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16.1

SYNTHESIS OF ORGANIC COMPOUNDS LABELED WITH POSITRON EMITTERS AND THE CARRIER PROBLEM‡

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In the past problems associated with the synthesis of compounds labeled with carbon-11, nitrogen-13, oxygen-15, fluorine-18, and a few other positron emitters to be used in biomedical experiments included the time course of the synthesis, chemical, radiochemical, radionuclidic purity, sterility, and apyrogenicity. More recently the question of specific activity has gained increasing prominence because of the use of labeled organic compounds in probing metabolic (1) and other biochemical pathways in vivo (2,3,4) and in developing agents for studying neuroleptic action and neuroanatomical mapping. The term carrier-free has not been used in its most rigorous sense and examples of direct measurement of specific activity where no carrier has been added are rare (5). It has been proposed that three terms be applied to radiopharmaceuticals (2), CF for Carrier-Free, NCA for No Carrier Added, CA for Carrier Added, and that the use of CF be restricted to compounds where experimental verification is available. It is clear that the vast majority of compounds listed in the literature as CF do in fact belong to the NCA class. A number of aspects of this question will be addressed. Primary is the source of contamination in the production phase, i.e. dilution of the desired radionuclide with stable nuclide. Choice of production method, quality control of target materials, and production line conditions will be discussed. Precursor preparation, if not on-line, becomes a second area for introduction of unwanted dilution. Third is the method of synthesis and the requirements determined by time course and yield question, and the use to which the compound is put. Finally, in vivo dilution by naturally occurring materials must be considered, especially if metabolism or catabolism is involved if one is to utilize the tracer effectively.

Another aspect of the tracer problem becomes apparent from the data in Table I listing the number of atoms one is dealing with in a true CF situation. One approach to the question has been the production of multi milliCurie or Curie amounts of product so that concentrations accessible for assay by modern analytical methods could be applied. Of great interest at present are new ultra-sensitive methods of analysis such as laser IR, laser optoacoustic spectrometry, and mass spectrometry. As yet these methods have not been applied to labeled compounds but the capability of precision assay of as little as 10^8-10^{10} molecules makes the experimental verification of carrier state a distinct possibility in a number of cases.

We must not lose site of the ultimate use of the tracer. On the one hand when dealing with brain receptor sites which range from about 0.01 to 0.5 picomoles/mg of protein (6×10^9 - 3×10^{11} sites) it can be seen that the concentration demands on the neuroleptics coupled with the detection capability of the instrumentation impose severe restrictions. However, in many other examples of tracer application the requirement that there be no detectable physiological response (i.e. drug action) from use of the radioactive compound is determining thus usually allowing greater latitude in the specific activity required.

The question of specific activity of compounds labeled with positron emitters has gained prominence primarily because of their application in neuroscience research. We can expect to see greater emphasis on this question and its relation to synthesis and use in the coming years.

‡Research carried out at Brookhaven National Laboratory under contract with the U. S. Department of Energy and supported by its Office of Basic Energy Sciences.

(1) M. Reivich, D. Kuhl, A. P. Wolf, J. Greenberg, M. Phelps, T. Ido, V. Casella, J. Fowler, E. Hoffman, A. Alavi, P. Som, and L. Sokoloff, Circ. Res. 44, 127-137 (1979).
(2) A. P. Wolf and J. Fowler, "Radiopharmaceuticals II", Proceedings 2nd Int. Symp. Radiopharm., Soc. Nucl. Med., N.Y. (1979), p. 73.
(3) M. J. Welch and T. J. Tewson, Ibid., p. 201.
(4) B. M. Gallagher, J. S. Fowler, R. R. MacGregor, R. M. Lambrecht, A. P. Wolf, E. J. Crawford, and M. E. Friedkin, "Principles of Pharmacology", CRC Press, Boca Raton, 1979, Vol. II, p. 135.
(5) cf. a. D. R. Christman, R. D. Finn, K. I. Karlstrom, and A. P. Wolf, Int. J. Appl. Radiat. Isot. 26, 435 (1975);
b. G. Berger, M. Maziere, J. Sastre, and D. Comar, private communication, (1978). $H^{11}CHO$.
c. M. Maziere, J. M. Godot, G. Berger, and D. Comar, J. Radioanal. Chem., in press, (1979). $^{11}CH_3I$.

TABLE I

Nuclide	Carbon-11	Carbon-14	Nitrogen-13	Oxygen-15	Fluorine-18
atoms/Ci	6.5×10^{13}	9.6×10^{21}	3.2×10^{13}	1.1×10^{12}	3.5×10^{14}

16.2

CARBON 11 LABELLED RADIOPHARMACEUTICALS FOR BRAIN RECEPTOR STUDIES

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One of the most exciting challenges of modern nuclear medicine is the technique by which brain specific receptors for neurotransmitters can be detected "in vivo". The association of drugs labelled with short-lived positron-emitting radioisotopes and positron emission tomography (PET) might in the near future demonstrate its potential to evaluate in human beings, in an atraumatic way, the amount of these receptors in the brain in various pathological states.

Receptors are defined as macromolecules having the function of recognizing an effector molecule in a specific manner. The binding between the receptor and the effector molecule or "ligand" induces the activation of a biological response. Specific receptors are characterized by their low density in biological tissues (10 to 100 pmoles of ligand bound per gram of tissue) and their low dissociation constant (10^{-9} to 10^{-10}M).

The differentiation between specific and non-specific binding sites, the latter being in high concentration with a high dissociation constant, is generally achieved by displacement of a radioactive ligand with a high dose (therapeutic dose) of cold material competing for the same receptors. Under these conditions, and if the specific activity of the radioactive ligand is high enough (in order to have a significant labelling of the receptor sites), the saturation effect will lead to a decrease in the radioactivity of the specific receptors, while non-specific receptors which are not saturable because of their high concentration, will still be labelled by the radioactive ligand. Furthermore, as specific binding sites are locally concentrated while non-specific sites are more diffusely distributed, this displacement should be observed only in those regions where specific receptors are present (1).

The design of a positron-emitter-labelled ligand has to fulfill a number of criteria in order to be usefull for human investigation. These criteria are related to the choice of the labelled agent, detection sensitivity, radiation dose, metabolism of the ligand and of the displacing agent.

1) The radioactive ligand has to be labelled with a positron emitter with a specific activity such that the concentration at the receptor site is at a maximum (of the order of magnitude of the dissociation constant of the receptor-ligand reaction) and the radioactivity high enough for PET detection. Usually, with the PET systems available today, the radioactive concentration at the site of the tissue of interest should be in the range of 0.1 to 0.5 μCi/g to be detected accurately. If the dissociation constant of the reaction is 10^{-9}M, the specific activity of the radiopharmaceutical should be in the range 100 to 500 Ci/mmole at the time of the measurement.

2) The radioactive ligand, which is generally intravenously injected, should reach the brain and cross the blood-brain barrier (BBB) freely in order to reach the receptor sites. This criterion is of importance since the higher the BBB permeability, the lower will be the required dose of radioactivity and the resulting radiation dose to the patient.

3) The choice of the labelling position of the radioactive ligand is generally imposed by the necessity of obtaining the pure product ready for injection in a time compatible with the use of short lived radioisotopes. Enzymatic degradation in vivo might affect the integrity of the ligand and release the radioactive isotope.

4) After displacement of the radioactive ligand by high doses of cold material, redistribution of the tracer taken up by peripheral tissues(liver,kidney,lung..)might modify the kinetics of the brain ligand-receptor reaction.

We have prepared three different ligands labelled with ^{11}C : for the study of opiate receptors, ^{11}C-etorphine ; for dopamine receptors, ^{11}C-pimozide and for benzodiazepine receptors, ^{11}C-Flunitrazepam (2).

All three were obtained in high radioactive yields : 20 to 100 mCi with specific activities ranging from 300 to 900 Ci/mmole at the time of use.

Flunitrazepam was obtained by $^{11}CH_3I$ methylation of the nor-derivative of etorphine by reductive methylation using $H^{11}CHO$, and pimozide by ring closure using $^{11}COCl_2$.

Animal distribution of ^{11}C-pimozide was investigated, showing a sufficient uptake by brain to encourage its use in humans. ^{11}C-Flunitrazepam specific binding in brain of baboons was demonstrated by positron emission tomography after displacement by Lorazepam. Other cold benzodiazepines failed to show displacement of flunitrazepam (Chlordiazepoxyde, clonazepam) in brain due to release of activity by other organs.

^{11}C-Flunitrazepam metabolism in humans was also studied, showing a distribution pattern which varied with the region and specific activity.

References :

(1) Neuro-transmitter Receptor Binding ; edited by H.I. Yamamura, S.J. Enna and M.J. Kuhar, Raven Press 1978.
(2) S.H.F.J. cyclotron Progress report 1979. C.E.A. France "In Press".

16.3

ULTRAMICRO-SCALE ORGANIC SYNTHESIS OF ^{11}C LABELLED MOLECULES FOR MEDICAL RESEARCH : A THEORETICAL AND PRACTICAL APPROACH

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It is often necessary to obtain ^{11}C labelled radiopharmaceuticals at very high specific radioactivities (> 1000 Ci/mmole). This is the case when toxic molecules have to be injected or when "in vivo" specific receptor binding is to be observed.

As the amount of labelled precursor which is handled is very small (concentrations often lower than M/1000) the organic synthesis is likely to be difficult or even impossible. This work deals with the study of the influence of the specific activity of the labelled precursor (A_1^*) on the synthesis yield of the radiopharmaceutical ($A_1^{*\prime}$) in the general equation

$$\nu_1 A_1^* + \nu_2 A_2 \rightleftharpoons \nu'_1 A'^*_1 + \nu'_2 A'_2$$

This yield $\dfrac{\text{radioactivity in } A_1^{*\prime} \text{ at equilibrium}}{\text{initial radioactivity introduced by } A_1^*}$ is calculated from the mass action law, when initial products, only present at the beginning of the synthesis, are not in stoechiometric proportions (1).

An increase in the precursor specific activity of A_1^* (which corresponds to a decrease of the mass of A_1^*) may result in an increase, a decrease or be without effect on the final yield of A'^*_1, according to the respective values of ν_1, ν_2, ν'_1, and ν'_2.

The following table shows for different types of reactions the evolution of the yield of labelling when the mass of A_1^* decreases, with A_2 remaining constant.

	$\nu_1 = 1$		$\nu_1 = 2$	
	Reaction	Yield	Reaction	Yield
$\Delta\nu = 0$	$A_1^* + A_2 \rightleftharpoons A'^*_1 + A'_2$ $A_1^* \rightleftharpoons A'^*_1$	$\nearrow 1$ $\rightarrow \neq 0$	$2A_1^* \rightleftharpoons A'^*_1 + A'_2$	$\rightarrow \neq 0$
$\Delta\nu = -1$	$A_1^* + A_2 \rightleftharpoons A'^*_1$	$\rightarrow \neq 0$	$2A_1^* + A_2 \rightleftharpoons A_1^{*\prime} + A'_2$ $2A_1^* \rightleftharpoons A'^*_1$	$\rightarrow \neq 0$ $\searrow 0$
$\Delta\nu = -2$	$A_1^* + 2A_2 \rightleftharpoons A'^*_1$	$\rightarrow \neq 0$	$2A_1^* + A_2 \rightleftharpoons A'^*_1$	$\searrow 0$

$\nearrow 1$: the yield tends towards 1 when A_1^* decreases.

$\rightarrow \neq 0$: the yield η tends towards a limit $0 < \eta < 1$, depending on the initial concentration of A_2, the mass constant coefficient and the solution volume.

$\searrow 0$: the yield tends towards 0.

$$\Delta\nu = \nu'_1 + \nu'_2 - \nu_1 - \nu_2$$

The kinetics of the reaction, also an important parameter since the half-life of ^{11}C is short (T = 20 mn) is often a complex function of the specific activity of A_1^* and can be determined only by experiment ; however, in some cases, the percentage of radioactivity incorporated per unit time is independent of the specific activity.

The influence of different parameters on equilibrium displacement, the importance of competing reactions and solvent impurities were also studied. This last parameter is of great importance since the amount of impurities in the solvent can be of the same order of magnitude as A_1^* and may interfere with the desired reaction.

These theoretical considerations were applied to the study of a number of compounds

$A_1^* + A_2 \rightleftharpoons A'^*_1 + A'_2$: methylation with ICH_3 of nor-derivative precursors (e.g. valium, flunitrazepam, methionine).

$A_1^* \rightleftharpoons A'^*_1$: $^*CH_3I$ synthesis using $^*CH_3OH$ and aqueous IH .

$A_1^* + A_2 \rightleftharpoons A'^*_1$: N-methylation with H^*CHO of nor-derivatives (chlorpromazine, imipramine).

$2A_1^* + A_2 \rightleftharpoons A'^*_1$: addition of $^*CH_3MgI$ on 3-3 ethylene dioxy-Δ^5 androstene 17-one (labelling of methyl testosterone).

$A_1^* + 2A_2 \rightleftharpoons A'^*_1$: acetone synthesis using $^*CO_2$ and $LiCH_3$.

The results of these syntheses will be discussed in detail.

(1) Emschwiller G., in Chimie Physique, Presses Universitaires de France, Paris (1951).

16.4

RADIOFLUORINATION WITH XENON DIFLUORIDE: L-6[F-18]FLUORO-DOPA

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The search is continuing for a simple and generally applicable method to synthesise fluoroaromatics with fluorine-18. Xenon difluoride (XeF_2) is known to fluorinate a wide variety of aromatic compounds rapidly and mildly (1). We have applied the XeF_2-method to the fluorination of L-dopa (L-3,4-dihydroxy-phenyl-alanine).

Radiofluorinated DL-dopa has already been shown to be a useful tracer for the investigation of the intracerebral dopamine metabolism in vivo (2). Also, it has been shown that the L-stereoisomer of [F-18]fluoro-dopa would be the preferred tracer (2).

