-82 limits the applicability of this isotope. However, gallium-68 with a half-life of 68 minutes, long enough to prepare a whole series of radiopharmaceuticals but short enough to allow the administration of millicurie quantities of radiopharmaceuticals, appears to be the isotope of choice for the widespread application of positron tomography. The advantages and disadvantages of the currently available generator will be discussed and the radiopharmaceuticals that can be labeled with gallium-68 compared and contrasted with those that can be labeled with the short-lived cyclotron-produced radionuclides.

No. 34

^{11}C-SEROTONIN: A TRACER FOR THE PULMONARY ENDOTHELIAL EXTRACTION OF SEROTONIN

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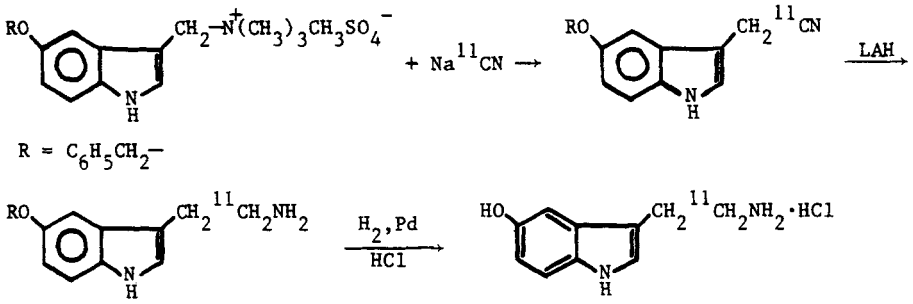
Serotonin is a potent vasoconstrictor, a neurotransmitter and is active in hemostasis. It arises in mammals from the hydroxylation and decarboxylation of the amino acid tryptophan. Normally this pathway accounts for only 1% of tryptophan metabolism in man but rises to 60% in patients with carcinoid resulting in abnormally high circulating levels of serotonin. Unusually high levels of serotonin can also be produced by the ingestion of foods with high serotonin content.

A number of enzymes including monoamine oxidase are responsible for the enzyme catalyzed degradation of serotonin. It has also been shown that the pulmonary endothelium very efficiently removes serotonin from the blood. This has been shown in isolated perfused lungs of animals and in humans during thoracic surgery. In studies with isolated perfused lungs, serotonin uptake has been shown to be an active transport process and to be effected by anesthesia and various metabolic inhibitors. The relationship of serotonin uptake by the lung to various disease states has not been determined largely due to the lack of a safe non-invasive technique for such an evaluation.

We have developed a rapid synthesis leading to ^{11}C-labeled serotonin for probing the lung uptake and physiological significance of pulmonary endothelial transport and metabolism of serotonin in normal and disease states.

^{11}C-Serotonin is synthesized by a modification of the original serotonin synthesis reported by Speeter using H^{11}CN as the isotopic precursor. The synthesis involves three steps (displacement, reduction of the nitrile and hydrogenolysis) as is shown in the reaction sequence. The ^{11}C-serotonin is purified by absorption of



the reaction mixture on a weakly acidic cation exchange resin (Biorex 70) washing with water to remove non-basic impurities and elution of the product with 0.25 M HCl. The overall yield is ∿ 5%, the synthesis time is 1 hour and the specific activity is ca. 2000 Ci/mmol. The radiochemical purity of the product was determined by thin layer chromatography and ion exchange chromatography with carrier serotonin.

Imaging studies in anesthetized dogs and rabbits show an initial lung uptake > 50% of the injected dose (100% = activity in entire field of view as the bolus moved through the chest) after i.v. injection of ^{11}C-serotonin. Studies are in

progress to refine and extend the techniques of radiopharmaceutical design and synthesis and to evaluate the utility of this technique for dynamically monitoring amine uptake and metabolism by the lung.

No. 35

OPTIMIZATION OF ^{11}C LABELLING OF RADIOPHARMACEUTICALS: ^{11}C CLOMIPRAMINE AS AN EXAMPLE

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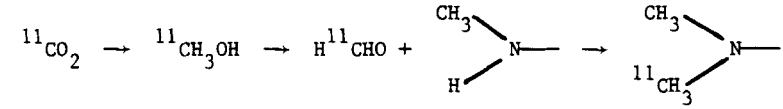
Using ^{11}C, a number of new radiopharmaceuticals which penetrate the blood brain barrier are now available in nuclear medicine. Some are natural products such as water, sugars, amino acids and allow metabolic studies within brain tissue. Others are synthetic products used as drugs acting on central nervous system. The later, injected in very low amounts, have no pharmacological effect but still localize in specific zones of the brain. It would be of great interest if it could be shown that their distribution or kinetics differs from normal to psychiatric patients. In order to know more about human brain uptake of some of these drugs, optimization of the labelling procedure with ^{11}C has to be studied. Because many of these drugs, for example clomipramine, have an accessible amino methyl group on the side chain, this particular molecule which is an antidepressant has been taken as an example.

Clomipramine-^{11}C is obtained by N-amino methylation using ^{11}C-formaldehyde of the corresponding nor derivative.

The synthesis scheme for ^{11}C labelling now in clinical use requires 4 steps:



some problems and proposed solutions will be studied for each step.

$^{11}CO_2$ and ^{11}C Methanol Preparation

The main problem which had to be studied at this stage is to obtain the highest possible radioactive concentration.

$^{11}CO_2$ is obtained by nitrogen irradiation with 17 to 20 MeV protons following the nuclear reaction $^{14}N(p,\alpha)^{11}C$. The ^{11}C being obtained by nitrogen transmutation, the mass amount of carbon produced is negligible. The measurements of this amount by gas chromatography in the form of the methanol obtained after water hydrolysis of the AL $Li(OCH_3)_4$ complex resulting from the reaction of $^{11}CO_2$ on H_4Li Al in THF solvent show:

--That for a 15-minute irradiation by a 15 µA beam current of 20 MeV protons, 2 µMoles of ^{11}C-methanol are produced with a specific activity of 150 mCi/µMole ten minutes after the end of the irradiation.

--That different parameters such as target gas purity containing known amounts of CO_2, hydrocarbons, etc. ..., target design and material, foils, o-rings, tubing material, time and mode of irradiation (static or dynamic, low or high pressure) do not change in a significant manner the specific activity of the carbon-11 which is very far from the theoretical value: 10^4 Ci/µMole.

In addition to these results, two observations have been made:

--The amount of non-radioactive methanol produced in the whole system with no irradiation is only ten percent of what is obtained when irradiating

the gas as described earlier.

--The specific activity of ^{11}C increases with time but reaches a maximum of about (150 mCi/μMole) when the irradiation is followed continuously for 6 hours. Beyond this time the specific activity remains constant.

No explanation can yet be given on the origin of this contamination which might be due to the sum of different phenomena such as absorption, radiolysis, leaks...

Formaldehyde-^{11}C Synthesis

The catalytic dehydrogenation of methanol to formaldehyde-^{11}C is carried out with a mixture of nitrogen oxygen using hot silver as a catalyst. The optimal conditions of dehydrogenation have been studied as a function of temperature, gas flow, oxygen concentration, silver amount, etc. ...

Methanol has been measured by gas chromatography, formaldehyde by Nash method and ^{11}C-formaldehyde by Dimedon precipitation. It is shown that ^{11}C-formaldehyde is produced with a 60 to 80% yield and is pure enough to be used as soon as it is produced. The total time of synthesis is 10 minutes.

Clomipramine Synthesis

The optimization of nor-clomipramine methylation by ^{11}C-formaldehyde in the presence of formic acid has been studied as a function of solvent, temperature, reaction time, agitation, formic acid and formaldehyde concentration. The amount of clomipramine synthetized is measured by HPLC.

100% yields are obtained by heating for 7 minutes at 110°C a mixture of 1 μMole nor-clomipramine, 1 μMole formaldehyde, 2 μl HCOOH and 500 μl dimethyl formamide. It has been shown that when water is present in the reaction mixture the labelling yield falls dramatically. To avoid this water which is used for the CH_3OH synthesis a $CaCl_2$ trap has been installed at the input to the silver furnace.

After purification by solvent extraction (80% yield) and sterilization by filtration on a millipore membrane, 20 mCi of ^{11}C-Clomipramine are obtained 35 minutes after the end of a standard irradiation. The specific activity of the product ready for injection is 70 mCi/μMole.

No. 36

SYNTHESIS OF CARBON-11 LABELLED DOPA

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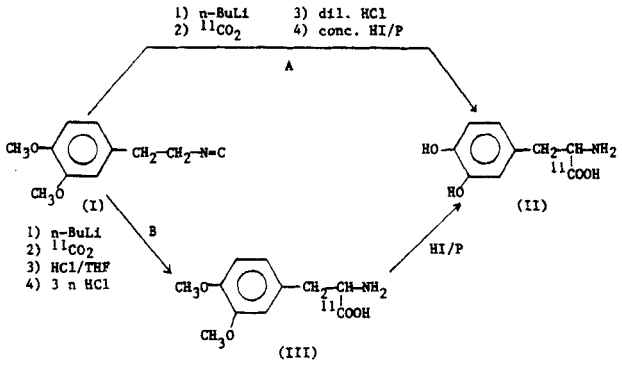
To investigate the distribution of 3,4-dihydroxyphenylalanine (Dopa, II) in brain structures by scintigraphy carbon-11 labelled Dopa was synthesized. As starting material was used 3,4-dimethoxyphenylethylisocyanide (I). Due to the presence of two hydroxyl groups in the Dopa molecule, the scheme used previously for the production of ^{11}C-phenylalanine and ^{11}C-phenylglycine required modification for this particular compound. The phenolic functions were protected as methoxy groups. The two routes A and B which differ only slightly, gave a remarkable difference in results.



Reaction path A:

Studies with $^{11}CO_2$ and $^{14}CO_2$ in this one pot reaction indicated that the radiochemical yields were substantially lower than expected from the results with previous investigated amino acids. The isocyanide I (0.25 mMol) was lithiated with n-BuLi at -80 to -70°C in a THF-hexane mixture. The resulting orange-red solution decolorized completely on reaction with CO_2 (20-30% excess) at the same temperature. After warming up to room temperature, dilute HCl was added and the solution evaporated to dryness. The residue was hydrolyzed with conc. HI for 10 min at 140°C. By means of preparative thin layer chormatography (Silica gel; n-BuOH:AcOH:H_2O = 4:1:1) ^{14}C-Dopa was isolated from the tarry mixture in a radiochemical yield of 1-4%.

The instability of Dopa, especially in basic solutions, does not permit the use of the purification method as developed for ^{11}C-phenylalanine employing an acidic Dowex column. Instead high pressure liquid chromatography of the reaction mixtures containing Dopa on various columns and packing materials gave promising results on analytical scale. However the results on the corresponding preparative columns were rather disappointing.