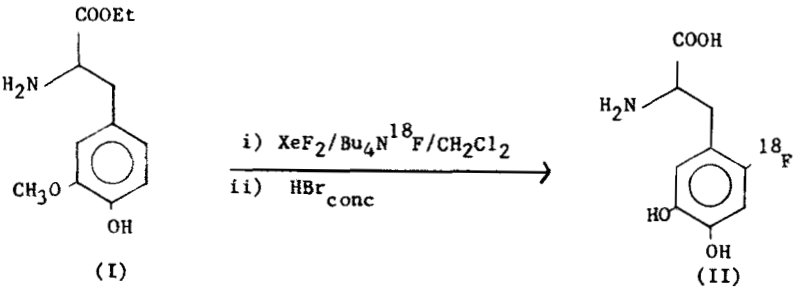
A derivative of L-dopa, L-3-methoxy-4-hydroxy-phenylalanine ethyl ester (I; 0.3 mmol) was reacted with XeF_2 (0.1 mmol) in methylene chloride at -60°C in vacuo for 15 min. The reaction product was hydrolysed immediately with 48% hydrobromic acid. The hydrolysate was chromatographed with HPLC (μBondapak/C-18, mobile phase: 0.1% acetic acid) and L-6-fluoro-dopa was separated. The chemical yield was 25% with respect to XeF_2. 6-Fluoro-dopa was identified by mass spectroscopy and ^{19}Fmr. In order to prove that 6-fluoro-dopa was in the L-form it was used as a substrate for the stereospecific enzyme L-aminoacid-decarboxylase (3). Over 90% of the L-6-fluoro-dopa was decarboxylated to 6-fluoro-dopamine.

The utility of this reaction for radiofluorinations was next investigated. The reaction mechanism of aromatic fluorination with XeF_2 predicts that fluoride, other than that from XeF_2 itself, can also enter the aromatic ring (1). [F-18]Tetrabutylammonium fluoride ($Bu_4N^{18}F$) was prepared from neutron-irradiated lithium carbonate (Scheme A). It served as F-18-donor. When then the fluorination reaction was carried out in the presence of $Bu_4N^{18}F$, L-6[F-18]fluoro-dopa (II; Scheme B) was obtained in radiochemical yields of <1%. The aromatic fluorination with XeF_2 follows a cationic as well as a radical mechanism simultaneously (1). The incorporation of [F-18]fluoride was the consequence of the radical mechanism operating. When experimental conditions were chosen to promote the radical route more F-18 was incorporated. No F-18 was incorporated when the radical reaction was quenched. Also, we found that this radical mechanism contributed very little to the formation of the fluoro-aromatic.

In order to achieve a useful radiochemical yield it is necessary to use proper [F-18]XeF_2 for both F-18-donor and fluorinating reagent. An important feature of this reaction is that chiral biomolecules can be radiofluorinated without racemisation.

A: neutron-irrad.$Li_2CO_3/^{18}F + H_2SO_4$ d $\xrightarrow{\text{distill}}$ $H_2O/H^{18}F$ $\xrightarrow[-H_2O]{+Bu_4NOH}$ $Bu_4N^{18}F$

B:



(I) (II)

(1) Filler R., Israel J. Chem., 17, 71 (1978).
(2) Garnett E.S., Firnau G., Nahmias C., Sood S. and Belbeck L., Am. J. Physiol., 238 (1980), in press.
(3) Firnau G., Garnett E.S., Sourkes T.L. and Missala K., Experientia (Basel), 31, 1254 (1975).

16.5

FLUORINE-18 FLUORINATION IN A CARRIER-FREE STATE BY CROWN ETHER

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In recent years synthesis of fluorine-18 labeled compounds were rapidly advanced with an interest in biological behavior and analogues as biological components in metabolism. Various method were developed in order to be suitable for use with fluorine-18. A large dose can not be administered to human in the case of meterials inducing intensely biological effect with a very small dose, such as hormones, vitamins and inhibitors, and positron camera needs appreciable radioactivity. It is an important subject to prepare fluorine-18 labeled compounds with high specific activity and high radioactivity in a practical application to nuclear medicine in vivo. Some preparation methods in a carrier-free state have been reported (1,2).

We have studied fluorine-18 fluorination by crown ether and potassium fluoride-^{18}F obtained from carrier potassium fluoride and aqueous fluorine-18 (3). Our experiments proved that there was limitation of specific activity in the previous method. We have developed carrier-free fluorine-18 labeling by the modified method using crown ether from aqueous fluorine-18. This method is as follows: potassium hydroxide and irradiated aqueous fluorine-18 were evaporated to dryness in a reaction vessel to give anhydrous potassium hydroxide containing carrier-free potassium fluoride-^{18}F, and this species was reacted with a substrate in a crown ether solution.

21-fluoroprogesterone-^{18}F was labeled in a carrier-free state, and several basic experiments were carried out in oder to achieve a good radiochemical yield in a short time. Evaporation to dryness of the potassium hydroxide solution containing carrier-free ^{18}F was done rapidly in a microwave oven and the specially designed vessel was used to stir effectively to solubilize fluorine-18.

It is the degree of solubilization of fluorine-18 that is the most important point to achieve high radiochemical yield. Therefore, solubilization of fluorine-18 activity from dried potassium hydroxide was examined under the condition of vauous solvents and different moles of the employed potassium hydroxide. These results showed solubilized fluorine-18 increased effectively when a teflon vessel was used instead of a glass one (Table I). A series of experiments of solubilization noted that percentage of solubilized fluorine-18 depended not only on moles of potassium hydroxide, but also on the total Coulomb value of irradiation and the employed volume of irradiated water; an increase in the latter value resulted in lowering solubilized fluorine-18 and an increase in the former value resulted in an opposite phenomenon.

Typical labeling was run using a teflon vessel under the following condition; 5μ moles of potassium hydroxide, 1 ml of 18-Crown-6 chloroform solution (100μmoles/ml), 40μmoles of mesylate of 21-hydroxypregn-4-ene-3, 20-dione were reacted at 60° for 2 hours. Radiometrical analysis of the reaction mixture by t.l.c. showed a good radiochemical yield (Table II).

This carrier-free labeling was also performed using aqueous fluorine-18 produced by the ^{18}O(p,n) ^{18}F nuclear reaction, in which case oxygen-18 water was recovered with a yield above 90%.

(1) Tewson T.J., Welch M.J. and Raiche M.E., J.Label. Compounde & Radiopharmaceuticals, 16, 10 (1979).
(2) De Kleijn J.P., Seetz J.F., Zawieko J.F. and Van Zanten B., Int. J. Appl. Radiat. Isotop., 28, 591 (1977).
(3) Irie T., Fukushi K., Ido T., Nozaki T. and Kashida Y., J. Label. Compounds & Radiopharmaceuticals, 16, 17 (1979).

Table I. Solubilization of the trapped ^{18}F in potassium hydroxide by crown ether solution

reaction vessel	solvent	added KOH (μ mole)	yield of solubilized ^{18}F (%)
glass	acetonitrile	1	4.4
		5	1.2
	benzene	1	0.7
		5	5.3
	chloroform	0	0.6
		1	1.6
		5	15.8
teflon	acetonitrile	1	16
		5	11.4
	benzene	1	48.6
		5	36.4
	chloroform	0	13.2
		1	71.1
		5	81.5

volume of crown ether solution: 1 ml (100μ moles/ml)
shaking for 2 hrs. at room temp.

Table II. Yield of carrier-free 21-fluoro-progesterone-^{18}F by potassium hydroxide-crown ether method

run no.	volume of irradiated water (ml)	Coulomb value of irradiation (mC)	^{18}F in reaction solution (%)	organic-^{18}F in solution (%)	final radiochemical yield (%)
1	15	1.86	26.8	1.05 # (3.9)	1.0
2	12	3.3	63.3	15.8 # (24.9)	10.8
3	18	3.9	46.1	20.5 # (44.5)	17.0
4	12	5.8	43.8	13.1 # (29.9)	9.4
5	12	2.7	55.3	25.1 # (45.4)	15.5

^{18}F production : $^{16}O(\alpha, pn)^{18}F$., added potassium hydroxide : 5μmoles
fraction of activity of organic layer obtained by washing out with water from the reaction solution

16.6

A novel method for the production of ^{11}C-phosgene.
Its use in the preparation of ^{11}C-labeled ethyl chloroformate,diethyl carbonate, diphenylurea, 5,5-diethyl barbiturate, 5,5-ethylphenyl barbiturate, ethylphenyl hydantoin and diphenyl hydantoin.

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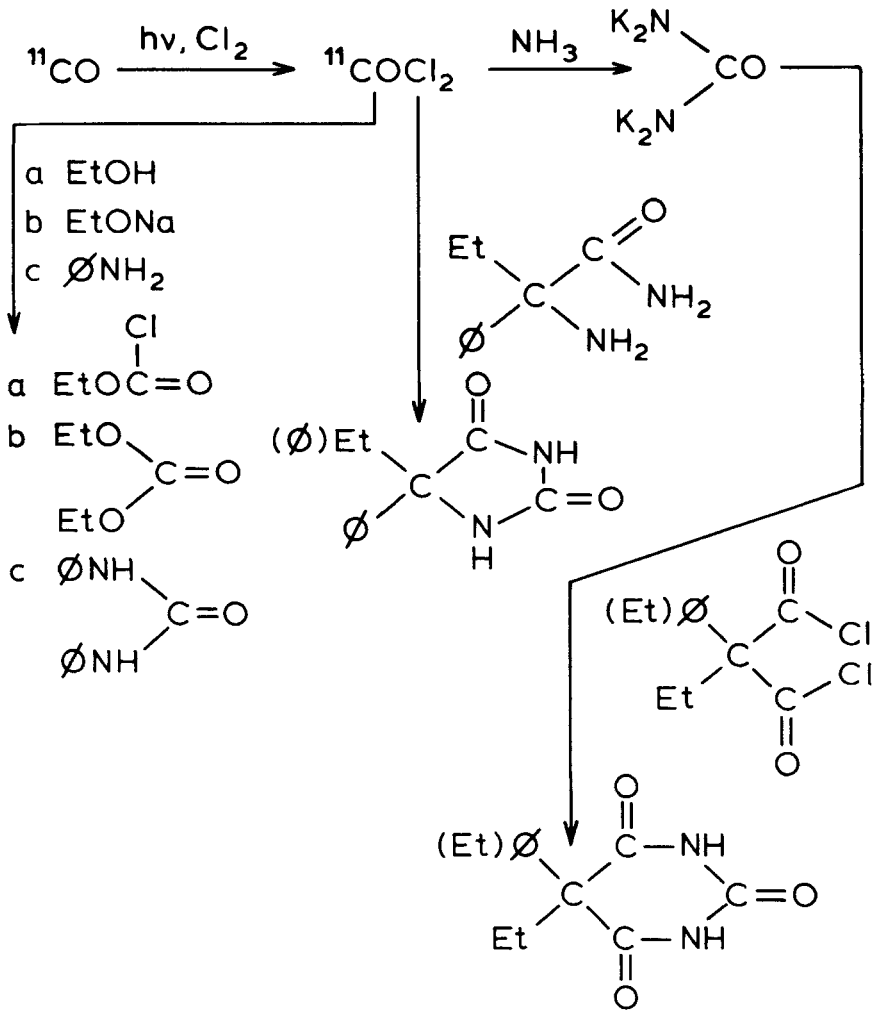
As reported earlier (1), ^{11}C-labeled phosgene ($^{11}COCl_2$) can be prepared from ^{11}CO using $PtCl_4$ as the chlorinating agent. Because from the $PtCl_4$ carrier carbon is introduced in the $^{11}COCl_2$ we adopted our flow system to the UV-light induced reaction of ^{11}CO with Cl_2 (2,3).
$^{11}CO_2$, transported in a flow of helium, is reduced in a Zn-oven,mixed with Cl_2-gas and led into a quartz spiral wound around a UV-lamp. Excess Cl_2 is removed by passing the gasstream through a bed of antimony. Thus $^{11}CO_2$ gives $^{11}COCl_2$ in 50% chemical yield within 10 minutes.
The $^{11}COCl_2$ was converted quantitatively into ^{11}C-labeled ethyl chloroformate, diethyl carbonate an diphenyl urea by leading the $^{11}COCl_2$ into ethanol (0^o), sodium ethanolate and aniline in toluene respectively.
The ^{11}C-labeled antiepileptic and hypnotic drugs 5,5-ethylphenyl barbiturate (luminal) and 5,5-diethyl barbiturate (veronal) were prepared from the corresponding malonic acid chlorides by adding a solution of these (1 μMole in pyridine) to ^{11}C-urea (prepared from $^{11}COCl_2$ by the method described previously (4)) and heating for 10 minutes at 150^o in a closed reaction vessel. The chemical yield after purification was 50–70%. Thus ^{11}C- luminal and veronal were prepared from $^{11}CO_2$ in about 60 minutes.
The ^{11}C-labeled hypnotic drug 5,5-ethylphenyl hydantoin (nirvanol) was prepared by reaction of $^{11}COCl_2$ with α-amino-α-phenyl butyric acid amide in xylene during 5 minutes at 140^o. After purification the chemical yield (with respect to $^{11}COCl_2$) was about 80%. The synthesis took about 40 minutes starting with $^{11}CO_2$.
Preliminary results on the preparation of 5,5-diphenyl hydantoin (phenytoin) by essentially the same procedure are promising.



1. D.Roeda, C.Crouzel, B. van Zanten, Radiochem. Radioanal. Letters 33(1978)175

2. M.Calvin, C.Heidelberger, J.C.Reid, B.M.Tolbert, P.E.Yankwich, Isotopic Carbon (Wiley, New York, 1949) page 155

3. G.A.Brinkman, I.Hass-Lisewska, J.Th.Veenboer, L.Lindner, Int. J. Appl. Radiation and Isotopes 29(1978)701

4. D.Roeda, C.Crouzel, P.J. van der Jagt, B. van Zanten, D. Comar, Submitted for publication to Int. J. Appl. Radiation and Isotopes

16.7

^{11}C-LABELED 1-AMINOCYCLOHEXANECARBOXYLIC ACID (^{11}C-ACHC), A POTENTIAL AGENT FOR STUDIES OF AMINO ACID TRANSPORT IN THE BRAIN

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We have previously reported the selective tumor uptake of the ^{11}C-labeled unnatural alicyclic α-amino acids, 1-aminocyclobutanecarboxylic acid (^{11}C-ACBC) (1) and 1-aminocyclopentanecarboxylic acid (^{11}C-ACPC) (2). The analogous agents, 1-aminocyclopropanecarboxylic acid and 1-aminocyclohexanecarboxylic acid (ACHC) showed much lower tumor specificities (3).

The brain uptakes of ^{14}C-ACBC, ^{14}C-ACPC, and ^{14}C-ACHC were compared and found to increase as the size of the alicyclic ring system increased. This seemed to be due to increased lipophilicity with increasing ring size and caused us to postulate that amino acids with larger alicyclic rings would have even higher brain uptakes. The ready availability of cyclic ketones with ring sizes of 7,8,9,10, and 12 carbon atoms led us to synthesize the ^{14}C-labeled alicyclic α-amino acids, 1-aminocycloheptanecarboxylic acid (ACHPC), 1-aminocyclooctanecarboxylic acid (ACOC), 1-aminocyclononanecarboxylic acid (ACNC), 1-aminocyclodecanecarboxylic acid (ACDC), and 1-aminocyclododecanecarboxylic acid (ACDDC) for determination of their tissue distributions in rats. These amino acids were synthesized by the Bücherer-Strecker technique, using 50% aqueous ethanol as solvent, a temperature of 110°C for both hydantoin formation and basic hydrolysis, and reaction times of 4 hr and 18 hr for the first and second step, respectively. Purification was by anion-exchange followed by cation-exchange chromatography (4). Chemical yields ranged from 17% to 73%. No attempts were made to optimize yields or to minimize production times, since our goal was to test the brain uptakes of the ^{14}C-labeled compounds.

Thin-layer chromatograms of each of these ^{14}C-labeled amino acids showed a single spot when visualized by use of a spark chamber immediately after synthesis. However, ACOC, ACNC, and especially ACDC showed gradual decomposition in solution, each yielding another ninhydrin-positive, zwitterionic compound, presumably an amino acid in which the ring had opened.

The eight ^{14}C-labeled alicyclic α-amino acids were each injected via the tail vein into 12-week-old male Fischer rats (4 animals per group). Each animal received 0.01 mmole (5 μCi) of ^{14}C-labeled amino acid per kg of body weight. At 30 min postinjection the rats were killed by exsanguination after light ether anesthesia. Samples of brain, blood, muscle, liver, spleen, kidney, lung, marrow, pancreas, small intestine, heart, and urine were removed. Weighed tissue samples were dissolved in NCS tissue solubilizer (Amersham/Searle Corp., Arlington Heights, Ill.) and assayed by liquid scintillation counting. Table 1 shows the tissue distribution of the compounds in four key tissues.

The hypothesis that increased ring size of alicyclic α-amino acids leads to increased brain uptake holds true only through ACHC. As the ring size is increased further, the uptake by brain then gradually falls off through ACDC and unexplainedly is near nil for ACDDC.

These studies indicate that ACHC has the greatest brain specificity of the alicyclic α-amino acids investigated and suggest that ^{11}C-ACHC may be of value in conjunction with positron emission computerized tomography as a tracer of amino acid transport in the brain. Neither ACBC or ACPC is metabolized. It is expected that ACHC will behave similarly and will remain intact in brain cells following transport through the plasma membrane subsequent to its passage across the blood brain barrier.

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Table 1

Effect of Ring Size on the Tissue Distribution of ^{14}C-Labeled Alicyclic α-Amino Acids in Male Fischer Rats at 30 Min Postinjection

Compound	Percent Administered Dose/g*			
	Brain	Blood	Muscle	Liver
ACBC	0.08 ± 0.00	0.33 ± 0.01	0.29 ± 0.01	0.66 ± 0.04
ACPC	0.23 ± 0.01	0.33 ± 0.00	0.38 ± 0.02	0.48 ± 0.03
ACHC	0.35 ± 0.02	0.42 ± 0.01	0.49 ± 0.02	0.40 ± 0.01
ACHPC	0.26 ± 0.03	0.34 ± 0.02	0.43 ± 0.05	0.34 ± 0.01
ACOC	0.29 ± 0.01	0.33 ± 0.01	0.42 ± 0.01	0.41 ± 0.02
ACNC	0.19 ± 0.01	0.28 ± 0.01	0.31 ± 0.01	0.40 ± 0.00
ACDC	0.13 ± 0.00	0.23 ± 0.01	0.24 ± 0.01	0.61 ± 0.02
ACDDC	0.01 ± 0.00	0.16 ± 0.01	0.18 ± 0.01	1.00 ± 0.09

* Average of 4 animals ± S.E.; normalized to a body weight of 250 g. (A uniform distribution thus would be 0.4%/g.)

(1) Washburn L.C., Sun T.T., Byrd B.L., Hayes R.L. and Butler T.A., J. nucl. Med. 20, 1055 (1979).

(2) Hayes R.L., Washburn L.C., Wieland B.W., Sun T.T., Turtle R.R., and Butler T.A., J. nucl. Med. 17, 748 (1976).

(3) Washburn L.C., Sun T.T., Anon J.B. and Hayes R.L. Cancer Res. 38, 2271 (1978).

(4) Washburn L.C., Sun T.T., Byrd B.L., Hayes R.L., Butler T.A. and Callahan A.P. "Radiopharmaceuticals II," proceedings of 2nd International Symposium on Radiopharmaceuticals, Soc. Nucl. Med., New York, 1979, p. 767.

16.8

ETORPHINE ^{11}C : A NEW TOOL FOR "IN VIVO" STUDY OF BRAIN OPIATES RECEPTORS.

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In order to study "in vivo" opiate receptors in animals and if possible in man, we have labelled etorphine, a morphine-like drug with Carbon 11.

Etorphine was chosen because this very potent narcotic agonist readily penetrates the B.B.B. (1) and is effective in very small doses and low total tissue concentration and consequently, the ratio between the amount of drug at receptor sites and at non specific sites may be more favourable (2). This point is very important for an "in vivo" study of opiate receptors by external detection with a positron camera.

Method of labelling : The method we used was derived from the Borch process (3) which we have routinely used to label other radiopharmaceuticals with Carbon 11 (4). Etorphine ^{11}C is obtained by reductive methylation of nor-etorphine by formaldehyde ^{11}C in acetonitrile in the presence of sodium cyanoborohydride.

Purification : The radiochemical and chemical purification of the labelled product was performed by H.P.L.C.

Materials : In order to avoid irradiation or contamination, all the manipulations were carried out semi-automatically in a closed lead-shielded cell (5). The H.P.L.C. was performed on a chromatograph (Waters Assoc.) with a 6000 A pump, U6K injector and a M 440 U.V. detector-Column Whatman Partisil Magnum 9 50 cm - Eluent = $CHCl_3$ 97 % + 3 % ethanol containing 1.5 % ethylamine and 2.5 % H_2O. Flow rate : 8 ml/min. λ 254 mn.

Experimental Formaldehyde ^{11}C (6) is trapped directly into a small conical tube cooled to - 5°C containing the nor- etorphine (1 µM) dissolved in acetonitrile (450 µl) with CH_3COOH (2 µl) and sodium cyanoborohydride (1 µM). The methylation of nor-etorphine is made by heating the hermetically closed tube to 70°C. For 5 minutes. After cooling the reaction mixture is injected into the chromatograph by air pressure introduced with a syringe. At the output of the column, the fraction corresponding to the chromatographic peak of etorphine is collected, under a current of nitrogen, in a pear-shaped tube heated in a water bath at 50°C in order to evaporate the eluent very quickly. The labelled residue corresponding to chemically and radiochemically pure etorphine ^{11}C is readily dissolved by 5 ml physiological saline buffered to pH 3.3 by sodium phosphate ($2.5\ 10^{-3}$M). The solution of etorphine ^{11}C is sterilized by remote control by filtration through a millipore membrane (0.22 µ) to an evacuated sterile flask using the pressure difference to accomplish the transfer.

Results : After chromatographic separation the labelled product is chemically and radiochemically pure and its identity is controled by H.P.L.C. compared with a standard of etorphine. The overall time for the preparation of etorphine-^{11}C ready to injection is 25 minutes (E.O.B). The chemical yield of methylation of nor-etorphine is close to 100 %. After a 30 minutes irradiation of nitrogen gas by 20 MeV protons at a 20 µA beam current, we generally obtain 80 MCi of injectable etorphine ^{11}C with a specific activity of 750 mCi/µmole. Under these conditions the quantity of etorphine which corresponds to 15-30 mCi, required for an "in vivo" study with the positron camera is 20-40 nanomoles (8 µg - 16 µg).

Conclusion : In view of the extremely interesting "in vitro" findings of this compound on rat brain homogenate using etorphine H^3 (3.3 Ci/mmol.) (7) etorphine labelled with carbon ^{11} should be a good ligand for studying "in vivo" opiate receptors by non-invasive means.

REFERENCES

(1) A. Goldstein - Life Sciences Vol 14 pp 615-623 (1974)
(2) C. Cerletti, L. Manara and T. Menini - Proceeding of the British and Italian P.S. 11th-13th september (1974)
(3) R.F. Borch, M.D. Berstein and H. Dupont-Durst - J. Am. Chem. Soc 93 2897-2904 (1971)
(4) G. Berger, M. Mazière, R. Knipper, C. Prenant and D. Comar. Int. J. Appl. Radiat. Isotopes 30 393-399 (1979)
(5) G. Berger, M. Mazière, J. Sastre and D. Comar. J. Label. Comp. and Radiopharm. vol XVII n° 1 (1980)
(6) M. Mazière, G. Berger, R. Masse, D. Plummer, D. Comar. "Electrophysiological Effects on Nicotine" A. Remond and C. Izard Ed. Elsevier Biomedical North-Holland Bromeddal Press pp 31-47 (1979)
(7) E.J. Simon, J.M. Hiller and I. Edelman. Proc. Nat. Acad. Sci., U.S.A. Vol 70 n°7 pp 1947-1949, July 1973.

16.9

[^{11}C]METHYL IODIDE IN THE SYNTHESIS OF ^{11}C-COMPOUNDS

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The usefulness of [^{11}C]methyl iodide and other labelled synthetic reagents in preparations of ^{11}C-compounds has aroused interest in the development of systems for the routine production of such compounds (1).

Our interest has been focused on the synthesis of [^{11}C]methyl iodide according to (2). By using this labelled alkyl halide (at a specific radioactivity of ca. 20 mCi (740 MBq)/μmol), we have carried out various alkylation reactions.

Sulphur alkylation. Various sulphide anions in liquid ammonia were alkylated with this [^{11}C]methyl iodide, giving [^{11}C-methyl]-L-methionine (3) and the tripeptide Gly-[^{11}C-methyl]Met-Gly (Scheme I) (4). This peptide was chosen as a model in the development of the synthesis of some labelled neurohormones.

Nitrogen alkylation. The alkylation of various nitrogen nucleophiles by [^{11}C]methyl iodide has been used for preparing methylated tertiary amines and quaternary ammonium salts (5). Reductive alkylation using [^{11}C]formaldehyde has been the generally preferred method for the synthesis of methylated secondary amines. However, under certain conditions, the alkylation of primary amines can also be performed by use of [^{11}C]methyl iodide and of the appropriate amine precursors. The concentration ratio between the amine precursor and [^{11}C]methyl iodide was in these preparations greater than 10^2, and in none of these cases was the dialkylated product observed. An example of this

is our synthesis of N-[^{11}C]methylaniline shown in Scheme II (6).

C-C bond formation. [^{11}C]Methyl iodide has thus been used in Corey-House coupling reactions for the synthesis of methyl-containing aliphatic and aromatic hydrocarbons (7), e.g., in the synthesis of [1-^{11}C]undecane (Scheme III). Furthermore, [^{11}C]methyl iodide has been used in the preparation of [^{11}C-methyl]thymidine according to Scheme IV (6), as well as in the asymmetric synthesis of [3-^{11}C]alanine using a chiral isocyanoacetic ester in an ion-pair alkylation giving the L/D enantiomers of the amino acid in the ratio 75/25 (Scheme V) (8).

The various ^{11}C-syntheses outlined here have stimulated us to develop a completely automated production unit - now under construction - synthesizing [^{11}C]methyl iodide.

(1) Iwata R., Ido T., Saji H., Susuki K., Yoshikawa K., Tamate K. and Kasida Y. Int. J. Appl. Radiat. Isotop., 30, 194 (1979); Berger G., Maziere M., Knipper R., Prenant C. and Comar D., Int. J. Appl. Radiat. Isotop., 30, 393 (1979).

(2) Marazano C., Maziere M., Berger G. and Comar D., Int. J. Appl. Radiat. Isotop., 28, 49 (1977).

(3) Långström B. and Lundqvist H., Int. J. Appl. Radiat. Isotop., 27, 357 (1976).

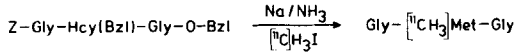
(4) Långström B., Sjöberg S. and Ragnarsson U., J. Lab. Comp. Radiopharmaceutical. (In press).

(5) Wolf A.P. and Redvanly C.S., Int. J. Appl. Radiat. Isotop., 28, 29 (1977) and references cited therein.

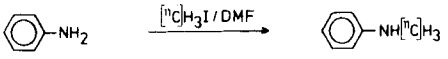
(6) Långström B., Thesis, University of Uppsala, 1980.