Reaction path B:

Since [^{14}C]-3,4-dimethoxyphenylalanine (III) could be converted almost quantitatively by HI hydrolysis into ^{14}C-Dopa, the synthesis and purification of this Dopa-derivative was investigated. Much better results were expected because III exhibits the stability of phenylalanine, unlike Dopa. Following carboxylation, 3 N HCl was used instead of concentrated HI in the hydrolysis step. When the reaction mixture was partially purified on acidic Dowex, amino acid III could generally be isolated in a radiochemical yield of 20 to 35%.

The radiochemical purity was established by thin layer chromatography and ranged from 85-91%. Next amino acid III was demethylated by HI to Dopa. The overall radiochemical yield was 18-32% while the radiochemical purity remained 90%. The chemical yield was 25 to 35% based on isocyanide (I).

In the ^{11}C-experiments the elapsed time between the end of the $^{11}CO_2$ production and the moment ^{11}C-Dopa was obtained, was reduced to 70 min. In addition to the $^{11}CO_2$ and carrier CO_2, in some experiments $^{14}CO_2$ was added for the TLC analysis. Further characterization of the final product was obtained by I.R. spectroscopy.

The reproducibility of the reaction leaves much to be desired. The lithiation of the isocyanide is very critical, determining the amount and number of byproducts.

No. 37

THE PREPARATION OF CARBON-11 LABELLED 17α-ETHYNYLESTRADIOL

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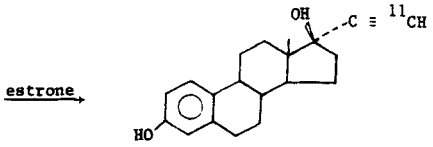
In the steroid hormone field hormonal action is synonymous with hormone binding. All hormone sensitive tumours are thought to bind their respective hormones by a mechanism involving receptor molecules in the target tissue. In many cases the introduction of an ethynyl group increases the hormonal activity of the compound. As an example, at molecular equivalent doses, the uptake of 17α-ethynylestradiol by target tissue is more than twice as great as the uptake of estradiol itself. If the ratio between the binding of hormone-responsive tumour tissue and surrounding tissue is high enough, gamma ray emitting steroid hormones are useful for the in-vivo detection of these tumours by scintigraphy. Information on the kinetics of these labelled drugs would quantify the tumour hormone response, and greatly enhance the usefulness of ablative therapy. It is essential for their use as radiopharmaceuticals, that steroids be labelled carrier free without destroying hormonal activity. The most obvious radionuclide is carbon-11. Carbon-11 labelled acetylide has previously been suggested as a useful precursor for steroid labelling[1].

The work presents the preparation of ^{11}C-labelled 17α-ethynylestradiol. The reaction sequence of the ethynylation reaction is given.

$$CaC_2 \xrightarrow{^{12}C(p,pn)^{11}C} Ca^{11}C_2 \xrightarrow{H_2O} HC \equiv {}^{11}CH \xrightarrow[THF]{BuLi} Li^{11}C \equiv CH$$



A convenient method for the preparation of $LiC\equiv{}^{11}CH$ was developed. Because of the instability of monolithiumacetylide, complexing agents such as liquid NH_3 or ethylenediamine have been reported. Not only stabilization but also deactivation of the LiC≡CH was noted. When n-butyllithium in hexane-THF was used the formation of LiC≡CH occurred smoothly at low temperatures. No appreciable formation of insoluble dilithiumacetylide was detected indicating that THF may act as a stabilizing agent. Recently Midland[2] described an extensive investigation concerning the preparation of LiC≡CH in THF. His results and ours are in close agreement. The production of ^{11}C-acetylene started with the irradiation of a CaC_2 target (0.5 cm diameter, 350 mg/cm^2 thick) with 30 MeV protons. The yield of 2 mCi/μA.min is considerably greater than that reported by Myers, for the $^{12}C(^3He,\alpha)^{11}C$ reaction on similar targets. After bombardment $H^{11}C\equiv CH$ was generated from the $Ca^{11}C_2$ by addition of a small amount of water. The radioactive acetylene was dried over magnesiumperchlorate, transported by a stream of helium and collected in a trap cooled with liquid nitrogen. It was then distilled onto a frozen homogenous substrate of n-BuLi in hexane and THF. On warming to room temperature $Li^{11}C\equiv CH$ was

formed in about 10 minutes. Estrone was introduced and the mixture was stirred at 50°C for 10 to 15 min. A 20% NH_4OH solution was added and the organic compounds were extracted with chloroform. High pressure liquid chromatography (silica gel; chloroform:hexane = 9:11) was employed to obtain an estrone-free sample. In a typical run 50 mg (0.185 mMol) estrone was used with 2 and 3 equivalents of n-BuLi and acetylene respectively. A radiochemical yield (not corrected for decay) of 3.5% was obtained. The chemical yield was 90%. The final product was characterized by NMR, IR and TLC. Thin layer chromatography (silica gel; benzene:methanol = 9:1) showed all of the activity concentrated in the spot of 17α-ethynylestradiol. The synthesis and the subsequent purification took less than 60 min.

References

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No. 38

DEVELOPMENT OF CYCLOTRON TARGETRY AND REMOTE RADIOCHEMICAL TECHNIQUES FOR THE CONTINUOUS LARGE-SCALE PRODUCTION OF ^{11}C-LABELED AMINO ACIDS

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A current ORAU/ORNL joint project is evaluation of the production of ^{11}C-labeled amino acids in batch quantities of ∿ 200 mCi each, produced at intervals of one hour or less.

The ORNL 86-Inch Cyclotron is being utilized to evaluate target efficiency and reliability in the production of ^{11}C-oxides by the $^{11}B(p,n)^{11}C$ reaction in B_2O_3 targets. Evolved radioactive gases are transferred by helium sweep gas from the target assembly to an adjacent remote-control manipulator cell for catalytic and chemical conversions.

Target designs consistent with constraints imposed by internal beam bombardment are being tested at beam currents up to 200 μA for the purpose of approaching an optimum combination of thick-target geometry and maximum recovery of ^{11}C-oxides. Specially shaped molybdenum and graphite substrates mounted inside a water-cooled target assembly utilize surface tension effects to retain molten B_2O_3 target material. Batch quantities of up to 4 Ci of ^{11}C-oxides are converted through $^{11}CH_4$ to $H^{11}CN$ at 90% efficiency preparatory to remote synthesis and purification of various ^{11}C-carboxyl-labeled amino acids (∿ 25 batches used clinically to date). In this manner we have produced up to 235 mCi of ^{11}C-1-aminocyclopentanecarboxylic acid for tumor imaging. No radiolytic decomposition has been observed.

We have shown that routine production of amino acids labeled with ^{11}C in ∿ 200 mCi batches produced at hourly intervals is feasible, permitting concurrent imaging of two patients to be performed up to three times per day. High patient throughput with ^{11}C-radiopharmaceuticals is an important economic prerequisite to their widespread clinical use, and we are studying the application of our techniques for large-scale continuous production to hospital cyclotron facilities. (This work is supported by USPHS Research Grants CA-14669 and 5-TO1-CA02587 from NCI. ORAU and ORNL are under contract with US ERDA.)

No. 39

^{11}C-LABELED AMINO ACIDS AS AGENTS FOR TUMOR AND PANCREAS VISUALIZATION

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A rapid, high-temperature, high-pressure modification of the Strecker amino acid synthesis has been developed as a general synthetic method for ^{11}C-carboxyl-labeled, natural and unnatural, amino acids. The appropriate carbonyl compound or derivative is heated with $(NH_4)_2CO_3$, NH_4Cl, and $K^{11}CN$ at 210°C for 10 minutes in a stainless steel pressure vessel to give the hydantoin, which is then hydrolyzed with NaOH at 210°C for 10 minutes. Purification is by anion exchange followed by cation exchange chromatography. The time required for synthesis from $H^{11}CN$ is approximately 45 minutes. Yields of five ^{11}C- and ^{14}C-labeled, neutral amino acids by this method have been 40-70%. The yield of ^{14}C-labeled DL-tryptophan has been 10%.

Prior tissue distribution studies of the analogous ^{14}C-labeled amino acids in test animals at early time intervals (15 min - 1 hr) have been used to assess the potential value of ^{11}C-labeled amino acids as agents for tumor and pancreas visualization. ^{11}C-Carboxyl-labeled 1-amino-cyclopentanecarboxylic acid (^{11}C-ACPC) has shown considerable potential as a tumor-scanning agent and is now being used clinically for that purpose. Analogs of ^{11}C-ACPC have also been tested in animals as tumor-localizing agents. ^{11}C-Carboxyl-labeled DL-valine and DL-tryptophan are of interest as agents for pancreas imaging. Clinical evaluation of DL-valine-1-^{11}C is now in progress.

We conclude that ^{11}C-labeled amino acids have significant potential as agents for tumor and pancreas visualization, especially in view of recent developments in positron emission computerized axial tomography.

(This work is supported by USPHS Research Grant CA-14669 from NCI. Oak Ridge Associated Universities is under contract with the US ERDA.)