(7) Långström B. and Sjöberg S., J. Lab. Comp. Radiopharmaceutical. (In press).

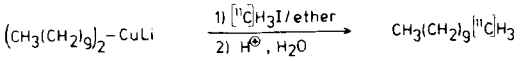
(8) Långström B. and Stridsberg B., Int. J. Appl. Radiat. Isotop. 30, 151 (1979).



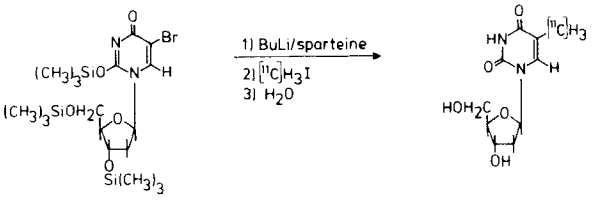
Scheme I



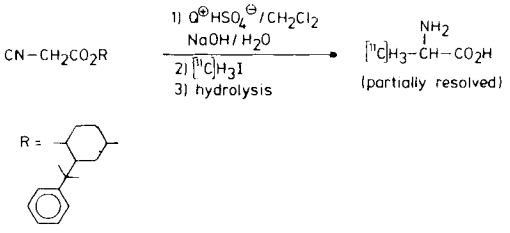
Scheme II



Scheme III



Scheme IV



Scheme V

16.10

NEW PATHWAYS TO AROMATIC FLUORINATION

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The introduction of ^{18}F into aromatic systems has only been successfully achieved in routine work by the Balz-Schiemann reaction (1). As this involves dilution with carrier, an inefficient use of fluorine and drastic conditions, other routes to fluorination were sought.

Modern electrophilic fluorinating agents (2) are impracticable (CF_3OF) and not regiospecific (3) (XeF_2, dilute F_2) and we sought a controlled and mild method for the generation of aryl cations in the presence of F^- and absence of radical inducing species (4), such as the nitrogen oxides, present in diazotisation reactions. The aryltriazenes (5) proved suitable and for many substrates, reaction of aryltriazene with HF-pyridine (Olah's reagent) provided a rapid (<5 min-0.5 h), mild (0^o-60^o) and efficient (60-95%) route to aryl fluorides (6). The general scope of the reaction has been elucidated and application to the synthesis of fluorinated aromatic amino-acids and fluoroestrone successfully achieved. The reaction can be run with stoicheiometric quantities of reactants and the pyridine is not, in principle, an essential component.

Developments in the synthesis of triazenes and alternative methods for their fluorinolysis will be discussed.

(1) A.J. Palmer, J.C. Clark, and R.W. Goulding, Internat. J. Appl. Radiat. Isotopes, 28, 53 (1977).
(2) M. Hudlicky, 'Chemistry of Organic Fluorine Compounds', 2nd edn., Ellis Horwood, Chichester, 1976.
(3) S.P. Anand, L.A. Quarterman, H.H. Hyman, K.G. Migliorese and R. Filler, J. Org. Chem., 40, 807 (1975).
(4) J.F. Bunnett and C. Yijima, J. Org. Chem., 42, 639 (1977).
(5) O. Wallach, Annalen, 235, 233 (1886).
(6) G.A. Olah, J.T. Welch, Y.D. Vankar, M. Nojima, I. Kerekes and J.A. Olah, J. Org. Chem., 44, 3872 (1979).

16.11

PREPARATION OF NO CARRIER ADDED ^{18}F-ARYL FLUORIDES: SCOPE AND CONDITIONS FOR THE REACTION

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We recently reported a high specific activity ($\sim 10^5$ Ci/mmol) synthesis of ^{18}F-aryl fluorides utilizing piperidyl triazenes as a source of anhydrous diazonium salts (1). It rapidly became apparent that the reaction was dependent on the nature of the triazene and that simple aromatic compounds were not necessarily suitable models for optimizing the yield for more complex molecules. The reaction has been performed on a variety of substituted aryl triazenes and while labeled aryl fluorides have been isolated in all cases, the yields vary from 0.5% to 50% of the available fluorine-18 activity.

The effects of the solvent and acid on the yield of haloperidol have been studied; these results are shown in Tables I and II. The effects of varying the concentration of reagents with the optimum acid and solvent are shown in Table III.

All these reactions were performed using fluorine-18 fluoride trapped on a silver wool-cesium carbonate plug. This suffers from several disadvantages. Firstly, the reaction of the cesium carbonate with the acid produces water, which can interfere with the reaction. Secondly, in the preparation of 3-deoxy-3-fluoroglucose (2) which utilizes the same target and trapping system, the reaction with $^{18}F^-$ was slower than that with $^{19}F^-$ because the washoff of the activity from the silver wool is the rate determining step. In the triazene decomposition reaction it is probable that the diazonium salt is completely decomposed before most of the activity has gone into solution. When the activity is trapped on a cold surface and then washed off with bromobenzene/sulphonic acid solution the yield expressed as the radioactivity in solution to that in the product is raised to 15% although this has little effect at present on production yields as the wash off procedure is inefficient. We anticipate that a target system which would allow the ^{18}F-HF to be added directly to the reaction solution (3) rather than intermediate trapping would result in overall production yields of 15% or better.

When benzene or bromobenzene are used as a solvent some labeled fluorobenzene or bromofluorobenzene is produced. These products are not formed when no triazene is added to the reaction mixture. These data are consistent with the diazonium salt decomposing to a transient and non-selective electron deficient aryl cation which can react with a fluoride ion if it encounters one, abstract a hydride or bromide ion from the solvent or react with any other nucleophiles present (Scheme I).

(1) Tewson T.J. and Welch M.J., J.C.S. Chem. Comm. 1149 (1979).
(2) Tewson T.J., Welch M.J. and Raichle M.E., J. Nucl. Med. 19, 1339 (1979).
(3) Winchell H.S., Wells D.K., Lamb J.F. and Beauchy S.B., U.S. Patent No. 501, 557 (1974).

TABLE I

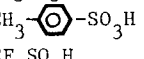
Reaction of Haloperidol Precursor with $Cs^{18}F$ and Methane Sulphonic Acid

Solvent	^{18}F-Haloperidol Yield*
THF	<0.1%
DME	<0.1%
Diglyme	0.1%
HMPA	NONE
DMF	NONE
Acetic Acid	<0.1%
Trifluoroacetic Acid	<0.1%
Benzene	0.7%
Bromobenzene	1.3%
Nitrobenzene	NONE
Methylene Chloride	0.3%
Acetonitrile	<0.1%

*Reaction times and temperatures were adjusted to give >95% decomposition of the diazonium salt.

TABLE II

Reaction of Haloperidol Precursor with $Cs^{18}F$ in Bromobenzene with Different Acids at 120-130°C

Acid	^{18}F-Haloperidol Yield
CH_3SO_3H	1.3%
	1.6%
CF_3SO_3H	0.15%*
CH_3CO_2H	0.1%
CF_3CO_2H	0.1%
H_2SO_4	<0.1%

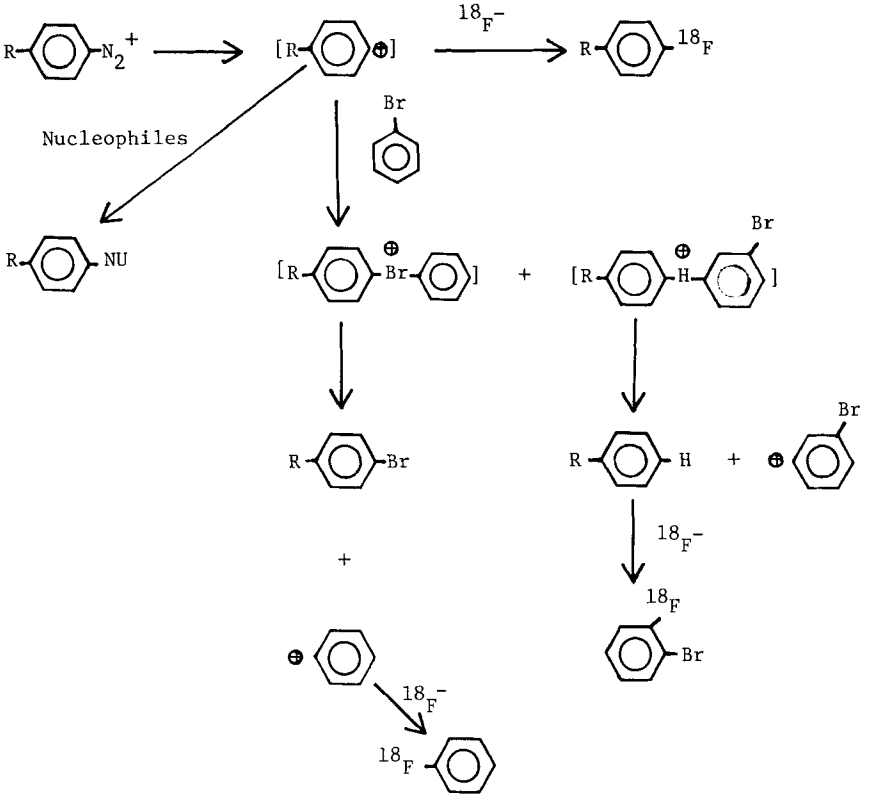
*Trifluoromethane sulphonic acid rapidly chars haloperidol in bromobenzene solution.

TABLE III

Reaction of the Haloperidol Precursor with $Cs^{18}F$ and Methane Sulphonic Acid in Bromobenzene at Various Concentrations

VOLUME OF SOLVENT (mls)	AMOUNT OF TRIAZENE (mgs)*	^{18}F-HALOPERIDOL YIELD
1.5	1	<0.1%
1.5	30	0.4%
1.5	50	0.6%
1.5	120	1.3%
0.5	50	0.9%
35	120	0.3%

*Triazene: acid was maintained at 1:4 molar ratio



SCHEME I

16.12

NUCLEOPHILIC SUBSTITUTION WITH FLUORIDE: EFFECTS OF SOLVENT, TEMPERATURE, IONS, LEAVING GROUP AND WATER.

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Nucleophilic substitution with fluoride is one of the main routes to fluorinated organic compounds, but reaction times have generally been considered too long to allow efficient synthesis of F-18 compounds because of the short half-life (110 mins). In the last two years, however, high yield and high specific activity syntheses of F-18 3-deoxy-3-fluoro-D-glucose (3-FDG) (1) and 2-fluoroethanol (2) have been reported by Tewson and co-workers, who use as a reagent F-18 fluoride trapped on CsOH dried onto silver wool. We have adapted their synthesis of 3-FDG to use F-18 tetraethylammonium fluoride (TEA^+ fluoride) produced from aqueous F-18 made in a reactor (3). As an extension of this work, we decided to systematically study the effects of some reaction conditions on the rate of the first step in its synthesis, displacement of triflate (trifluoromethanesulphonate) by fluoride from 1,2-5,6-di-isopropylidene-3-O-trifyly-D-allofuranose. This investigation would be useful, we felt, in helping us to make rational choices of solvent, temperature, leaving group, counter-cation, etc., in designing syntheses of other F-18 compounds. Although the synthesis of 3-FDG works at the no-carrier added level (3), we added carrier fluoride to facilitate interpretation of the present studies. Other investigators, some reviewed by Palmer et al (4) and by De Kleijn (5), have used widely differing reaction conditions for substitutions with F-18 fluoride.

Standard conditions were: 10 μmol of the triflyl compound, 10 μmol of TEA^+ and 5 μmol of F-18 fluoride in 0.3 ml of solvent in a silli-vial (Pierce Chemical Company). The excess TEA^+ was balanced by approximately equimolar amounts of hydroxide and sulphate, which resulted from distillation of F-18 HF from sulphuric acid into TEA^+ hydroxide during work-up of the irradiated lithium carbonate (3). Solvents were distilled and stored over molecular sieves 4A.

Solvent and Temperature. In initial experiments with dimethylformamide (DMF) as solvent, we had to lower the temperature from the originally used 150° to room temperature (22°) before the rate was slow enough to measure conveniently. The effect on the rate of changing a particular condition could then be determined. Under standard conditions at 22° the reaction was 50% complete in about 30 minutes, the rate was not linear (determined from 5, 10, 20 and 40 minute time points).

Rates decreased in the order DMF > dimethylacetamide, dimethylsulphoxide, hexamethylphosphoric triamide > acetonitrile > tetrahydrofuran >> dioxane, toluene. There was a factor of 2-4 between DMF and acetonitrile and of about 10 between the latter and THF. Rates in dioxane and toluene were too slow to measure. However, at high temperatures (>80°), acetonitrile gave higher yields than DMF. This could be due to destruction of the triflyl compound in DMF, and not to a primary effect of temperature. In DMF at 150° even a slight excess of triflyl compound over TEA^+ decreased the yield markedly, probably because of formation of triflic acid leading to premature removal of the leaving groups.

Water. This was expected to lower reaction rates by hydrating fluoride and decreasing its nucleophilicity. Reactions in acetonitrile were almost stopped by addition of 30 μmol of water and rates were about halved by 10 μmol. We have not measured the amount of water which enters our reactions from flask walls, solvent and reactants. According to De Kleijn (6), TEA^+ dried in a vacuum dessicator is the dihydrate, so that at least 20 μmol of water may normally be present. Conceivably, some of the rate differences observed between solvents could be due to their differing ease of dehydration.

Cations. No differences in rate were found when TEA^+ in acetonitrile was replaced by tetramethylammonium or tetrabutylammonium. Previously (3), we were unsuccessful

in using Cs^+ instead of TEA^+ in DMF at 150°.

Anions. Five reactions were done for 15 minutes in 1 ml of refluxing acetonitrile with 50 µmol of the triflyl compound to determine effects of hydroxide and sulphate on the decay-corrected radiochemical yield: (a) with 50 µmol of TEA^+ fluoride the yield was 96%; (b) with an additional 100 µmol of TEA^+ fluoride the yield decreased as expected; (c) however, when 100 µmol of TEA^+ hydroxide was used (i.e. in addition to 50 µmol of the fluoride), the yield only dropped to 79%, indicating that hydroxide does not compete well with fluoride; (d) the yield with 50 µmol of TEA^+ sulphate was 52%, suggesting good competition of sulphate with fluoride, and (e) finally, when 50 µmol of TEA^+ sulphate plus an additional 100 µmol of the triflyl compound were added, the yield rose to 97%.

Leaving Group. When the triflyl leaving group was replaced by tosyl (toluene-p-sulphonyl), no measureable reaction occurred in 45 minutes using DMF at 150°. This was unexpected, although Bentley (7) has commented that tosyl is about 10,000 times less active than triflyl, this must surely depend on the substrate, and the tosyl group was used by Foster et al (8) in their original synthesis of (unlabeled) 3-FDG by this route. They reported a 69% yield in 10 hours in refluxing acetonitrile. We have not tried other leaving groups in this reaction. Christman et al (9) compared iodo, tosyl and mesyl (methanesulphonyl) groups for the synthesis of 6-deoxy-6-fluoro-D-galactose and concluded that tosyl gave the best results. However, we have found iodo to be superior to tosyl in a study of the syntheses of several simple alkyl fluorides. We have also found the latter reactions to proceed best in acetonitrile at 120-150° (higher temperatures and other solvents gave lower yields) and to be catalysed by silver oxide. No effect of the latter on the synthesis of 3-FDG could be demonstrated. Ingold (10) has discussed the mechanism of silver salf assisted nucleophilic substitution.

We conclude that, at least in the reaction of 1,2-5,6-diisopropylidene-3-O-triflyl-D-allofuranose; the use of tetramethyl, tetraethyl and tetrabutylammonium as counter cation gives identical reaction rates; the presence of anions other than fluoride is unimportant as long as the total anions are not in excess over the triflyl compound, and that at higher temperatures acetonitrile appears to be the best solvent.

(1) Tewson T.J., Welch M.J. and Raichle M.E., J. Nucl. Med. 19,1339 (1978).
(2) Tewson T.J. and Welch M.J., J. Nucl. Med. 20,663 (1979).
(3) Gatley S.J. and Shaughnessy W.J., Int. J. Appl. Radiat. Isotopes (in press).
(4) Palmer A.J., Clark J.C. and Goulding R.W., Int. J. Appl. Radiat. Isotopes 28,53 (1977).
(5) De Kleijn J.P., J. Fluorine Chem. 10,341 (1977).
(6) De Kleijn J.P., Ariaansz R.F. and Van Zanten B., Radiochem. Radioanal. Lett. 28, 257 (1977).
(7) Bentley T.W., Ann. Reports (D) 72,71 (1975).
(8) Foster A.B., Hems R. and Webber J.M., Carbohyd. Res. 5,292 (1967).
(9) Christman D.R., Orhanovic Z. and Wolf A.P., J. Label. Comp. 13,283 (1977).
(10) Ingold C.K., Structure and Mechanism in Organic Chemistry (2nd Ed.), Cornell University Press, Ithaca (1969).

16.13

NUCLEAR DATA FOR MEDICAL ISOTOPES

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The enormous growth in the amount of nuclear data measured, evaluated and then presented to the user in publications of a utility varying from essential to worthless over the past 20-30 years has mainly been due to the importance of nuclear energy. As a direct result of this, data related to nuclear power, such as neutron cross-sections and nuclear structure data for the fission products and actinides are, in the main, better organised and treated than are charged particle cross-sections and nuclear structure data for neutron deficient nuclides.

There is a world-wide structure of neutron data centres, organised by the International Atomic Energy Agency in Vienna and consisting of 4 International Data Centres and a network of interlocking national centres and data committees. The I.A.E.A. maintains a comprehensive bibliography (CINDA) of neutron cross-section data and the data centres are steadily expanding their files of nuclear structure data.

For a long time there was no real international organisation of non-neutron nuclear data and we have depended largely on un-coordinated efforts by many laboratories. These have given us such excellent publications as the Table of Isotopes, Nuclear Data Sheets, the Jülich Gamma-ray catalogue and the Karlsruhe charged particle cross-section data published in Landolt-Börnstein. The coverage has, however, been very patchy. In the last few years collaboration has improved markedly with the allocation of groups of mass numbers to laboratories all over the world for continuous nuclear structure data evaluation and with attempts by the I.A.E.A. to co-ordinate available effort in other parts of the field.

Fortunately, the nuclear data needed for the development of a new neutron deficient isotope for radiopharmaceutical use is fairly simple and quite readily available. We can divide the operation into two stages. In the first we make a list of possible nuclides and then examine their relevant nuclear structure properties. For this we need only:-

(a) A wall chart of the nuclides (Karlsruhe or Knolls Atomic Power Laboratory).
(b) Nuclear Data Sheets OR Table of Isotopes (7th Edition) OR Gamma Ray Catalogue Part II (U. Reus et al, G.S.I. Darmstadt Report 79-2 1979).

Having produced our short list, the second stage consists of finding a suitable production route. For this we need only:-

(c) I.N.I.S. Abstracts OR Bibliography of Integral Charged Particle Nuclear Data (Burrows and Bint, BNL-NCS-50640 1977) OR Landolt-Börnstein, Group 1, Vol. 5, Part b.
(d) Original publication OR Landolt-Börnstein, Group 1, Vol. 5, Part c.

Nuclear data can be treated as a subject of extreme complication and at great depth. The ordinary sporadic user is strongly advised to restrict his usage to simple publications like those mentioned above. If you want more detail - ask a specialist.

16.14

DEUTERON BEAM PENETRATION IN A NEON GAS TARGET FOR PRODUCING FLUORINE-18‡

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Lack of proportionality between radionuclide yield and particle beam current at a fixed entering energy in gas targets has been a common observation among researchers concerned with optimizing yield in a production mode, particularly for carbon-11, nitrogen-13, oxygen-15, and fluorine-18 compounds. These density reduction effects have been described relative to radiopharmaceutical production (1,2), and nuclear physics applications (3,4). There is a lack of information on the quantitation and mechanism of such density reduction. Thus, tedious trial and error approaches have been used in order to optimize a specific application. In order to better understand a particular system, deuteron interactions with neon have been studied.

Penetrations of a 10 cm target at entering energies of 5.4, 10.7, and 14.1 MeV, beam currents of 1, 5, 10, 15, and 20 μA, and pressures which produce thick and thin target yields were used. The method is rapid, not dependent on the measurement of radionuclide yields, and is useful for studying most important target gases.

The experimental target used duplicates the geometry of the $^{20}Ne(d,\alpha)^{18}F$ target used at the Brookhaven National Laboratory 60-inch cyclotron (2,5) with dimensions of 2.54 cm diameter by 10 cm long with a sandwiched entrance window of 220 mg/cm^2 aluminum followed by 24 mg/cm^2 nickel on the neon side. The test target differs from the production target in that the water-cooled body and window flange are aluminum instead of nickel, and a 3.45 mg/cm^2 aluminum exit window followed by a 1.3 mm gas gap and electrically-isolated water-cooled aluminum beam stop have been incorporated. The target body and gas gap are connected to a common source of neon pressure, which is solenoid controlled to permit rapid increase, decrease, or isolation. Target body and beam stop leads operate in two alternate modes: (a) connected together for measuring incident deuteron beam current with an electrometer, and (b) connected in series with a 300 volt D.C. power supply and an electrometer for measuring ion current produced in the gas gap by deuteron penetration.

Figure 1A shows voltage gap current as a function of neon pressure with the beam on. The break points where the gap current rises sharply is a pressure that will insure thick target conditions since the 1.1 MeV deuteron energy required to penetrate the exit window matches the $^{20}Ne(d,\alpha)^{18}F$ threshold. Parameters influencing the increase of penetration as pressure is lowered include: (a) spread in deuteron energy incident on the front window, (b) distribution of deuteron energy due to straggling in the windows and neon, and (c) radial and longitudinal density distribution in the beam strike.

Figure 1B is a linear plot of 1A which emphasizes that the break points signaling the onset of penetration are caused by approximately 0.1% of the deuteron beam. The 15 μA curve in 1B shows the fit of a computer model which separates and quantitates the three penetration parameters mentioned above. Important information obtained from the model calculations are: (a) verification that the shape and magnitude of the experimental curves can be accounted for by ionization in the voltage gap due to deuterons penetrating the exit window, and (b) quantification of the density reduction and its distribution.

Figure 1C combines the break point pressures described in 1A and 1B with measurements made at two additional energies, giving neon pressure required (beam on) for a 10 cm thick target at any desired deuteron energy and beam current combination.

The model was used to assess the effect of pressures below the break point on reducing the yield of $^{20}Ne(d,\alpha)^{18}F$ at 14.1 MeV entering energy and 15 μA beam current. Using the excitation function of Nozaki (6), results were 56% of theoretical yield at 370 psia and 86% at 480 psia. Corresponding results based on $^{18}F\text{-}F_2$ actually recovered from the production target were 50% and 85% (2). The 480 psia condition is routinely used, and is seen to be a good combination of achieving an acceptably high radionuclide yield with a minimum target surface area to enhance $^{18}F\text{-}F_2$ recovery efficiency. Using a model in which density reduction is due purely to thermal heating, the calculated temperatures at 480 psia are 307°C average and 388°C maximum in the beam strike, and 70°C average outside the beam strike.

Parameters identified for further study include beam optics, impurity gases, gas temperature measurements, pressure rise and fall rates, and flowing target gas. The basic method of penetration measurement can be extended to a wide variety of target gases, geometries, and operating conditions. The mechanism of density reduction in these studies has not been unequivocally identified.

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*Research Collaborator from Medical and Health Sciences Division, Oak Ridge Associated Universities, Oak Ridge, Tennessee, 37830, which operates under contract number DE-AC05-760R00033 with the U. S. Department of Energy, Office of Health and Environmental Research.

(1) Oselka, M., Gindler, J. E., Friedman, A. M., Int. J. Appl. Radiat. Isot. 28, 804 (1977).
(2) Casella, V. R., Ido, T., Wolf, A. P., Fowler, J. S., MacGregor, R. R., and Ruth, T. J., Anhydrous $^{18}F\text{-}F_2$ for Radiopharmaceutical Preparation, accepted for publication.
(3) Robertson, L. P., White, B. L., Friedman, K. L., Rev. Sci. Instr. 32, 1405 (1961).
(4) McDaniels, D. K., Berquist, I., Drake, D., and Martin, J. T., Nucl. Inst. Meth. 99, 77 (1972).
(5) Casella, V. R., Ido, T., Wolf, A. P., J. Label. Cmpds Radiopharm. XIII, 209 (1977), abstract.
(6) Nozaki, T., Iwamoto, M., and Ido, T., Int. J. Appl. Radiat. Isot. 25, 393 (1974).

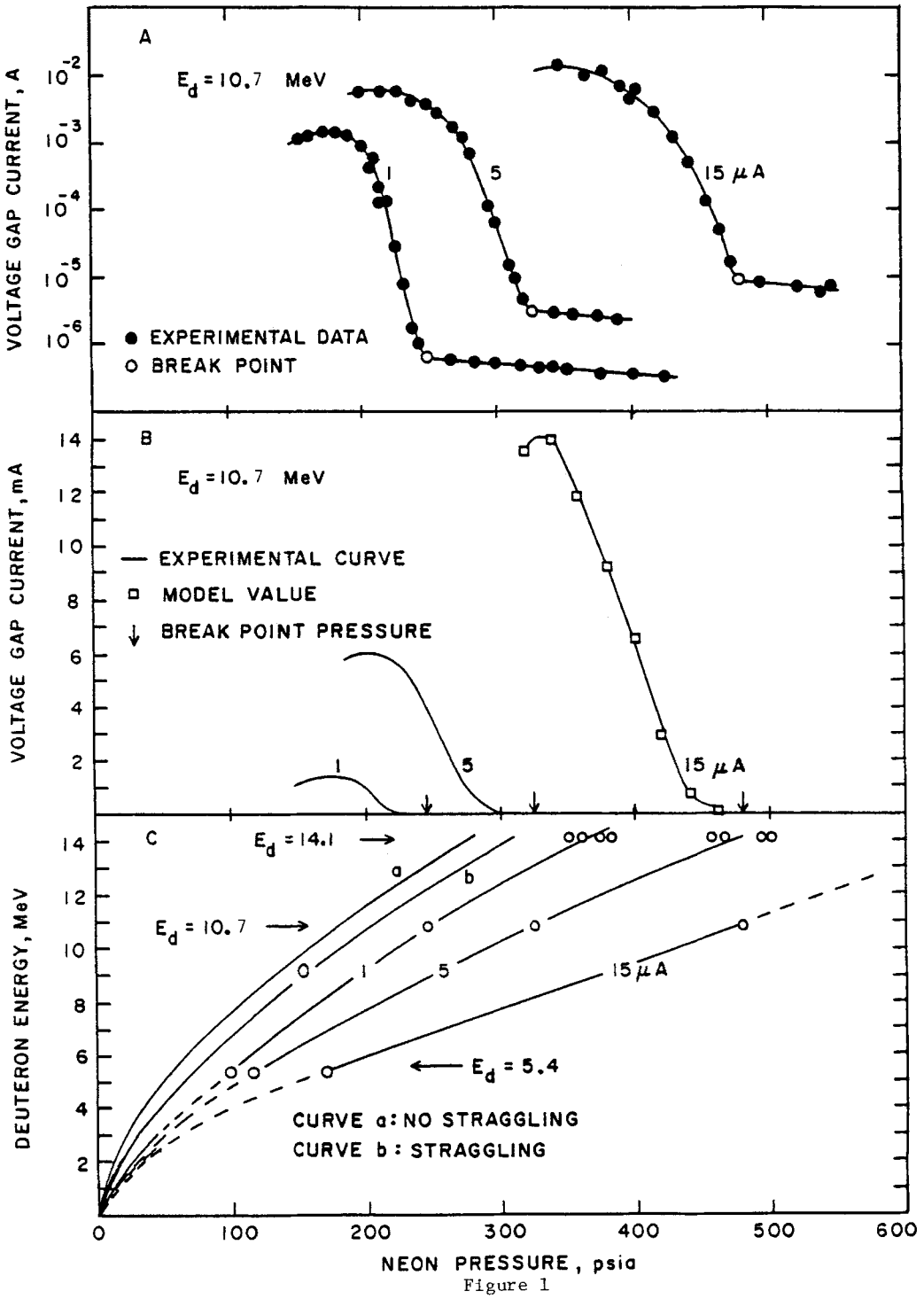
A
E_d = 10.7 MeV
1
5
15 μA
● EXPERIMENTAL DATA
○ BREAK POINT
VOLTAGE GAP CURRENT, A
B
E_d = 10.7 MeV
— EXPERIMENTAL CURVE
□ MODEL VALUE
↓ BREAK POINT PRESSURE
VOLTAGE GAP CURRENT, mA
C
E_d = 14.1
a
b
E_d = 10.7
E_d = 5.4
CURVE a: NO STRAGGLING
CURVE b: STRAGGLING
DEUTERON ENERGY, MeV
NEON PRESSURE, psia

Figure 1

16.15

CARRIERFREE LABELLING WITH RECOILING ^{18}F-ATOMS

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Without doubt cyclotrons are the predominant machines for the production of short lived positron emitting radionuclides and herewith labelled substances (1). At locations however, where there is only access to a linear accelerator in future it may turn out that these linearaccelerators will give help for usefull production of some radionuclides as well as some labelled compounds. So we have got under nonoptomized irradiation conditions out of a 20 gram sample of sodium hydroxide 0.5 mCi of a ready to use physiological saline solution of ^{18}F (2). ^{11}C-carbonmonoxide or carbondioxide can be produced by bombarding an appropriate target consisting of charcoal flushed with pure oxygen with high energy quanta. The yield is far more than 10 millicuries within few minutes.