No. 40

SEMI-AUTOMATED SYNTHESIS AND PURIFICATION OF RADIOPHARMACEUTICALLY PURE ^{13}N-L-ALANINE IN A CONTINUOUS FLOW SYSTEM

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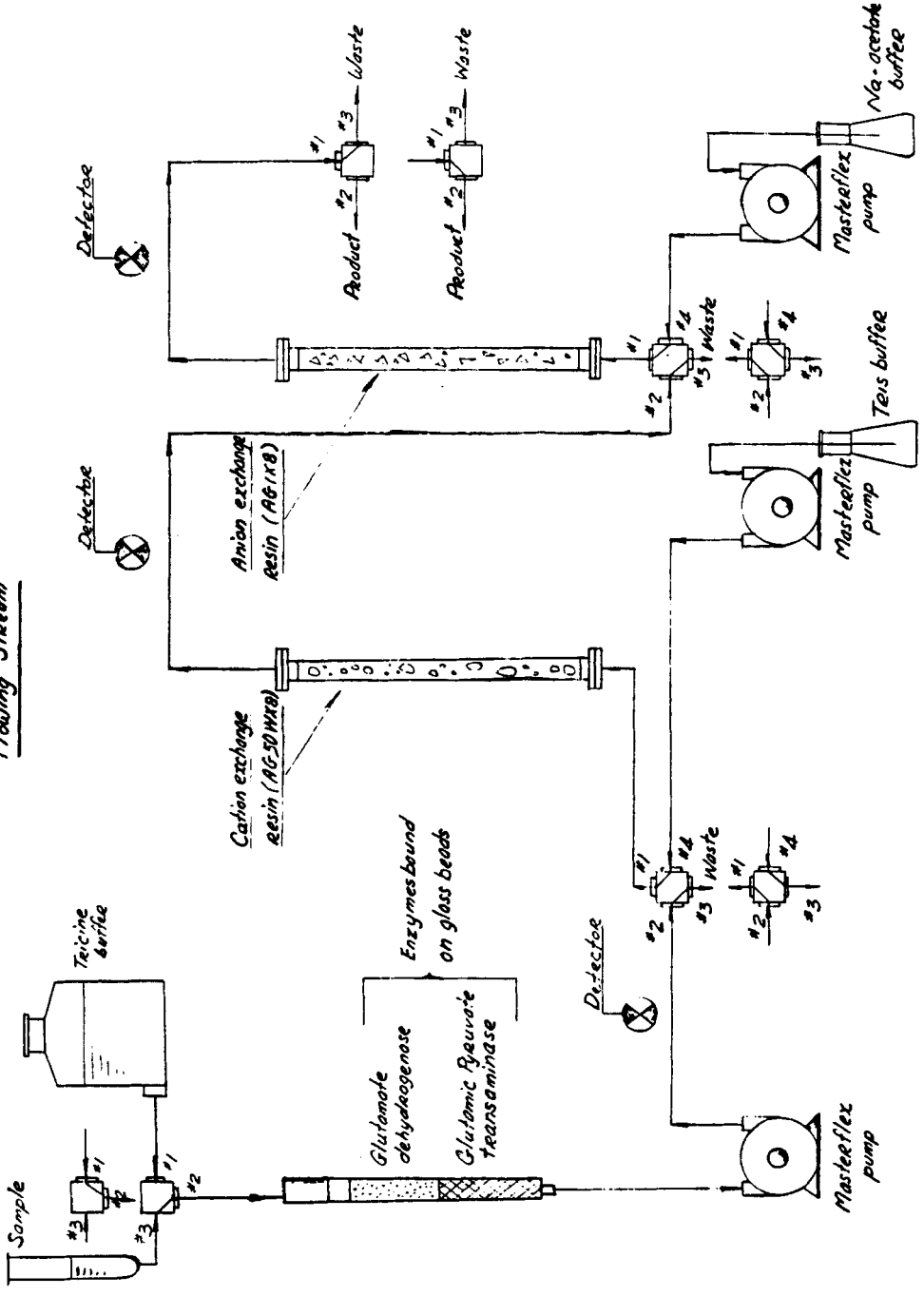
The synthesis of radiopharmaceuticals labelled with short-lived radioisotopes requires relatively large amounts of radioactivity at the start of the synthetic procedure to insure production of useable quantities of labelled product. Complex procedures that involve a great deal of manipulation present serious radiation exposure problems for the chemist. This exposure may be reduced by conducting the synthesis in a closed system without direct involvement of the chemist in the intermediate steps.

Our previous procedure for the synthesis and purification of ^{13}N-L-alanine involved several intermediate steps which limited the level of radiolabel that could be used, because an initial activity of 50 mCi of ^{13}N produces an exposure of about 10 R/hr at 6 cm from the reaction vessel. In order to permit synthesis of a greater amount of labelled product, a semi-automated system for the synthesis of ^{13}N-L-alanine has been developed. The process is based on our previously reported utilization of enzymes immobilized on glass beads for the synthesis of pure amino acids labelled with ^{13}N.

The semi-automated system includes three chromatographic columns connected through 4-way valves as shown in the attached Figure. Column 1 (0.35 x 25 cm) contains glutamate dehydrogenase bound to glass beads in the top half and glutamic-pyruvic transaminase bound to glass beads in the bottom half. Column 2 (0.2 x 15 cm) contains cation exchange resin (AG 50W x 8). Column 3 (0.2 x 15 cm) contains anion exchange resin (AG1 x 8). Prior to use, column 1 was equilibrated with 0.1 M tricine buffer, pH 7.6, column 2 with 0.01 M Tris buffer, pH 4.9, and column 3 with 0.05 M acetate buffer, pH 7.4. ^{13}N-L-alanine synthesis was initiated by trapping ^{13}N ammonia in 0.1 M tricine buffer, pH 7.4. To this solution 10 µmoles of α-keto-glutarate, 1 µmole of NADH and 2 µmoles of pyruvate were added to give a total volume of 1 ml. This solution was added to the funnel feeding column one. The radioactive solution was pumped through columns 1, 2, and 3. The radioactivity passing through the columns was monitored with a survey meter. The flow rate thru the columns was about 0.8 ml/min. The maximum yield of ^{13}N-L-alanine thus far obtainable is 55% of the $^{13}NH_3$ used (corrected for decay), in a volume of 19 ml and requires about 21 minutes for collection. The most optimal yield is 35% of $^{13}NH_3$ used obtained in about 9 minutes in a volume of 7 ml. The yield probably could be improved by the addition of a greater amount of enzyme to the glass beads in column one.

The system described greatly reduces handling of the intermediate fractions and permits an increase in the amount of starting radioactivity and product with reduced radiation exposure to personnel.


Semi-automated Synthesis of ^{13}N-alanine in a continuously flowing Steam
Sample
Tricine buffer
Glutamate dehydrogenase
Glutamic Pyruvate transaminase
Enzymes bound on glass beads
Cation exchange resin (AG-50 W8)
Anion exchange resin (AG1-X8)
Detector
Product
Waste
Masterflex pump
Tris buffer
Na-acetate buffer

No. 41

SYNTHESIS AND ANALYSIS OF ^{13}N-ASPARAGINE FOR MYOCARDIAL SCANNING

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L-Asparagine, prepared by amidation of aspartic acid with $^{13}NH_3$ preferentially localizes in the dog heart (J. Nucl. Med., 15:1223, 1974) and appears to be a useful myocardial scanning agent. Asparagine synthetase, which incorporates NH_3 into aspartic acid has been purified from E. coli K-12 (ATCC 25288) by a modified procedure originally described by Cedar and Schwartz (J. Biol. Chem., 244:4112, 1969). The maximum velocity for conversion of aspartic acid to asparagine was found to be 6.5 μmoles $min^{-1}mg^{-1}$ of enzyme protein.

$^{13}NH_3$ was produced by the $^{16}O(p,\alpha)^{13}N$ reaction by bombardment of H_2O with 15 MeV protons and subsequent reduction of $^{13}NO_2$ and $^{13}NO_3$ to $^{13}NH_3$ by titanous hydroxide. $^{13}NH_3$ thus produced was absorbed in a reaction mixture containing Tris-HCl, 5 mM, pH 8.2; Mg-acetate, 7.5 mM; ATP, 6 mM and L-aspartic acid 6 mM. The reaction mixture was then incubated with 40 μg/ml of the enzyme for 10 minutes at 37°C. After removing $^{13}NH_3$ by evaporation to dryness under vacuum the nonvolatile radioactivity was redissolved in a small volume and analyzed by high voltage electrophoresis with authentic ^{14}C-asparagine on a thin layer cellulose plate. The electrophoresis was carried out in 0.07 M borate buffer pH 9.2 under 140 volts/cm for 7 min. The cellulose strip was kept cool by circulating cold water during electrophoresis. Sixteen to 24% of $^{13}NH_3$ was converted to nonvolatile substances when incubated with asparagine synthetase. No significant radioactivity was detected in nonvolatile material without incubation with the enzyme (> 0.5%). When the non-volatile material was further analyzed by electrophoresis it was observed that 80% of the radioactivity was associated with asparagine. The entire process requires 18-20 minutes and the final preparation contains only normal metabolites.

No. 42

ADAPTING THE OLD TO NEW NEEDS: ^{13}N-LABELED UREA

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^{13}N-labeled urea is potentially useful in studying the mechanism by which infusion of urea reduces brain swelling. Since the amount of urea used for this procedure is large, high specific activity is not required. Therefore the following procedure, in which the Wöhler synthesis (Pogg. Ann., 12:253 (1828)) proceeds rapidly enough because of the high concentration of carrier ammonium cyanate, can be used.

^{13}N-labeled urea was made by adapting the method of Walker and Hambly (J. Chem. Soc., 67:746 (1895)). In a typical run silver cyanate, 1.0 gram (6.7 mmole) was magnetically stirred with 6.7 ml water while 3.45 ml solution containing 6.7 mmoles ammonium chloride was added. Stirring was continued for 1 hour. Air was bubbled for several minutes through 7.4 ml filtrate from this mixture, after which time 5 ml water containing ^{13}N-labeled ammonia was added and the solution was boiled 3 min., followed by cooling in ice-water. One gram urea was dissolved in the solution and 1 gram mixed bed resin (Bio Rad AG 501-X8(D) 20-50 mesh) was added. Deionization was completed by adding the mixture to the top of a 1 cm i.d. column containing 3 grams of the mixed bed resin and, after discarding the first 3 ml, collecting eluate (using water as the eluant) at the rate of 5 ml/min. Time from addition of ^{13}N-ammonia was 10 min.; yield 56% (corrected for decay) in 15 ml.

A preparation containing 12 mCi of ^{13}N with approximately 1 gram of urea was injected intravenously into a baboon. Exclusion of the labeled urea from the brain was demonstrated by imaging.

No. 43

N-13 SPECIES PRODUCED BY PROTON IRRADIATION OF WATER

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The irradiation of water with 14.5 MeV protons has been shown to produce as principal radioactive products nitrogen-13 labeled nitrate, nitrite and ammonium ions. The yield of $^{13}NH_4^+$ has varied from 100% at low integrated doses (0.08 μAh) to 0.4% at high integrated dose (10 μAh), but NH_4^+ was always detectable in the solution. $^{13}NO_3^-$ varied from non-detectable levels at low dose to 85% at high doses, but was never 100%. $^{13}NO_2^-$ varied from zero at low dose to approximately 14% at high dose. The radioactive constituents were measured by high pressure liquid chromatography using a "Partisil-SAX" anion exchange column, potassium dihydrogenphosphate eluent at pH 3.5, and a well NaI crystal coupled to a rate meter and strip chart recorder. NO_2^- and NO_3^- were detected, with added carrier, by UV absorption at 215 mμ. The presence of $^{13}NH_4^+$ was confirmed by gas chromatography using a "Poropak-Q" column. A number of wet chemistry tests were also used to obtain quantitative results in agreement with the chromatographic results. A striking feature of these measurements was the variability of the results, dependent on the recent use of the target chamber, which was made of titanium and had a stainless steel window foil.