By irradiation of monofluoro acetic acid (MFA) one amongst other radioactivities, free fluorine or fluoride e.g., some labelling of the target molecule, the MFA. Especially for this highly toxic substance the specific activities are far too low for medical use.

But if one irradiates a saturated solution of potassium fluoride in glacial acetic acid it turns out that some of the ^{18}F is to be found as radioactive MFA. This has been done using the n,2n-reactions (3). Our experience with the γ,n-reaction is demonstrated in figure 1 showing a TLC-separation.

Instead of potassium fluoride as fluorine donor one can use fluorinated paraffines, e.g. freons. Amongst other products you get radioactive MFA, too. This is shown in figure 2. With freon or similar substances one can mix other substances than acetic acid. We have done some experiments with longer chained fatty acids and amino acids. Especially the experiments with amino acids seem to be very promising. There are labelled products of biological interest, which are easy to separate from the target amino acid and the halogenated paraffin.

The specific activities of these newly formed ^{18}F-products are very high and, because of the possibility of starting with large amounts of target substance, some millicuries should be available (4). Preparative HPLC will additionally help to prepare such activities from a large target mixture. Most of the time needed for preparation belongs

therefor to the HPLC-separation and the following concentration.

(1) Wolf A.P. and Redvanly C.S., Int.J.Appl.Radiat.Isotop. 28, 29 (1977)
(2) Donnerhack A. and Sattler E.L., Int.J.Appl.Radiat.Isotop. in press
(3) Mantescu C., Genuche A., Simionescu L., Radiopharmaceuticals and Labelled Compounds, Proceedings of a Symposium, IAEA, Vienna, Vol. 1, p. 395 (1973)
(4) Donnerhack A. and Sattler E.L., Europ.J.Nuc.Med., in press

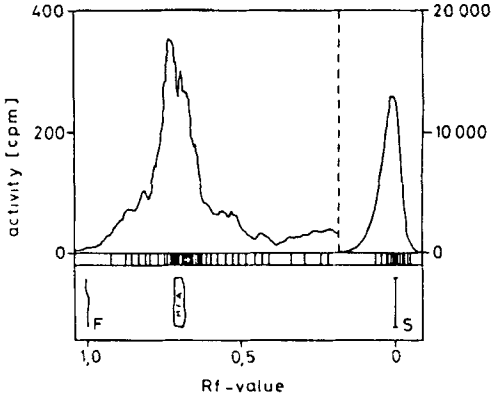

Fig. 1: TLC on silica of a KF/acetic acid mixture; solvent: ethanol, ammonia (26%) and water (80:4:16)

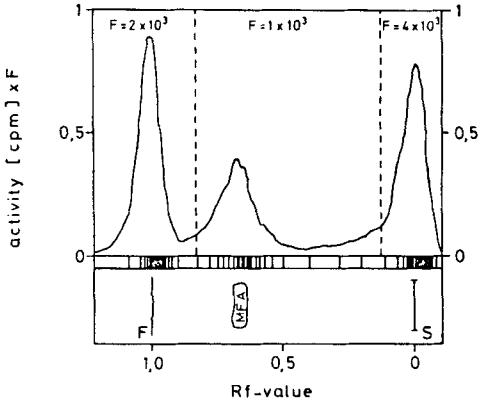

Fig. 2: TLC on silica of a Freon-11/acetic acid mixture; solvent: as fig. 1

16.15a

CARRIERFREE ^{11}C-RECOIL-LABELLING AT A LINAC

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There is no doubt that the predominant machine for the production of short lived positron emitting radionuclides and therefrom labelled compounds is the cyclotron (1). But it may turn out that under circumstances where instead of a cyclotron a linear accelerator is available, some radionuclides and labelled compounds can be produced in useful amounts. For example continous degasing during irradiation of a sample of alumina of some hundred gram delivers some millicuries of ^{15}O within minutes. Pulsing this system by interrupted flushing with a carrier gas leads to still higher activities.

Depending on the substance (chemical and physical form) high energy gamma irradiation brings about a more or less broad spectrum of labelled products. The original substance is labelled to a tiny degree by exchanging ^{12}C-atoms against ^{11}C-atoms. So one gets very low specific activities (2). But all other labelled products should be more or less carrier free depending on radiochemical "coproduction".

So despite the fact that in irradiated amino acids there is a relatively high retention of ^{11}C the achievable specific activity remains very low. This situation should be tolerable for turnover measurements of sugars but not of amino acids. However using sugars as target molecules one will never reach such high retention as with amino acids.

We have begun to analyse some radioactive products originating from the amino acid irradiation. A rapid separation method is HPLC. Recently the separation technique for amino acids has been improved (fig. 1). After HPLC-separation we analysed one fraction (fraction 8, fig. 1 right) by TLC and high voltage electrophoresis. TLC on silica is quite snother system than the reversed phase, which we used for HPLC. Electrophoresis is just another one. The results are given in figure 2. In RP-HPLC fraction 8 with the longer retention time should be a longer chained or more lipophilic molecule. According to the electrophoretic behavior the molecule has the attributes of an amino acid. It migrates like a cation in acid buffer. It is immobile in buffer where the normal amino acids are uncharged. It is not an amine, for these are mobile in the less acid buffer and much faster in the

acid one. In strong acidic electrophoresis the new substance moves more slowly than the target molecule. In this case, starting with valin, leucin displays the same behaviour. This product of obvious ^{11}C-addition is comparable to newly produced leucin or isoleucin. There is no ninhydrin-positive substance peak originating from measureable radiochemical formation. So, by irradiation an one gram sample of amino acid one should get 1 to 10 mCi of a carrier free substance, which migrates on electrophoresis like a neutral amino acid. Growth experiments with bacteria support this assumption. The preparation time lies within one half life of ^{11}C.

(1) Wolf A.P. and Redvenly C.S., Int.J.Appl.Radiat.Isotop. 28, 29 (1977)
(2) Trampisch W. and Sattler, E.L., Kerntechnik 18, 222 and 18, 307 (1976)

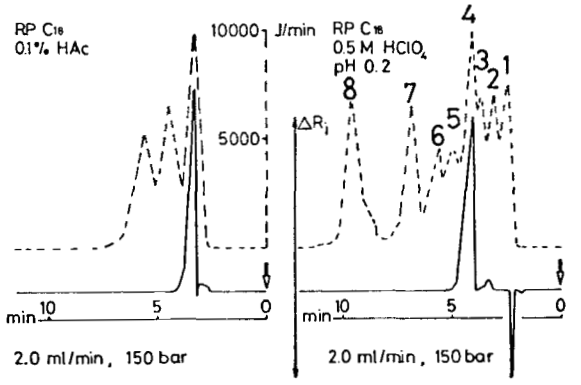

Fig. 1: Radio-HPLC of irradiated valine

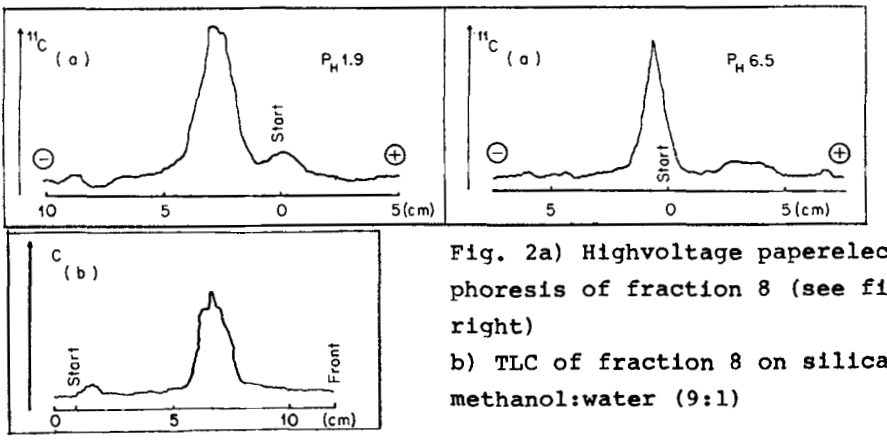

Fig. 2a) Highvoltage paperelectrophoresis of fraction 8 (see fig. 1 right)
b) TLC of fraction 8 on silica with methanol:water (9:1)

16.16

A NOVEL TARGET FOR THE PREPARATION OF ANHYDROUS $H^{18}F$ WITH NO ADDED CARRIER.

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An all copper target for irradiating neon with deuterons to produce $H^{18}F$ has reproducibly given yields of 10 mCi/μAmp EOSB (end of a saturation bombardment). The F-18 with no added carrier, is extracted from the target by purging with 10% hydrogen in helium (purge gas) while heating the target to 300°C. Seventy five percent of the total activity induced in the target is extracted. The target is not passivated.

Targets for the preparation of F-18 by the charged particle bombardment of neon have usually been made of nickel or monel metal (1,2). This choice is based on available data regarding the safe handling of fluorine and protection of apparatus against corrosion by passivation with fluorine. Available reports (3,4) indicate the absence of exchange between $H^{18}F$ and passivated metals at temperatures up to 500°C. The preparation of materials of biologic interest frequently demands high specific activities. For example, the use of ^{18}F-4 fluoroestradiol to study estrogen receptors requires the steroid not saturate the available binding sites. One curie of F-18 contains 3.5×10^{14} atoms of F-18, about 0.6 nmoles. Since the coating on the walls of a fluorine passivated target involves millimolar amounts of fluorine, losses as small as one part per million will isotopically dilute the F-18 produced. Therefore ultra high specific activity preparations will be difficult to obtain from a passivated target system. Preliminary experiments with an existing nickel target showed variable low amounts of F-18 released to the purge gas while heating the target to temperatures up to 500°C. Due to the lack of data regarding the retention of F-18 by metals and the difficulty of removing F-18 from the existing nickel target, experiments were undertaken to find which metals most readily released F-18 to the purge gas (5). A frame to hold metal foils was constructed so the foils were held in the neon out of the path of the beam. Foil strips (1 cm x 12 cm) were arranged on the frame so the surface was parallel to the beam axis. The frame was placed inside a nickel target tube closed at each end with nickel plates. The foils were treated by heating to 300°C while purging. After cooling, the target was filled with neon and irradiated with deuterons (~6 Mev on the neon). After irradiation, the neon was exhausted by purging while heating to a preselected temperature. Aluminum was found to retain more F-18 than any other tested material and was used to monitor the variable distribution of activity along the target axis. The foils were examined with a Ge(Li) detector to show they were not struck by the deuteron beam. A value of activity per unit area was derived to permit comparison of the F-18 retained by the various materials as shown in Table I. Based on these data copper was selected as the material of choice to contain neon for production of F-18 by charged particle bombardment. A prototype target was constructed from which 75% of the total activity induced in the target was recovered. This target was of all copper construction with exterior silver soldered joints. A 2.5×10^{-2} cm Cu foil silver soldered in place as the entrance window was subject to frequent failure. A new (Mod. 1) target was developed with an entrance window consisting of a 2.5 mm thick plate with a hand-lapped seal to the target tube to clamp the foil in place. Operation of the new target involves initial preparation by purging the target while heating to 320°C. The target is stored with purge gas until use. For irradiation it is filled with neon to a pressure of 1.2 ATM, closed, and irradiated in a static condition. After irradiation the target gas is exhausted to the atmosphere and the target purged while heating to 320°C. The purge gas passes directly from the target through a short insulated tube to a copper or polyethylene collection trap immersed in an ice-alcohol bath (-5°C) or liquid nitrogen. F-18 retained quantitatively in the trap distills out at about room temperature when the cooling is removed. KOH evaporated from methanol onto copper retains activity in the cooled trap. The F-18 activity on the KOH can be removed along with the KOH, by water or methanol. Polyethylene traps containing pyridine or N, N-dimethylformamide and cooled by immersion in liquid nitrogen also retain the activity. The activity is qunatitatively recovered with the solvent from the trap after the trap is allowed

to warm to room temperature. A sample of the activity trapped with pyridine followed a straight line decay for >1400 minutes with a half-life of 110.4 minutes. Examination of the entire samples from DMF, pyridine and KOH collections with a Ge(Li) detector and spectrometer showed no detectable radioactive impurities. The identity of the compound as $H^{18}F$ was confirmed by synthesizing ^{18}F-4-fluoroestrone methyl ether via a Schiemann type reaction (6) and ^{18}F-haloperidol via the diazonium fluoride salt. The identity of these products was established by thin-layer chromatography and mass spectroscopic analysis. Based on these syntheses and the distillation of the activity near room temperature, the form of the F-18 is identified as no carrier added $H^{18}F$. Table II shows the average performance of the target which has been used at sustained beam currents up to 20 μAmp. The Mod. 1 target has operated trouble free for over 15 consecutive cyclotron irradiations.