No. 44

PRODUCTION OF ANHYDROUS FLUORINE-18 FOR NUCLEAR MEDICINE*

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A means of preparing large quantities (> 1 Ci) of anhydrous fluorine-18 for routine use in the production of radiopharmaceuticals has been developed using the $^{20}Ne(d,\alpha)^{18}F$ nuclear reaction. The radioactivity was produced using the Brookhaven 60" variable-energy cyclotron providing 23 MeV deuterons on the target window which were degraded inside the target from 14→0 MeV. Thick-target yields for 13.8- and 9.4-MeV deuterons were measured to be 82 mCi and 67 mCi per microampere saturation, respectively: A special gas-handling system, made from monel and nickel, and target chamber, also made from nickel, were used so that the very reactive fluorine-18 produced would not react or exchange with the container walls. The yield of fluorine-18 removed from the target chamber was found to depend on the target gas pressure and on the amount of carrier gas added to the target. For routine production runs 350 psig Ne(0.1% F_2) target gas is contained in a water cooled 10 cm nickel target chamber with a helium cooled front window of 1 mil nickel (inside) and 31 mils aluminum for irradiation with 15-25 μA of deuterons (14→0 MeV). These runs provide removal (^{18}F-F_2) of 80-90% of the fluorine-18 produced, and > 600 mCi (EOB) of the ^{18}F-F_2 (sp. act. - 10 Ci/mmole) has been removed from the target chamber after a 36 μAH irradiation. Labelling of radiopharmaceuticals with the fluorine-18 produced is accomplished with a remote control system which delivers the radioactivity to a reaction vessel in a hot lab adjacent to the cyclotron vault. We use fluorine from this target system to produce 2-fluoro-2-deoxy-D-glucose by the reaction of the ^{18}F-F_2 with triacetyl glucal (radiochemical yield -6%). Different carrier gases, including SF_6, SF_4, COF_2, and NOF, have been used to produce other possible fluorinating agents[6] labelled with fluorine-18.

* This research was performed under contract with the U. S. Energy Research and Development Administration, and partially supported by NIH Grant No. 5-P41 RR0067.

No. 45

^{18}F-DAST AS A REAGENT IN THE SYNTHESIS OF AN ^{18}F-SUGAR

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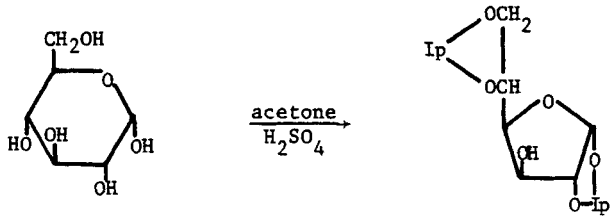
^{18}F-Diethylaminosulfur trifluoride (^{18}F-DAST), an agent which is known to effect F-for-OH substitution, has been used in the production of a labeled fluorosugar. The precursor DAST was synthesized by the addition of trimethylsilyldiethylamine in freon-11 to a stirred solution of SF_4 in the same solvent at -78°C. After purification by reduced pressure distillation, the unlabeled DAST was placed in a glass trap at -196°C, part of the circulating cyclotron target system. In a Monel target $H^{18}F$ was produced by the $^{20}Ne(d,\alpha)^{18}F$ nuclear reaction in a gas mixture of Ne + 15% H_2. At the end of bombardment, the trap is sealed and removed. The ^{18}F-DAST is formed as a result of exchange with $H^{18}F$ at room temperature. The ^{18}F-DAST was then used as a reagent in the synthesis of ^{18}F-3-fluoro-3-deoxyglucose. All the hydroxyl groups of glucose with the exception of that in the 3-position were protected by the formation of the derivative 1,2:5,6-di-O-isopropylidine glucofuranose by the following reaction sequence:



The derivative, along with unlabeled DAST, was prepared in large batches and stored for use when needed. Labeling conditions and various methods to remove the protecting groups will be evaluated. Purification methods and liquid chromatographic separation of the final labeled compound from other components of the mixture using a Waters Associates μ-Bondapak/carbohydrate column will also be discussed.

No. 46

THE PREPARATION OF ^{18}F-LABELED TOBACCO SMOKE*

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An important question in tobacco and health research is the relationship of the pulmonary deposition and clearance of particulate matter in cigarette smoke to the medical problems associated with smoking. Previous investigators have used ^{14}C to label smoke or have used γ-emitting radionuclides to label aerosols or inert particles which, at best, only approximate the size and shape of the particulate phase of smoke and are non-physiologic. Wehner et al.[1] used a neutron activation method to give smoke labelled with very high energy γ-emitters. We have developed methods to label smoke with ^{18}F which will allow inhalation studies in experimental animals and man using non-invasive nuclear medicine techniques.

Several compounds with different volatilities have been evaluated for their labelling effectiveness of the particulate and gas phases of mainstream cigarette smoke. Using ^{18}F produced by the ^{20}Ne(d,α)^{18}F reaction at our Van de Graaff accelerator we have adapted a method reported by Bergmann et al.[2] for the preparation of fluoroethanol by the reaction of ethylene carbonate and K^{18}F to produce ^{18}F-labelled fluoroethanol of specific activity 250 μCi/g. Similarly ^{18}F-labelled fluoropropanol (specific activity 2 mCi/g) was prepared by the reaction of propylene carbonate and K^{18}F.

Fluorine-18 labelled 3-fluoro-p-menthane, a fluorine analogue of menthol was prepared by the direct substitution reaction of K^{18}F and the tosylate ester of menthol; the volatile fluoromenthol was continuously distilled under vacuum from the reaction mixture and collected in a dry ice trap.

Using standard cigarettes and a vacuum smoking machine, studies of the distributions of activity between the different smoke phases were carried out with ^{18}F-labelled compounds. The compounds were injected at the filter interface of filter (f) cigarettes or at a corresponding point in regular (r) cigarettes. The results of the distribution experiments for mainstream smoke are as follow:

	particulate (%)	vapor (%)
^{18}F-fluoroethanol (r)	7	10
(f)	3	12
^{18}F-fluoropropanol (r)	2	14
^{18}F-fluoromenthol (r & f)	24	1

In initial smoking experiments with mice sacrificed immediately after smoking, ^{18}F-fluoroethanol gave significant activity in the lungs, head and liver.

References

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(2) E. D. Bergmann and I. Shahalc, J. Chem. Soc. C (1966) 899.

*This investigation was supported by the University of Kentucky Tobacco and Health Research Institute Project No. KTRB 124-05-7H900.

No. 47

LABELLING WITH REACTOR-PRODUCED FLUORINE-18

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Fluorine-18 labelled organic compounds are potentially useful tools in nuclear medicine. Specific biological processes, i.e. the transport and metabolism of organic compounds can be traced with the ^{18}F-labelled fluorinated analogues, if the fluorine position is not involved in biochemical pathways. This concept is based on the fact that fluorinated and non-fluorinated organic molecules are nearly isomorph.

Fluorine-18 produced by accelerator irradiation is very suitable for ^{18}F-chemistry as anhydrous and very reactive ^{18}F-labelled fluorinating species can be obtained. There are, however, several reasons to consider ^{18}F-production by reactor irradiation:

1. Per mCi of ^{18}F the costs of production are much lower in the case of reactor irradiation;
2. Nuclear reactors operate nearly continuously and the irradiation of one or more samples is possible at any time. Accelerators must be turned on for every irradiation and are much less reliable with regard to their operation;
3. In some parts of the world reactors are, but accelerators are not available for irradiation purposes;
4. The reactor-production of carrier-free ^{18}F up to 70 mCi per irradiated sample has been reported.

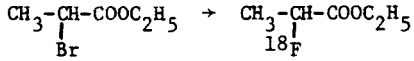
However, neutron-irradiation of oxygen containing lithium salts yields ^{18}F in an undefined chemical and physical state imbedded in a solid matrix. Because its separation from this matrix involves the dissolving in a suitable solvent, usually water, the main drawback of this manner of ^{18}F-production is the fact that only $^{18}F^-$ can be obtained. This limits the ^{18}F-chemistry to nucleophilic substitution at activated centers.

We developed a fast procedure for the separation of ^{18}F from irradiated Li_2CO_3. Dissolving in water and agitating with a cation exchange resin (H^+-form) yields in a short period of time an aqueous solution of carrier-free ^{18}F:

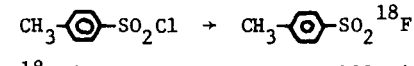
$$2\,\text{(P)}H + 2Li^+ + (CO_3)^{2-} \rightarrow 2\,\text{(P)}Li + Co_2\uparrow + H_2O$$

Subsequently,

A. After adding a known amount of KF and removal of the water, the formed $K^{18}F$ (radiochemical yield >90%, 80 min. after E.O.B.) was used in the following conversions:

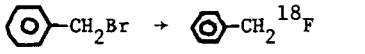


I



II

B. In a similar way $(C_2H_5)_4N^{18}F$ (radiochemical yield >90%, 45 min. after E.O.B.) can be produced. This compound was used in the following reactions:

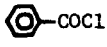
I and 

III

C. An anion exchange resin in the F^- -form was rinsed with the carrier-free ^{18}F-solution. After drying ^{18}F-labelled resin was obtained in a radiochemical yield of >95%, 45 min. after E.O.B. The labelled resin was used in the following reactions:

I and III and $n\text{-}C_6H_{13}OTos \rightarrow n\text{-}C_6H_{13}{}^{18}F$ IV

$CH_3\text{-}COCl \rightarrow CH_3\text{-}CO^{18}F$ V

 VI

The use of  -^{18}F as a fluorine donating agent was first reported by G. D. Robinson Jr. (1973), but his results could not be reproduced by us. Therefore, we investigated systematically the use of anion exchange resins in the F^--form in nucleophilic displacement reactions. We observed that a well defined resin in the F^--form only can be prepared in all-Teflon equipment and that only this resin yields reproducible and good results.

All aforementioned reactions were carried out at an 0.1 mmole scale within 2 half-lifes. If 50 mCi of ^{18}F is present at E.O.B. and the synthesis time amounts to 2 x $t_{\frac{1}{2}}$ (= 220 min.) with a chemical and radiochemical yield of 25%, the specific activity of the product will be 125 mCi/mmole (total activity about 3 mCi). This indicates that further chemical conversions of the prepared compounds are within our reach.

No. 48

SYNTHESIS AND APPLICATION OF ^{73}Se-SELENOMETHIONINE

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^{73}Se-selenomethionine was prepared by the biochemical process. ^{73}Se was prepared by the nuclear reaction of Ge(^{3}He,xn)^{73}Se process and separated as H_2SeO_3 for the selenium source of the biosynthesis. Saccharomyces cerevisiae and a screening strain (TO-1) for the purpose of the selenomethionine production (its identification is under study) were used. In order to shorten the preparation time, the H_2SeO_3 was added to the fermented liquor in which the above strains had been grown for 8-10 hours. The protein fraction of the strain was hydrolyzed and then selenomethionine-^{73}Se was separated chromatographically.

The TO-1 strain gave a better incorporation of ^{73}Se and a higher yield of selenomethionine than those of Sacch. cerevisiae. The preparation time was about 20 hours after the cyclotron irradiation and the over-all activity yield was about 7% based on starting activity. Scintigram from the positron detector will be shown.

No. 49

PREPARATION, SCINTIGRAPHIC EVALUATION, AND DISSOCIATION STUDY OF ^{11}C-2-N-PHENYLETHYLAMINOALKANENITRILE HYDROCHLORIDES

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Nine carrier-containing 2-N-phenethylaminoalkanenitrile hydrochlorides were prepared as follows incorporating the positron-emitting 20.4 min half-life radionuclide ^{11}C:

$$RCHO + C_6H_5CH_2CH_2NH_2 \xrightarrow[10\text{-}37^{\circ};\ 20\text{-}25\ min]{Na^{11}CN + aq\ NaHSO_3} \xrightarrow[Ether]{HCl} RCHNHCH_2CH_2C_6H_5 \cdot HCl \quad (^{11}CN)$$

$$R = H, CH_3, C_2H_5, n\text{-}C_3H_7, iso\text{-}C_3H_7, n\text{-}C_4H_9, iso\text{-}C_4H_9, n\text{-}C_5H_{11}, n\text{-}C_6H_{13}$$

Total synthesis time was 51-66 min and 6-35 mCi of product was obtained. The ^{11}C compounds dissolved in 0.5-1.5 ml of DMSO were administered intravenously to dogs, and in vivo distribution patterns were serially imaged scintigraphically. ^{11}C-2-N-Phenethylaminobutanenitrile hydrochloride showed rapid initial accumulation of activity in brain, lungs, and heart-blood pool, progressing to some concentration in liver, kidney, and bile, followed by diffuse whole body distribution. Quantitative in vitro studies with this compound at 6 min confirmed the localization of activity in the cerebrum and parotids of the dog. The lower molecular weight compounds showed rapid diffuse whole body distribution of activity with no evidence of organ concentration of activity. The branched-chained 2-N-phenethylamino-3-methylbutanenitrile hydrochloride showed a distribution of radioactivity similar to that of free cyanide. The long-chained lipid-soluble 2-N-phenethylaminooctanenitrile hydrochloride showed initial liver activity, progressing to diffuse whole body distribution. H^{11}CN was identified in the expired breath of dogs administered with these compounds. The rate of dissociation of these aminonitriles in aqueous buffered solutions of pH 5 and pH 7.3 is dependent upon structural changes in the molecule. Lower rates of dissociation in blood plasma reflect protein bonding sites for cyanide.

No. 50

PHOTOAFFINITY LABELING OF ESTROGEN RECEPTORS FROM UTERINE TISSUES. FACTORS INFLUENCING LABELING EFFICIENCY

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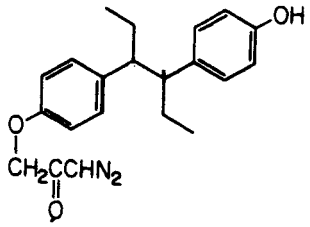
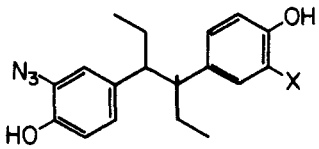
A systematic approach has been taken in the preparation and evaluation of photoaffinity labeling reagents for the estrogen receptor from rat and lamb uterus. Several derivatives of estradiol and non-steroidal estrogen hexestrol, containing photoreactive diazocarbonyl or azide functions, have been synthesized. The receptor binding affinity of these compounds and their capacity to photointeract with the estrogen binding site (inactivate) can be assayed indirectly by competition assays. Several of the compounds that showed both reasonably high binding affinities and inactivation efficiencies (in particular hexestrol 4-O(3'-diazo-2'-ketopropyl) ether (1) and hexestrol 3-azide (2) have been prepared in high specific activity,



1.8 [15]

Compound 1



X=H 70 [15]

Compound 2

tritium-labeled form. Direct binding measurements with these derivatives in unpurified rat uterine receptor preparations show that these compounds bind to the receptor, but they also show considerable non-specific binding to non-receptor proteins. Irradiation of these derivatives in unpurified rat uterine cytosol preparations results in incorporation of large amounts of radioactivity into protein in a covalent fashion. Electrophoretic fractionation of these preparations (under denaturing conditions-8 M urea) show several bands of radioactivity; the labeling of some of these is protected by estradiol. Analysis of labeled proteins can be made more reliably using a partially purified receptor preparation from lamb uterus. The receptor in this preparation has been disaggregated by mild trypsinization and can be electrophoresed with binding activity preserved. Electrophoretic analysis of the proteins in this preparation, radiolabeled by irradiation in the presence of the photoreactive reagents, shows some covalent incorporation into the receptor region of the gel with compound 2 (as evidenced by the solvent inextractability), but not with compound 1. The effectiveness of the photoaffinity labeling reagent prepared thus far is assessed, and suggestions are made for the design of new, more effective reagents.

No. 51

THE PREPARATION AND EVALUATION OF β ADRENERGIC DERIVATIVES. I. DEVELOPMENT OF AN IN VITRO SCREENING PROCEDURE

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It will be difficult to follow the natural pathway of a biochemical or a drug with a gamma emitting isotope if the radionuclide is bound directly to the biologically active compound. The native functional groups will probably be required to interact with the specific biological site responsible for physiologic action. If the radiolabel interferes with the binding step, the normal behavior of the molecule will be altered and true tracer studies will not be possible. Furthermore, the radionuclide may bind to the molecule with insufficient affinity to produce a stable compound. In either case, the desired behavior of the labeled compound will not be achieved.

In an attempt to circumvent these problems, derivatives which contain a site directing molecule and a radionuclide carrying group would be of interest.

An appealing class of compounds to trace are the β adrenergic agents, specifically the β adrenergic antagonists. If derivatives of these β blockers could be prepared and labeled with gamma emitting radionuclides, an unique procedure for tracing their kinetics would be available. In order to efficiently explore this approach, a reliable screening method for derivatives of cardiospecific β blockers would be helpful. A great deal is now known about the action of β adrenergic receptors in vitro. If the efficacy of the new derivatives can be judged by an in vitro receptor system, a suitable method to test these derivatives will be available. The development of an in vitro system is necessary before the newly synthesized derivatives can be evaluated. The following properties were studied in order to assure that the in vitro test is valid:

1. Retention of β adrenergic receptor in turkey erythrocyte membranes (TEM). TEM separated by standard techniques produced cAMP when stimulated by isoproterenolol. This stimulation was blocked by propranolol.
2. Specific binding of the radiolabeled antagonist. Three common radiolabeled antagonists were studied to determine the amount of specific binding to TEM. Tritiated alprenolol (^{3}HDHA) gave the highest specific binding (65%).
3. Effect of the quantity of TEM on ^{3}H DHA binding. The specific binding increased in a linear fashion from 0.2 to 2.0 mg protein in 0.5 ml. Because of the difficulty in obtaining large amounts of TEM, 1.0 mg of protein ($\sim$ 2% specific binding) was used.
4. Saturability and affinity of ^{3}H DHA binding sites. The binding of ^{3}H DHA was shown to be a saturable process. Half maximal saturation occurred at $\sim$ 5 nM which is in close agreement with other determinations of the dissociation constant.
5. Kinetics and reversibility of binding. At 37°C the binding of ^{3}H DHA was rapid with $\sim$ 70% bound in 20 min. On addition of excess propranolol, the ^{3}H DHA was displaced in 10 min.
6. Effect of D- and L- agonist of ^{3}H DHA binding. Stereospecificity as an important factor in determining specific interaction with a receptor. The comparison of D- and L- agonists indicate that the binding is indeed to the β receptor. The order of reactivity indicated a β_1 adrenergic receptor.

If lung microsomes are used this order reversed indicating that β_2 adrenergic receptors are present. The relative effectiveness of each drug in interacting with the β_1 or β_2 receptor paralleled literature findings obtained by physiological tests such as heart beat and muscle contraction ($R = 0.94$).

7. Antagonistic properties of derivatives which have tertiary or quaternary nitrogen atoms. Four compounds have been synthesized to determine the effect of charge on the nitrogen atom at physiological pH. These data indicate that the positive charge on nitrogen is critical for receptor binding.

The data presented here demonstrate the presence of ^3H DHA binding sites in the TEM preparation which have the characteristics of β adrenergic receptors. Binding is rapid, reversible, saturable and stereospecific. This system will allow the in vitro screening of β adrenergic derivatives by a simple and rapid method.

No. 52

SITES OF DIRECT AND INDIRECT HALOGENATION OF PROTEINS

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Iodinated proteins vary in biologic activity and rate of deiodination with the iodination conditions used. Proteins, peptides and amino acids have been labeled with radioiodide and radiobromide under a variety of pH conditions and labeling methods, including iodine monochloride, chloramine-T, electrolytic and enzymatic methods of direct labeling, and indirect acylation with N-succinimidyl-3-(4-hydroxyphenol) propionate (SHPP). A proteolytic enzyme (pronase) was used at pH 7 to degrade the labeled proteins and peptides to single labeled amino acids with minimal hydrolysis of carbon-halogen bonds. The resulting compounds were separated by ion exchange chromatography and detected with a flow through radioactivity detector and a ninhydrin colorimeter. Variations in the yield of monohalotyrosine, the most stable product, can be correlated with differences in in vivo stability of halogenated protein preparations prepared by the different methods. Other products observed included di-halotyrosine, mono-halohistidine and oxidation products of halotyrosine. Attachment of halogenated SHPP to a protein provides a stability toward hydrolysis equivalent to forming only monohalotyrosine residues. SHPP conjugates mainly with lysine, tyrosine and histidine residues in proteins to various degrees. At low levels of labeling SHPP does not affect the biologic properties of fibrinogen.

No. 53

PREPARATION OF I-123 LABELED 16-IODO-9-HEXADECENOIC ACID AND DERIVATIVES FOR HEART PERFUSION IMAGING

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16-Iodo-9-hexadecenoic acid is a structural analog of a biologically active, long chain fatty acid. Myocardial extraction of the radioiodinated compound ($\tilde{=}$ 70%) is comparable to that of K-43 and C-11 labeled long chain fatty acids. The I-123 labeled compound and several related derivatives are being evaluated as agents for myocardial perfusion imaging in experimental animals and man.

I-123 labeled 16-iodo-9-hexadecenoic acid is synthesized by halogen interchange between 16-bromo-9-hexadecenoic acid and I-123-iodine in 2-butanone (MEK). I-123 is produced by the Te-124(p,2n)I-123 reaction using 22 MeV protons. The irradiated Te-124 (96% enriched) target is dissolved in acid peroxide, I-123 is distilled into base, and I-123-iodide is extracted from 4 N NaOH with seven successive portions of MEK. The combined MEK extracts, containing 85-95% of the I-123, are reduced to 2 ml total volume by gentle heating. One milligram of 16-bromo-9-hexadecenoic acid is added to the MEK solution and the labeling reaction procedes under reflux for 90 min. A labeling yield of 75-85% is usually achieved. Residual MEK is evaporated with a nitrogen gas stream and the labeled product is taken up on 2 ml of 25% human serum albumin. I-123-iodide is removed by passing the HSA solution through a 1 cm diameter x 3 cm "Dowex 1-8X" column eluted with 6 ml of physiologic saline. Terminal sterilization is by "Millipore" filtration (0.22 μ). Five hr are required to produce 20 mCi of labeled 16-iodo-9-hexadecenoic acid in 6% HSA from a target containing 50 mCi of I-123 at EOB. The specific activity, based on total fatty acid, is 20 mCi/mg.