TABLE I

F-18 ACTIVITY RETAINED PER UNIT AREA FOR DIFFERENT PURGE TEMPERATURES

Arbitrary units normalized to aluminum = 100

Final Purge Temp.	Cu	Ni	W	Mo
Ambient	68	95	64	75
100°C	60	77	63	73
200°C	28	31	25	45
300°C	15	24	16	23

TABLE II

ALL COPPER TARGET PERFORMANCE (AVG.)

Target Type	Integrated Beam Cur.	Length of Irradiation	Yield mCi/μAmp EOSB
Prototype	1 μA hr.	1 hr.	8.4
Mod. 1	1 μA hr.	3/4 hr.	11.2
Mod. 1	5 μA hr.	1/2 hr.	11.1
Mod. 1	7.5 μA hr.	<1/2 hr.	9.8
		Average	10.1 ± 1.3

(1) Lambrecht, R.M., Neirinckx, R., Wolf, A.P. Int'l. J. Appl. Rad. & Isot. 29, 175-183, 1978.
(2) Straatman, M.G., and Welch, M.J. J. Nucl. Med. 18, 151-158, 1979.
(3) Rogers, M.T., and Katz, J.J. JACS 74, 1375-1377, 1952.
(4) Boggs, J.E., Van Artsdalen, E.R., and Brossi, E.R. JACS 77, 6505-6506, 1955.
(5) Dahl, J.R., and Tilbury, R.S. Abstract #381, 31st S.E. Regional ACS Meeting, Roanoke, Va., Oct. 24-26, 1979.
(6) Schmall, B., Tilbury, R.S. and Nisselbaum, J.S. The synthesis of ^{18}F labeled 4-fluoroestradiol and haloperidol for studies in nuclear medicine, ACS 4th Winter Fluorine Conf., Daytano Beach, Fla., Jan. 29-Feb. 2, 1979. (Abstract).

16.17

NITROGEN - 13 HOT ATOM REACTIONS APPLIED TO AMPHETAMINE SYNTHESIS

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The chemical products, resulting from the interaction of N-13 atoms produced via the $^{16}O\ (p,\alpha)^{13}N$ nuclear reaction on a water target, are believed to be a complex function of radiation chemistry and hot atom chemistry (1,2,3). The products include nitrogen-13 labelled nitrate, nitrites and ammonia. Utilizing this nuclear reaction, coupled with a catalytic reduction of the nitrogen-13 moities, reliably high yields of "no carrier added" nitrogen-13 labelled ammonia are available for synthetic radiopharmaceutical preparations.

The application of hot atom products to the synthesis of nitrogen-13 labelled compounds of potential clinical utility will be presented. A relatively simple procedure for the preparation of amphetamine and nitrous oxide labelled with nitrogen-13 produced via the reaction of protons with oxygen or water, will be detailed.

1. Krohn, K.A. and Parks, N.J., Second Intern. Symposium on Radiopharmaceutical Chemistry, St. Catherine's College, Oxford, 3-7 July, 1978.
2. Tilbury, R.S., Dahl, J.R. and Marano, S.J., First Intern. Symposium on Radiopharmaceutical Chemistry, Brookhaven National Laboratory, New York, 21-24 September, 1976.
3. Krohn, K.A., Ninth Intern. Hot Atom Chemistry Symposium, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, 18-23 September, 1977.

* Research carried out at Brookhaven National Laboratory under contract with the U.S. Department of Energy and supported by its Divisions of Basic Energy Science and Biomedical and Environmental Research and PHS Grants NINCDS 9 R01 NS15380 and Mt. Sinai Medical Center by NINCDS 1 P01 NS 15639.

A1

QUANTITATIVE LYMPHOSCINTIGRAPHY. RADIOPHARMACOLOGICAL AND KINETIC STUDIES OF VARIOUS COLLOIDS IN THE PARASTERNAL LYMPH NODES IN RABBITS.

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The status of the lymphatic system is usually investigated by contrast lymphography. In some cases, however, such a procedure cannot be used because of the lack of suitable lymph vessels. Thus it is not possible to examine the lymph flow from the primary tumor site of a malignant melanoma or in the parasternal lymphatics in breast carcinoma. Moreover, it is not possible to perform quantitative dynamic studies with this method. Some clinical contraindications also limit its use.
The search for complementary and alternative methods has led to the use of a number of compounds labeled with radionuclides for lymphoscintigraphy (1). In the search for compounds suitable for scintillation camera imaging giving small absorbed doses, substances labeled with Tc-99m have been explored (2,3,4).
The aim of the present investigation was to set up an experimental model for lymphoscintigraphic studies, in which the radiochemical and biokinetic characteristics of different compounds could be compared.
Experiments were performed on rabbits. The animal was fixed in supine position in a special made coach. The colloid in a total volume of 0.5 ml was injected bilaterally just below the xiphoid process. With a scintillation camera sequential images were taken every 15 s after the injection and stored on magnetic tape. Regions of interest of the lymph nodes were selected in the image and time activity curves were generated.
The sample to be analyzed was applied at the top of a column filled with Sepharose^{R} 4B and developed with 10.0 ml 0.9% NaCl (5,6). Thereafter the column which still retains all the radioactivity was scanned with a slit collimated NaI(Tl)-crystal detector. The radioactive zones as shown in Figure 1 give information on the size distribution of the labeled colloid. The scanning profiles for some $^{99}Tc^{m}$-colloid kits commercially available and for some colloids prepared by ourselves were obtained.
To estimate the flow rate of the colloid into the lymph nodes an open two compartment model was used to evaluate the time activity curves. In the model the rate constant k_1, represents the uptake to the parasternal lymph nodes, whereas the rate constant k_2 indicates the disappearance from the injection site to other parts of the body. Results are given in Figure 2. The most rapid uptake show the ^{198}Au and $^{99}Tc^{m}Sb_2S_3$ colloids also with the smallest out flow to other organs.
The percentage uptake in the parasternal lymph nodes was calculated at different times after the injection. At 4 hours the uptake values differ between 0.5% to 9% with highest values for the ^{198}Au and $^{99}Tc^{m}Sb_2S_3$ colloids.
From the activity profiles of the Sepharose^{R} 4B columns the distance of mean activity in the column was calculated. In Figure 3 below the percentage uptake at two hours after injection is plotted as function of this distance. There is a strong dependence of the uptake in the lymph nodes and the mean activity particle size. With the ^{198}Au-colloid of 5 nm particle size the highest uptake values are registered. Both larger and smaller particles reduced the uptake (7).
Previous studies in patients using large particle $^{99}Tc^{m}$-sulphur colloid showed lack of correlation between uptake in the lymph nodes and the histopathology (4). However, by using $^{99}Tc^{m}$-antimony sulphide colloid a better correlation is achieved. This type of studies has up to now found to be of great value in the staging procedure of patients with malignant melanoma (8).

(1) Zum Winkel K., "Lymphologie mit Radionukliden", Berlin, Verlag Hildegard Hoffman, 1972.
(2) Ege G.N., Internal mammary lymphoscintigraphy. The rationale, technique, interpretation and clinical application: A review based on 848 cases. Radiology 118:101-107, 1976.
(3) Ege G.N., Internal mammary lymphoscintigraphy in breast carcinoma: A study of 1072 patients. Int. J. Radiat. Oncol. Biol. Phys. 2:755-761, 1977.
(4) Aspegren K., Strand S.E. and Persson R.B.R., Quantitative lymphoscintigraphy for detection of metastases to the internal mammary lymph nodes. Biokinetics of $^{99}Tc^{m}$-sulfur colloid uptake and correlation with microscopy. Acta Radiol. Oncol.

17:17-26, 1978.
(5) Persson R.B.R. and Strand S.E., Labelling processes and short-term biodynamical behaviour of different types of $^{99}Tc^{m}$-labelled complexes. In Proceeding of Symposium on New Developments in Radiopharmaceutical and Labelled Compounds. Copenhagen, IAEA, Vienna 1973, pp. 169-188.
(6) Persson R.B.R., Strand S.E. and Knöös T., Radioanalytical studies of ^{99m}Tc-labelled colloids and macromolecules with gel chromatography column scanning technique. J. Radioanal. Chem. 43:275-286, 1978.
(7) Strand S.E. and Persson R.B.R., Quantitative lymphoscintigraphy I. Basic concepts for optimal uptake of radiocolloids in the parasternal lymph nodes of rabbits. J. Nucl. Med. 20:1038-1046, 1979.
(8) Jönsson P.E., Strand S.E., Bergqvist L., Hafström L.O. and Persson R.B.R., Quantitative lymphoscintigraphy II. Imaging technique, dosimetry and histopathology in patients with malignant melanoma. To be published.

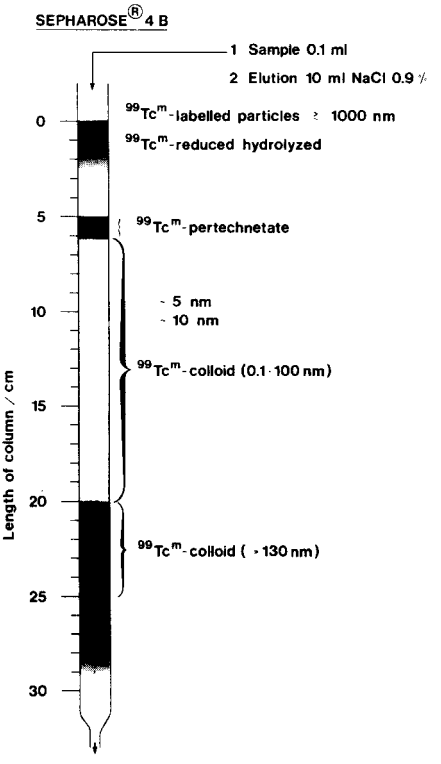

Figure 1. Activity-size distributions obtainable with GCS technique on columns filled with Sepharose 4B.

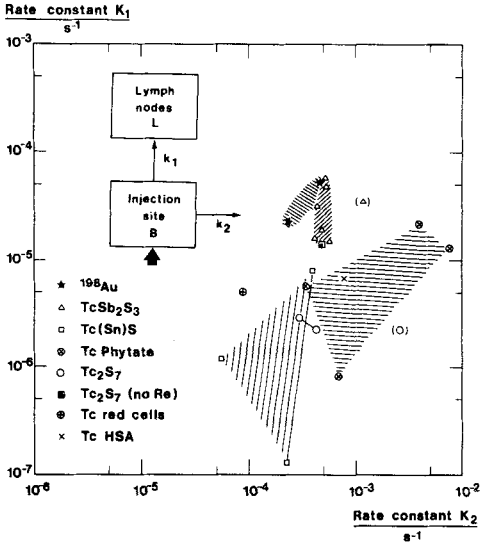

Figure 2. Scatter diagram of the rate constants k_1 and k_2 obtained from the two-compartment analysis of the time-activity curves for the parasternal lymph nodes.

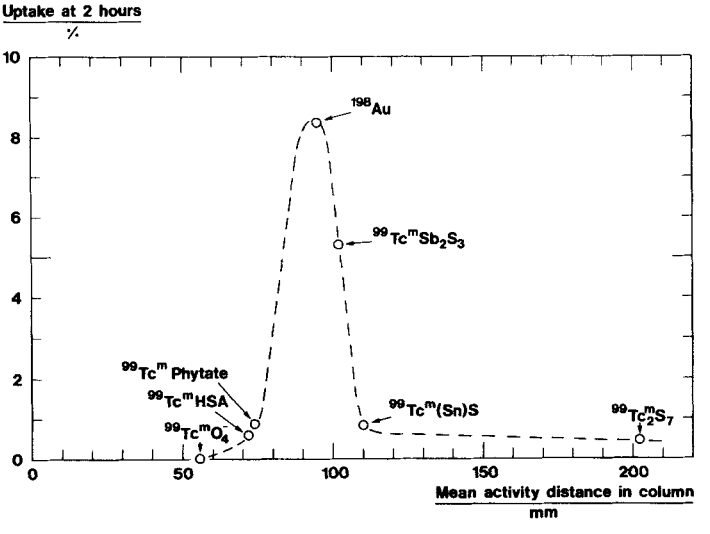

Figure 3. The percentage uptake 2 hours after injection as a function of the mean activity-size distance in the column for the different compounds investigated.

A2

HEXAAQUORUTHENIUM(II) - A REACTIVE INTERMEDIATE FOR PREPARATION OF RADIOPHARMACEUTICALS

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The most stable ruthenium coordination complexes are formed from +2 and +3 oxidation states. As part of a program to investigate potential synthetic routes to ruthenium-97 radiopharmaceuticals, the Ru^{+2} state was examined (1). Hexaaquoruthenium(II) was chosen for the study because it is reactive and can be prepared (2-4) from either $RuCl_3$ or other ruthenium compounds like RuO_4. It should be possible to prepare desired ruthenium-103 chelates from the hexaaquo complex by ligand substitution of the labile water molecules. Such a possibility was investigated, and this paper describes the preparation of high specific activity hexaaquoruthenium-103(II) complex and its successful conversion to a stable tris (bipyridyl) ruthenium(II) complex. Ru-103 isotope was used because of the convenience offered by its long half-life (39.6 days).