Radioiodinated 16-iodo-9-hexadecenoic acid has been shown to be extracted from the coronary circulation in proportion to blood flow. I-123 is an attractive label because of its favorable physical characteristics. This agent may prove to be a useful alternative to K-43, Cs-129, or Tl-201 when high information density, sequential myocardial perfusion images are required.

No. 54

PREPARATION AND QUALITY CONTROL OF ^{11}C-, ^{34m}Cl-, ^{77}Br- and ^{123}I-LABELLED FATTY ACIDS FOR KINETIC STUDIES OF HEART MUSCLE METABOLISM

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^{123}I- and ^{11}C-labelled long-chain fatty acids have recently been prepared[1] and used for the measurement of myocardial extraction[1,2]. For noninvasive kinetic studies of heart muscle metabolism we have labelled several fatty acids with the neutron deficient radionuclides ^{11}C ($T_{½}$ = 20.3 min), ^{34m}Cl ($T_{½}$ = 32.0 min), ^{77}Br ($T_{½}$ = 56 hrs) and ^{123}I ($T_{½}$ = 13.2 hrs). In order to obtain a high specific incorporation rate together with a high chemical stability, comparative studies with fatty acids labelled in different positions with various radionuclides have been carried out. The radionuclides have been prepared at the Jülich Isochronous and Compact Cyclotrons via the following reactions: $^{14}N(p,\alpha)^{11}C$, $^{35}Cl(p,pn)^{34m}Cl$, $^{79,81}Br(d,xn)^{76,77}Kr \xrightarrow{\beta^+,EC} {}^{76,77}Br$ and $^{127}I(d,6n)^{123}Xe \xrightarrow{\beta^+,EC} {}^{123}I$. In the case of ^{34m}Cl Szilard-Chalmers separation was applied using K_2ReCl_6 as target material.

The α-halostearic acids and the 17-^{123}I-heptadecanoic acid were prepared practically carrier-free by nucleophilic halogen exchange at the corresponding bromo- or iodofatty acids with radiochemical yields ranging from 50 to 100%. A comparison of the kinetics of the ^{123}I-for-Br exchange in α-bromo stearic acid in the melt, the solution and the decay-induced labelling in the melt shows that exchange of carrier-free $^{123}I^-$ in molten α-bromostearic acid at 87°C is the fastest process and is completed (100%) within 20 minutes. Palmitic and stearic acid labelled with ^{11}C in the carboxylic groups were prepared from the corresponding Grignard compounds with yields of 50 to 60%. The carrier-free products were identified and purified by high pressure liquid chromatography and then transferred into a 6% human serum albumin solution for animal experiments. Chromatographic quality control was essential in all cases studied, since both radioactive and inactive impurities are formed during synthesis in non-tolerable amounts, a fact which has often been neglected.

As expected on the basis of the greater C-halogen bond energy the α-chloro and α-bromofatty acids show higher stability than the corresponding iodofatty acids, and for kinetic studies the 17-haloheptadecanoic acids are much more superior to the α-halofatty acids. Comparative kinetic studies in mice demonstrate that myocard incorporation doses of 4.5% (43%/g) and 2% (19%/g), respectively, are obtained for the 17-iodoheptadecanoic and α-iodostearic acid, the ratio myocard to blood being 7 and 1.7, respectively at the maximum of specific incorporation which is observed within only about 2 min. Obviously, the 17-^{123}I-heptadecanoic acid is particularly useful for the study of fast kinetics of heart muscle metabolism. Preliminary double tracer (^{11}C,^{123}I) experiments have also been carried out in order to obtain information on the question of catabolism versus deiodination.

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No. 55

HEART UPTAKE OF RADIOIODINE LABELED CARDENOLIDES

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Radioiodine labeling of cardenolide derivatives was attempted in hopes that the labeled compounds might serve for tracing the heart uptake of cardiac glycosides in vivo clinically. Optimal clinical use of cardiac glycosides is complicated by lack of reliable means to predict individual cardiac response or intoxication, which seems closely associated with the cardiac concentration of the drug. Cardiac glycosides have been found to accumulate mainly in the kidneys and, to a lesser extent, in the liver and heart, given normal renal function. In renal failure, the highest concentration is seen in the heart, and lesser concentration in the liver and kidneys. Other multifarious factors influence the uptake also.

Now that iodine-123 is available from a cyclotron, it is feasible to employ it as a tag and measure it in a scintillation camera. What needs to be sought out are the appropriate chemical modifications of the cardenolide structure that help to make the labeling process easy and prompt, and at the same time give them similar pharmacokinetics to the original drugs. We picked out tyramine as an iodine-trapping principle and conjugated it to various forms of cardenolides, so that it was merely required to label the "ready-made" conjugate with iodine before administration.

We tried at first to prepare digoxin-tyramine conjugate by condensing the amino group of tyramine with the terminal digitoxose, after opening its ring structure to expose two vicinal hydroxyl groups by periodate oxidation, of digoxin (cf. 1). Two main products were obtained in this reaction; one composed of a digoxigenin, two digitoxose moieties and a terminal digitoxose-tyramine couple (Compound I), and the other, its analog, containing only one digitoxose bridge (Compound II). Both compounds were easily labeled with carrier-free ^{125}I by the conventional chloramine-T method. The second trial was to prepare a digitoxin derivative --- 3-O-succinyldigitoxigenin tyramine complex (Compound III) which is devoid of sugar moieties (cf. 2,3). The compound was obtained at a high yield and could be labeled with iodine as well (Fig. 1).

Each labeled compound was mixed with control ^{3}H-digoxin (I and II) or ^{3}H-digitoxin (III) in dilute alcohol and injected intravenously in dogs, then the body distribution measured after dissection. Pieces of organs were isolated and the radioactivity of ^{125}I was measured in a well-type scintillation counter. The same samples were dried in Visking tubes, burnt in an oxygen flask, and vapored tritium was collected with added carrier water. After addition of sulfite, the water was distilled for removal of radioiodine, and the radioactivity of the water was measured in a liquid scintillation counter.

Cardiac uptake of ^{125}I-cardenolides was higher for each compound as compared to the uptake in skeletal muscles, regardless of the difference in the whole distribution. The closest correlation of the uptake between ^{125}I-labeled derivative and corresponding control cardenolide was found with Compound II, serving our purpose, only with exception of the too much uptake of (II) in the lungs (Table 1). The localization of (II) in lung tissues is being examined by microscopic autoradiography.

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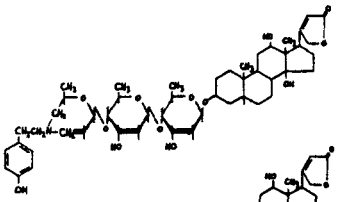
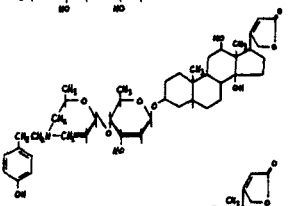
Compound I



Compound II



Compound III

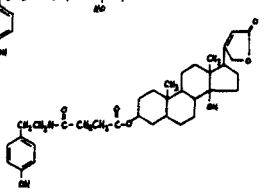


Fig.1 Tyramine-cardenolide conjugates

Table 1. Body Distribution of ^{3}H- and ^{125}I-Cardenolides in Dogs
(% Dose / kg of tissue)

Organ	Compound I				Compound II				Compound III			
	1 h		6 h		1 h		6 h		1 h		6 h	
	^{3}H	^{125}I	^{3}H	^{125}I	^{3}H	^{125}I	^{3}H	^{125}I	^{3}H	^{125}I	^{3}H	^{125}I
Blood	2.6	4.7	.7	1.8	4.4	5.2	1.9	5.9	7.5	21.5	3.4	13.1
Lungs	9.0	28.2	7.9	6.6	6.9	32.4	5.5	19.2	25.6	163.4	11.3	45.9
r. Atrium	21.7	7.9	7.5	2.4	6.0	6.7	6.0	6.6	15.4	10.3	11.4	6.9
l. Atrium	17.6	7.0	8.3	2.4	6.5	6.8	6.8	6.7	15.1	7.8	11.3	6.0
r. Ventricle	22.5	8.2	12.9	2.8	11.2	10.1	9.8	8.7	21.1	6.0	20.1	4.3
l. Ventricle	26.0	8.3	14.8	3.0	10.6	11.2	10.9	9.6	25.1	6.2	21.3	4.3
M. intercostalis	4.9	3.1	2.4	.9	3.7	2.7	5.9	5.8	6.9	3.0	3.8	2.9
M. pectoralis major	4.9	3.1	2.7	.9	5.5	2.8	4.5	5.3	5.5	3.0	3.5	2.2
Liver	18.9	24.7	11.2	6.5	11.3	18.0	18.5	15.8	52.1	12.0	21.8	6.7
Kidneys	140.0	55.0	130.4	19.4	24.0	20.4	48.9	30.3	133.6	13.4	135.9	10.7
Body weight of dog (kg)	12.0		12.5		18.5		12.0		12.0		9.5	

Chemical dose of injection was 0.2-0.25 mg.
Each value is the mean of five separate determinations.

No. 57

COMPARATIVE STUDIES OF PRACTICALLY CARRIER-FREE IODINATION METHODS WITH IODINE-123 AND IODINE-125

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We have carried out comparative studies on the following three methods of practically carrier-free iodination:

1) Decay-induced direct labeling by simple gas exposure of the substrate with the ^{123}Xe and ^{125}Xe precursor, respectively.

2) Decay-induced preparation of an iodination reagent by gas exposure of KIO_3 with ^{123}Xe and ^{125}Xe, respectively.

3) Non-isotopic halogen exchange in melt and in solution.

Basic studies on the selectivity and reactivity of the iodination of simple liquid monosubstituted benzenes via the Xe-gas exposure technique reveals the pattern of relatively high reactivity and low selectivity to be expected from homolytic iodination processes. I-for-H substitution yields range from about 8 to 14% and I-for-X substitution increases from 3.6 to 20% when going from fluorobenzene to bromobenzene. In all systems studied no difference is observed between $^{123}Xe/^{123}I$ and $^{125}Xe/^{125}I$ within the experimental error, again indicating that in both cases neutral iodine atoms lead to the final product formation.

The reactive iodination reagent which is obtained by $^{123,125}Xe$ gas exposure of solid KIO_3[1] is essentially identical with the classical iodide-iodate method. Small molecules such as tyrosine, thyronine and desoxyuridine are iodinated with yields of 82, 29 and 90%, respectively. Macromolecules such as fibrinogen, hypatitis-antibody and insulin can also be labelled under mild conditions with 52, 40 and 60%, respectively. The iodination strongly depends on the polarity of the solvent, e.g. the yield of iodotyrosine decreases from 80 to about 30% when going from aqueous solution to dioxane. Unlike in the direct gas exposure technique the reactivity and selectivity exhibited by the $^{123}Xe/KIO_3$-iodination method clearly shows the pattern of an electrophilic substitution. In phenol 84% is directed into the ortho-position, while in aniline 70% of p-iodoaniline is obtained.

Iodination by isotope exchange in melt is a well established method[2,3] which however, does not lead to carrier-free products. We have therefore applied the I-for-Br element exchange in the melt in order to prepare carrier-free α-iodofatty acids and iodohippuric acids. The kinetics of these processes are presented and compared with those of corresponding exchange processes in solution.

In all cases high pressure radio liquid chromatography was used for quality control and some relevant cases of its importance are presented[4].

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No. 58

A METAL-CATALYZED RADIOIODINATION REACTION

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Recent studies in this laboratory have dealt with a synthesis of radioiodinated stilbesterol by exchange labeling of iodinated stilbesterol. We confirmed that a cuprous ion catalyzed exchange in various organic solvents produced good yields of radioiodinated stilbesterols. The best conditions were achieved using either dichloromethane of dimethoxyethane as solvents, with CuCl 10^{-6}M, the iodinated substrate $5x10^{-5}$M and 1 mCi of ("no carrier added") $Na^{125}I$. Total yields of labeled materials were 70%, with yields of 35-40% of the purified mono-radioiodostilbesterol. Purification was usually achieved by HPLC (μ-porasil, chloroform, 1 ml/min). Similar results were obtained with iodinated estrogens and iodinated stilfosterols; in addition, the kinetics of these reactions were determined.

In the course of these studies we also noted that cupric ion appeared to catalyze direct labeling, without the need for a preexisting leaving function. Direct, cupric catalyzed labeling has been achieved using $CuCl_2$ 10^{-6}M and ("no carrier added") $Na^{125}I$ in dichloromethane or acetontrile as solvents and estradiol, testosterone and estrone as substrates. The total labeling yields were of the order of 50-60%, and the mixture of radioiodinated products could again be purified by HPLC, either using direct or reverse phase chromatography. Neither Zn, Ag, or Pt in any of their oxidation states induced any exchange, but antimony, used as $SbCl_5$ led to yields and purities better than $CuCl_2$.

Further details of these studies and its possible reaction mechanism will be discussed.

(This work was supported, in part, by grant 5-P01-CA-14089).

No. 59

ON SOME RADIOHALOGEN DERIVATIVES OF CHOLESTEROL

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For studying the effect of the substituent and its position on biological properties of halogenated steroids, we have undertaken to prepare the following radiohalogen derivatives of cholesterol and to compare their behaviours in animal body: cholesteryl fluoride, bromide and iodide; 3-acetoxy-5-fluoro-6-hydroxy-cholestan, 3-acetoxy-5-hydroxy-6-fluoro-cholestan, and the corresponding bromine compounds; 19-iodo-cholesterol and 6-iodomethyl-19-nor-cholest-5[10]-en-3β-ol; and excitation-labelled ^{123}I-cholesterol and ^{77}Br-cholesterol. The labelled cholesteryl halides were prepared by the isotopic exchange and halogen interchange reactions. They showed slow clearances from blood, reflecting their hydrophobic character. The fluorohydrins, which were synthesized by the reaction of $B^{18}F_3$ on epoxide, demonstrated high adrenal concentrations shortly after i.v. injection. The excitation labelling in the $^{123}Xe \rightarrow ^{123}I$ decay gave an ^{123}I organic yield of about 50%, with its one third being of fairly stable iodosteroids. The ^{123}I-steroids, after being separated into two portions by TLC, was injected into mice; the adrenal concentration of radioiodine was found to be much lower than in the injection of 19-iodo-cholesterol and 6-iodomethyl-19-nor-cholest-5[10]-en-3β-ol, though no marked differences were observed in the distribution pattern over other organs. Similar labelling with ^{77}Br via the $^{77}Kr \rightarrow ^{77}Br$ decay gave poorer and less reproducible organic yields than in the ^{123}I labelling. In general, the carbon-fluorine bonds in the halogenated steroids have shown a much higher stability than the other carbon-halogen bonds. Making use of experiences and information obtained in the present study, we are going to prepare radiohalogen-labelled steroids of high hormonal activities.

No. 60

RADIOACTIVE 6β-HALOGENOMETHYL-19-NORCHOLEST-5(10)-en-3β-ol AS AN ADRENAL SCANNING AGENT

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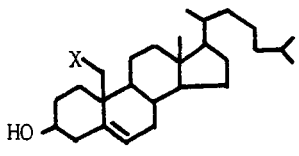
Our previous studies demonstrated that 6β-iodomethyl-19-norcholest-5(10)-en-3β-ol-I-131 (NCL-6-I-131) concentrates in rat adrenal gland ten times more than 19-iodocholest-5-en-3β-ol-I-131 (CL-19-I-131) which has been recognized as a clinically useful diagnostic agent for photoscanning of the adrenal gland. [M. Kojima, et al., J. Nucl. Med. 16 (1975) 666]. The finding has suggested that NCL-6-I-131 is more suitable as an adrenal-scanning agent than NCL-19-I-131, and the clinical diagnostic usefulness of NCL-6-I-131 for adrenal imaging has also been reported. [S. Fukui, et al., Clin. End. 23 (1975) 1051].

Furthermore, the structural requirement essential for adrenal uptake of NCL-6-I-131 was ascertained from the fact that rat adrenal accumulates four and eight times more 6β-methyl-19-norcholest-5(10)-en-3β-ol-^{3}H, which was the basic skeleton of NCL-6-I-131, than cholesterol-^{3}H at 3 and 7 days, and retains a higher concentration through 7 days. These results suggest strongly that the radioactive 6β-halogenomethyl analogues of NCL would be applicable to diagnosis as adrenal photoscanning agents.

In the case of iodine-131 which has been used so far, the reduction in radiation exposure that can be safely administered to the patient often restricts its application. Among the numerous radioactive iodine, iodine-123 is the most suitable nuclide for in-vivo studies, but it takes usually 5-7 days to make a clear image of adrenal gland with radioactive NCL-6-I; iodine-123 is not suitable for the purpose of the adrenal photoscanning because of its too short half-life ($T_{\frac{1}{2}}$ = 13.3 h).

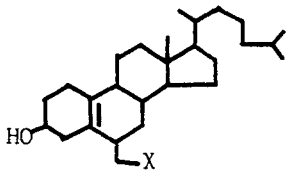
On the other hand, Bromine-77 ($T_{\frac{1}{2}}$ = 56 h) has convenient and not too short half-life, and its major γ-lines [239 KeV (30%) and 521 KeV (24%)] are suitable for detection with present-day γ-photoscanner. Thus we intended to prepare the 6β-bromomethyl analogue of NCL which would be readily applicable to the formation of a radioactive tracer.

With the knowledge of our earlier work concerning the formation of NCL-6-I, the initial step includes the preparation of 19-bromocholest-5-en-3β-ol (CL-19-Br). CL-19-Br was conveniently prepared by the nucleophilic displacement of cholest-5-en-3β,19-diol 19-toluene-p-sulfonate 3-acetate with LiBr in isopropanol followed selective hydrolysis of its 3-acetate with NaOH. Smooth conversion of NCL-6-Br was achieved by heating of CL-19-Br in AcOH or acetonitrile in good yield.



CL-19-I X=I

CL-19-Br X=Br



NCL-6-I X=I

NCL-6-Br X=Br

No. 61

A RADIOIODINATED BRETYLIUM ANALOG AS A POTENTIAL AGENT FOR IMAGING THE ADRENAL MEDULLA AND ASSOCIATED TUMORS

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While it is now possible to image the adrenal gland and diagnose adrenal cortical diseases with agents that selectively concentrate in the adrenal cortex (e.g. 19-iodocholesterol-^{131}I), attempts to find agents which concentrate in the adrenal medulla and which would be potentially useful in the diagnosis of neural crest tumors such as pheocromocytoma and neuroblastoma have been thus far unsuccessful.

Previous studies with isomeric radioiodinated bretylium analogs suggested that the p-isomer (p-RIBA) was capable of concentrating in adrenal medulla. Studies with radioiodinated and carbon-14 labelled material confirmed this property in both rats and dogs. High target-to-nontarget ratios were observed within two days and persisted for as long as 8 days. Studies are now in progress with p-RIBA-^{131}I to determine its potential as an adrenal medulla imaging agent. The synthesis and tissue distribution properties of this agent will be presented.

No. 62

RADIOIODINATED PYRIDINES - POTENTIAL ADRENOCORTICAL IMAGING AGENTS

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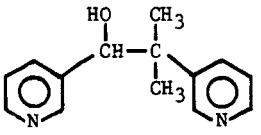
Many enzyme inhibitors possessing aniline or pyridine moieties are known to reversibly bind with enzymes of the adrenal cortex. A series of these ^{3}H-labeled inhibitors were recently synthesized in our laboratory and their tissue distribution determined in dogs. Metyrapol-^{3}H showed the highest uptake in the dog adrenal cortex (8.9% kg dose/gm at 1 hr); similar levels have been observed in the human adrenal cortex. Thus we have focused efforts on radioiodinating metyrapol (I) in hopes of obtaining a superior adrenal imaging agent.

Pyridines are highly resistant to electrophilic substitution and the presence of two unsymmetrical pyridine rings in metyrapol makes selective reaction with nucleophilic agents unfeasible. However, pyridine N-oxides are readily functionalized by a diverse number of reactions.

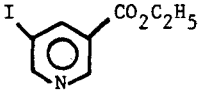
Ethyl nicotinate N-oxide (II) was adopted as a model for developing a radioiodination approach to metyrapol. Compound II was converted to 5-nitro-ethyl nicotinate by known methods. Consecutive treatment with PCl_3 and Pt/H_2 reduced both the N-oxide and the nitro group to give 5-amino-ethyl nicotinate (III). Reaction of the diazonium salt of III with NaI produced 5-iodo-ethyl nicotinate (IV). Compound IV failed to undergo exchange labeling with $Na^{125}I$ in refluxing ethanol at various acid pH's. However, the N-oxide of 5-iodo-ethyl nicotinate gave 10% exchange in refluxing ethanol after 20 hr. and 95% exchange in propylene glycol at 120° after 12 hr. The radioiodinated N-oxide was easily reduced to ^{125}I-5-iodo-ethyl nicotinate by treatment with Raney nickel/H_2.

Metyrapone was therefore selectively converted to its mono-N-oxide (V) by reaction with an equivalent of peracid. Treatment of V with p-$NO_2C_6H_4COCl/AgNO_3$ gave the 5-nitro-N-oxide derivative. Pt/H_2 reduction of the nitro group followed by diazotization in the presence of iodide yielded 5-iodo-metyrapone-mono-N-oxide (VI). Compound VI was exchange labeled with $Na^{125}I$ in propylene glycol at 135° in 80% radiochemical yield. Subsequent treatment with Raney nickel/H_2 gave ^{125}I-5-iodo-metyrapone (VII). Reaction of VII with LiH (O-Bu-t)$_3$ produced ^{125}I-5-iodo-metyrapol.

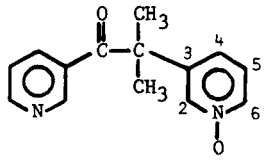
Nitration of metyrapone-mono-N-oxide (V) with HNO_3/H_2SO_4 at 125° gave the 4-nitro-mono-N-oxide analog. Catalytic reduction of the nitro group resulted in an intramolecular condensation reaction. However, treatment of the 4-nitro derivative with acetyl iodide at 50°C yielded 4-iodo-metyrapone-mono-N-oxide (VIII) directly. Exchange labeling of VIII is presently under investigation.



I



IV



V

No. 63

RADIOIODINATED ORGANOPOLYCATIONS AS POTENTIAL IMAGING AGENTS

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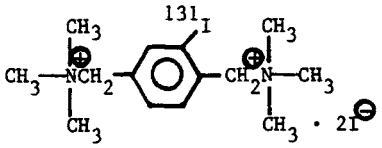
The ability of bisquaternary drugs such as hexamethonium and decamethonium to accumulate preferentially in cartilaginous tissues of the body suggested the use of such drugs as possible carriers for a γ-emitting nuclide. A radiopharmaceutical capable of localizing in cartilaginous tissue would have potential value in the diagnosis of abnormalities associated with the spinal column and joints.

Tissue distribution studies in rats with hexamethonium-^{14}C and a radioiodinated analog (NM-194) confirmed the ability of such polycations to bind to cartilage as well as other tissues having a high mucopolysaccharide content (e.g. meninges and lung). The relationship of this binding to the presence of polyanionic chondroitin sulfate will be discussed. Scans obtained in dogs with a ^{131}I-labeled analog will also be presented.



NM-194

No. 64

COMPACT CYCLOTRON PRODUCTION OF I-123 IODIDE FOR RADIOPHARMACEUTICAL SYNTHESIS

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I-123 labeled radiopharmaceuticals are desirable because they give a high flux of 159 KeV photons and a low dose of radiation per mCi. We have developed a procedure for the CS 22 compact cyclotron to produce 100 mCi batches of radiochemically pure I-123 iodide suitable for high yield labeling.

Twenty mg of aluminum powder mixed with 180 mg of 96% enriched Te-124 powder is pressed (20,000 psi) between two thin 1 inch diameter aluminum foils to insure good heat transfer during a 4 hr irradiation with 22 MeV protons at 25 μamp in the external beam of the cyclotron. Production yield is 125 mCi I-123 and 0.9 mCi I-124 at EOB. The elemental Te target which contains I-123 is dissolved in 25 ml 7 M sulfuric acid and 2 ml 30% hydrogen peroxide. Carrier iodide (4 μg) and water (25 ml) are added to insure distillation of more than 90% of the I-123 into a dilute NaOH trap. I-123 collected in the trap exists as a mixture of iodide and iodate; this iodate is reduced to iodide by the addition of thiosulfate prior to further steps. Aqueous iodide is readily obtained by thiosulfate reduction at pH 3. Anhydrous iodide is extracted from 4 N NaOH with methyl ethyl ketone. (The sulfuric acid solution of Te-124 is neutralized to 1 M acid. Te(VI) is reduced to Te(IV) with 5 g NaBr. Elemental Te suitable for pressing into new targets is precipitated with sulfur dioxide, washed and dried.)

These procedures for isolating radiochemically pure iodide are trouble free, convenient and reliable. The mCi yield is higher for Te-124 (p,2n) I-123 than for Te-122 (d,n) I-123. The Anger camera system gives acceptable spatial resolution when used with I-123 produced from Te-124. The cost of Te-124 is substantially less than the cost of Te-122. I-123 labeled o-iodohippurate and 16-iodo-9-hexadecenoic acid prepared from this I-123 iodide are now being used in clinical trials in humans.

No. 65

A NEW APPROACH TO TARGET CHEMISTRY FOR THE IODINE-123 PRODUCTION VIA THE $^{124}Te(p,2n)^{123}I$ REACTION

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A project was started to produce iodine-123 for local users on a routine scale with the AVF cyclotron of the Eindhoven University of Technology. Both the $^{123}Te(p,n)$ reaction and the $^{124}Te(p,2n)$ reaction may produce large quantities of iodine-123 in the energy range of the cyclotron. In order to enable routine production of about one Ci of iodine-123, we have formulated a set of criteria to be met with respect to target composition, target chemistry and radiochemical separation of the iodine:

a) Thermal and radiation stability of the target and target support.
b) Simple and almost complete separation of radioiodine from the tellurium within a short separation time.
c) Simple and almost complete reprocessing of the target.

We have aimed at a radiochemical separation of iodine from tellurium via a dry-distillation procedure and maintaining at the same time the integrity of the target. For this purpose we have studied tellurium dioxide as a possible target material. We found that heating of the tellurium dioxide just above its melting point (735°C) for only a few minutes, results in a release of more than 99.7 percent of the iodine.

A three oven separation system purifies the iodine-123 from tellurium and fluorine isotopes.

Since an oxygen containing atmosphere at 750°C and molten tellurium dioxide are rather corrosive, there is only a limited choice for a target support material. The total time required for irradiation, target handling, radiochemical separation and packing for shipment amounted to less than 3 hours.

Labeling experiments with the iodine-123 produced have been carried out and will be discussed.

No. 66

NEW EXCITATION FUNCTIONS FOR THE PRODUCTION OF MEDICALLY USEFUL HALOGEN RADIOISOTOPES

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Short-lived neutron-deficient radionuclides of halogens, such as ^{34m}Cl, $^{76,77}Br$ and ^{123}I are of great interest for labelling biomolecules and for producing radiopharmaceuticals. However, for an optimization of the procedures with respect to yields and impurities a knowledge of the relevant excitation functions is needed.

For the production of carrier-free ^{34m}Cl, the excitation function of the reaction $^{35}Cl(p,pn)^{34m}Cl$ was measured up to proton energies of 45 MeV. Szilard-Chalmers separation using K_2ReCl_6 was applied to obtain a carrier-free product.[1] Recoil yields of 15% were obtained. Taking this into account, the yield of carrier-free $^{34m}Cl^-$ lies in the same range as that from the other reactions, e.g. $^{32}S(\alpha,pn)^{34m}Cl$, giving rise directly to carrier-free ^{34m}Cl.

For the production of the $^{75-77}Br$ radioisotopes via their krypton precursors, the relevant excitation functions for $^{75-77}Kr$ production by high-energy deuteron activation of natural bromine up to deuteron energies of 90 MeV have been measured. A comparison with other known production methods is given with particular emphasis towards the adaptation to a routine production at a compact cyclotron.

Finally, the excitation functions of the α-particle induced reactions on natural iodine leading to the precursors of ^{123}I were measured up to α-energies of 170 MeV. The known low-energy data of these reactions[2] were completed to the more production-relevant energies above 100 MeV. Although the cross sections are more or less in the same range as for high-energy proton and deuteron activation, the production rate is lower because of the lower ranges of the α-particles in the target material.

References

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(2) R. M. Lambrecht, A. P. Wolf, F. Helus, M. Untucht, and W. Maier-Borst, Int. J. Appl. Radiat. Isotopes, in press.

No. 67

DEVELOPMENT OF A METHOD FOR PRODUCTION OF ^{123}I ON A LARGE SCALE USING THE (p,5n) REACTION AND A FLOWING LIQUID TARGET

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The Harwell Variable Energy Cyclotron is capable of producing an extracted 58 MeV proton beam of at least 30 μA. At this beam energy the best 123-I production route is via 123-Xe from 127-I by the (p,5n) reaction. We decided that the convenience of a flowing liquid target was such that we would develop the use of CH_2I_2, first tried by Lindner with low beam currents in 1973.

Our target system consisted of a thin window titanium cell through which the CH_2I_2 was pumped by a peristaltic pump, and a stripping section in which a flow of He removed the 123-Xe from the liquid. The gas stream passed through a CO_2 trap to remove entrained liquid and then through a glass nitrogen cooled trap to condense the Xe.

Early experiments with currents below 1 μA revealed no special problems other than those inherent in the use of a target liquid so corrosive that the only suitable construction materials are titanium, viton rubber and glass. However, once we started using higher currents, normally between 10 and 20 μA, we rapidly ran into major difficulties, of which the most important were:

1) The CH_2I_2 tended to polymerise, forming a viscous fluid which blocked the system.
2) Radiation induced chemical reactions occurring in the CH_2I_2 resulted in the formation of volatile organic and inorganic iodides which were not condensed in the CO_2 trap but were deposited in the nitrogen trap, causing blockages and contamination of the final product with 127-I. These reactions are dose rate dependent and so are not noticed at low currents.
3) The trapping efficiency of both the CO_2 and nitrogen traps was totally inadequate.

These problems were solved by the following means:

1) Dissolving elemental iodine in the CH_2I_2 prevents polymerisation and has the additional advantage of raising the density to 3.6 g/I/ml.
2) Introduction of a silver zeolite trap after the CO_2 trap, running at 150°C, removes all iodides from the gas stream.
3) Redesigning the CO_2 trap so that the copper coils are alternately cooled and then heated to 50°C greatly increases its efficiency.
4) Addition of 1% of Xe to the He carrier gas increases the trapping efficiency of the nitrogen trap to near 100%.

In two major series of trial runs we produced ^{123}I weekly for 8 weeks and delivered it to hospitals in the UK for patient trials. At the start of the first series we were only producing 30-40 mCi of 123-I at a rate of ∿ 2 mCi/μAh in a 2 hour run; by the end of the second series the total was more like 200 mCi and the production rate was ∿ 6 mCi/μAh. At the time of writing this abstract we are making major changes in the stripping section of the equipment and we will report on experiments with this in which we hope to raise the production rate still further. The radionuclidic purity of the 123-I has been consistently very high, ∿ 0.15% of 125-I by activity being the only detectable impurity.