High specific activity (6mCi/mg) $^{103}RuCl_3$ was refluxed with mercury in the presence of a non-complexing acid (HBF_4) to form the chloropentaaquoruthenium complex (see reaction scheme).

$$^{103}RuCl_3 + Hg \xrightarrow[\text{Reflux}]{HBF_4} [Ru(H_2O)_5Cl]^{2+} + Hg_2Cl_2\downarrow$$

$$[Ru(H_2O)_5Cl]^{2+} \xrightarrow{\text{Zn-Hg (or } H_2/Pt)} [Ru(H_2O)_6]^{2+} + Cl^-$$

$$[Ru(H_2O)_6]^{2+} \xrightarrow[-AgCl]{1.\ Ag^+} [Ru(H_2O)_6]^{3+} \xrightarrow{2.\ O_2}$$

Reduction of the chloropentaaquoruthenium complex with either zinc amalgam or by catalytic hydrogenation gave the reactive hexaaquoruthenium(II) complex. After precipitation of the chloride ions as silver chloride, it was oxidized by oxygen to the more stable Ru(III) complex for purification on an ion-exchange (Dowex AG 50W x2) column. The pure hexaaquoruthenium(III) complex was eluted from the column with 2M fluoboric acid and characterized by comparison of its UV spectrum with that published in the literature (3). The yield of pure product was 40% based on $^{103}RuCl_3$.

The hexaaquoruthenium(III) fluoborate was then quantitatively reduced by H_2/Pt to the +2 state and reacted with excess 2,2'-bipyridine at pH 4.0. The 2,2'-bipyridine ligand was chosen because of the ability of heteroaromatic nitrogen atoms to form very stable ruthenium(II) complexes (5). The resulting tris(bipyridyl)ruthenium(II) complex was purified by ion-exchange chromatography, then characterized by comparison of its UV spectrum (6) and its retention time in HPLC with that of a non-radioactive sample prepared independently by a different method (5). Radiochemical purity of the complex was determined to be greater than 95% by HPLC. In vivo behavior of the complex in mice was determined (see Table 1) which was markedly different from that of $RuCl_3$ (7).

This work establishes a route to prepare ruthenium chelates such as tris (bipyridyl) ruthenium(II) fluoborate from the title compound.

(1) Subramanyam V., Pratt F.P., Mysliwy, T., et al, "Use of Chloropentaammine-ruthenium(III) Dichloride for Preparation of Radiopharmaceuticals" (manuscript in preparation). Ruthenium +3 state was examined
(2) Mercer E.E. and Buckley R.R., Inorg. Chem., 4, 1692 (1965).
(3) Cady H.H. and Cannick R.E., J. Am. Chem. Soc., 80, 2646 (1958).
(4) Kaller T.W. and Earley J.E., Inorg. Chem., 10, 1149 (1971).
(5) Burstall F.H., J. Chem. Soc., 173 (1936).
(6) Paris J.P. and Brandt W.W., J. Am. Chem. Soc., 81, 5001 (1959).
(7) Srivastava S.C., Richards P., Meinken G.E., Som, P., Atkins H.L., et al, "Radiopharmaceuticals II", Second International Symposium on Radiopharmaceuticals, Seattle, Washington, March 1979.

TABLE 1
Biodistribution of $[Ru(bipy)_3]Cl_2$ in Mice

Organ	Percent Injected Dose Per Organ* Time in Hours 0.25	1	4	24
Blood†	1.2 ± 1.4	0.1 ± 0.0	0.1 ± 0.0	0.0
Liver	28.3 ± 5.4	29.6 ± 5.1	29.4 ± 3.0	21.4 ± 4.3
Kidneys	38.7 ± 4.9	41.2 ± 4.1	32.1 ± 0.5	25.0 ± 8.2
Carcass & Head	19.4 ± 17.3	11.1 ± 9.2	0.0 ± 0.0	0.0
Intestines‡	6.9 ± 1.1	9.6 ± 1.2	6.2 ± 2.7	1.2 ± 0.3
Urine	21.9 ± 4.0	20.6 ± 11.0	4.1 ± 5.9	0.3 ± 0.5

* Mean ± std. dev. of three mice.
† Total blood volume considered to be 5% of body weight.
‡ Includes contents.

A3

ACETYL-METALLOCENES-LABELLING, ORGAN-DISTRIBUTION, BIOCHEMISTRY

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Acetyl-Metallocenes were labelled with ^{103}Ru and ^{191}Os by exchanging the central atom of acetylferrocene (1) and with ^{3}H by exchange in Tritium-water.
The metabolism of acetyl-[Ru^{103}]-ruthenocene was studied in mice and rats. It was hydroxylated in the side chain and excreted as the corresponding glucuronide. Acetyl-ruthenocene and the hydroxycompound were accumulated in adrenals as measured by organ-distribution (2) and confirmed by autoradiography. Micro-autoradiography shows a high concentration in the zone glomerulosa of the adrenals, which corresponds to the corticoid synthezising tissne.
Since the injection of $^{103}RuCl_3$ to mice showed a very different organ-distribution, there must be a distinct affinity of the metallocene molecule itself for the tissue. This conclusion was confirmed by measuring the organ-distribution of double labelled ruthenocen derivatives ($^{3}H/^{103}Ru$ or $^{14}C/^{103}Ru$).
In conctrast to the affinity of acetylruthenocene to adrenals no similar high affinity of acetyl-[^{191}Os]-osmocene could be detected, although the stability of osmocene under biological conditions was better than the other metallocenes tested:

osmocene > ruthenocene > ferrocene

1) D. Langheim, M. Wenzel and E. Nipper,
Chem. Berichte 108, 146 (1975).

2) A. Taylor, J. Macha and M. Wenzel,
J. Nucl. Med. 21, 63 (1980).

A4

STRUCTURE-DISTRIBUTION STUDY OF I-125-ω-IODO FATTY ACIDS

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Radioiodinated fatty acids have been evaluated for use as myocardial imaging agents. Two major disadvantages for clinical use lie in 1) short myocardial $T_{\frac{1}{2}}$ and 2) high blood activity levels. Both of these problems may be related to rapid β-oxidation of the fatty acids by the myocardium and liver. There are several approaches to decreasing the rate of β-oxidation, three of which are reported here.

One approach is to modify the carbon skeleton to promote storage as triglycerides (TG). Two *in vivo* studies offer clear support for the TG storage of erucic acid (22 carbon chain) in the myocardium up to 30 min. after injection (1,2). Several radioiodinated fatty acids of varying chain length [$I(CH_2)_nCO_2H$,n=18,21 and 26] were synthesized by a Li_2CuCl_4 catalyzed coupling of ω-olefinic Grignards with magnesium chloride salts of ω-bromo fatty acids. Addition of HBr in the presence of benzoyl peroxide and I-125-iodide exchange in refluxing methylethyl ketone gave the final products. Shorter fatty acids (n=10,12 and 15) were prepared by radioiodide displacement of commercially available ω-bromo fatty acids. Table I lists heart and blood concentrations at various time intervals. The initial myocardial uptakes for n=18 and 21 are approximately three-fold higher than for n=15 (I). The myocardial $T_{\frac{1}{2}}$ for n=18 and 21 are doubled relative to I and the heart-to-blood ratios are slightly higher.

A second approach is to manipulate the carbon skeleton to directly inhibit β-oxidation. Dimethyl substitution at both α and β positions has been shown to inhibit β-oxidation (3,4). Inhibition of β-hydroxyacyl dehydrogenase is theoretically affected by placement of a substituent at the β or incipient β position of fatty acids. Two such compounds, 13-iodo-3-methyltridecanoic acid (II) and 16-bromo-9,10-methylenehexadecanoic acid (III) were synthesized and evaluated. Compound II was synthesized via a malonic ester condensation using 2-bromo-11-dodecene. Deesterification and decarboxylation were followed by HBr addition and radioiodide displacement as described above. Compound III was obtained from a Simmons-Smith addition to 16-bromo-9,10-hexadecenoic acid ester followed by hydrolysis and radioiodide displacement. Myocardial concentrations for II are nearly 30% lower than for I but values for III are approximately 20% greater (0.42 for III vs. 0.36 for I at 10 min). No significant increase in myocardial $T_{\frac{1}{2}}$ was observed for II and III.

A third approach involving mechanism based irreversible inhibition, specifically suicide inhibition of acyl dehydrogenase or enoyl-CoA-isomerase, has been briefly explored. Bloch has shown suicide inhibition of E. Coli dehydrase with 3-decynoic acid (5). Compound IV, 14-iodo-9-tetradecynoic acid, was synthesized via condensation of ω-bromooctanoic acid with the dilithio derivative of hexyn-6-ol. The resulting alcohol was converted to the respective iodide by tosylation of the acid ester, iodide displacement and deesterification to IV. The concentration of IV by the dog myocardium was comparable to I at 5 and 20 min. but no increase in myocardial $T_{\frac{1}{2}}$ was observed. Longer chain alkynoic acids are under investigation.

TABLE I
HEART(H) AND BLOOD(B) CONCENTRATIONS*
FOR I-125- $I(CH_2)_nCO_2H$ IN RATS

TIME(MIN)	5		10		20		40	
n	H	B	H	B	H	B	H	B
10	.35	.21	.14	.18	.10	.17	-	-
12	.30	.18	.26	.14	.21	.17	-	-
15	.29	.08	.43	.10	.33	.09	-	-
18	1.05	.17	.76	.16	.67	.16	.70	.14
21	.79	.10	.50	.15	.47	.15	.34	.14
26	.25	.57	.21	.39	.14	.33	-	-

*Concentrations expressed in %Kg dose/g

1. Vasdev, S.C. and Kako, K.J., J. Mol. Cell Card., 9, 617 (1977).
2. Ong, N., Bezard, J., and LeCerf, J., Lipids, 12, 563 (1977).
3. Tryding, N. and Westöö, G., Acta Chem. Scand., 10, 1234 (1956).
4. Goodman, D.S. and Steinberg, D., J. Biol. Chem., 233, 1066 (1958).
5. Helmkamp, G.M. Jr., Rando, R.R., Brock, J.H. and Bloch, K., J. Biol. Chem., 243, 3229 (1968).

A5

POTENTIAL RADIOPHARMACEUTICALS FOR DIAGNOSIS AND TREATMENT OF CARCINOID TUMOR

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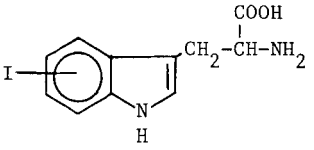
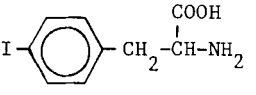
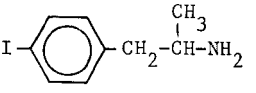
Carcinoid tumor is a neoplasm derived primarily from the enterochromaffin cells of the gut (1). Tumors of this type are characterized by their ability to produce and secrete significant amounts of serotonin (2). Therefore, iodinated compounds known to interfere with the biosynthesis and/or metabolism of serotonin were selected as potential radiopharmaceuticals for the diagnosis and treatment of carcinoid tumor. In this study, serotonergic agents of two general classes were prepared and tested: a) precursor antimetabolites (tryptophan hydroxylase inhibitors) - 5-iodotryptophan (I), 6-iodotryptophan (II), and 4-iodophenylalanine (III), and b) the end-product antimetabolite 4-iodoamphetamine (IV).



I (5-iodo)
II (6-iodo)



III



IV

Each of the 125-I compounds I - IV was produced by exchange-labelling of the corresponding 127-I compounds under aqueous conditions. Radiochemical yields of up to 70% and specific activities (as determined by ultraviolet spectroscopy) of 0.3 to 0.5 mCi/μmol were typically obtained.

The 127-I iodotryptophans I and II were synthesized from the respective nitroindoles in overall yields of 20 - 25% via a seven-step sequence modified from the procedure of Lambrecht, et al. (3). A simpler, four-step sequence toward I from 5-iodoisatin is currently under investigation. The cold iodoamphetamine IV was prepared via a three-step sequence from 4-iodobenzaldehyde. Efforts are also underway to obtain IV via direct iodination of amphetamine.

Compounds I - IV, each labelled with 125-I, are undergoing distribution studies using a mouse mastocytoma model (LAF-1 mastocytoma, Mason Research Inst., Worcester, Mass.). Preliminary data indicate that each of the compounds tested localizes in the model tumor with the amino acids attaining maximum levels much more quickly than the amphetamine IV.

(1) Oates, J. A. and Butler, T.C., Adv. Pharmacol., 5, 109 (1967).
(2) Brown, H. in "Serotonin in Health and Disease," Vol. 4, W. B. Essman, Ed., Spectrum Publications, New York, 1977, chap. 6.
(3) Lambrecht,R.M., Atkins, H., Elias, H., Fowler, J.S., Lin, S.S. and Wolf, A.P., J. Nucl. Med., 15, 863 (1974).

A6

RADIOIODINATED CHOLESTERYL ESTERS: SYNTHESIS AND TISSUE DISTRIBUTION

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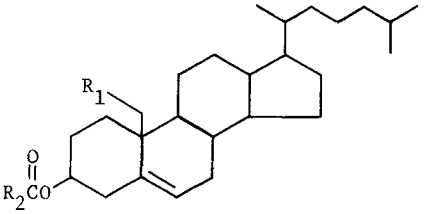
Radioiodinated derivatives of cholesterol have been widely used for adrenal imaging. Since cholesterol is transported in the plasma and stored in tissues largely in an esterified form (1), this study was undertaken to synthesize and evaluate the utility of several radioiodinated ester derivatives of cholesterol as potential imaging agents.

19-Iodocholesteryl esters were made by reaction of the fatty acyl chloride with 19-iodocholesterol. The palmitate (I), oleate (II), and linoleate (III) esters of 19-iodocholesterol were prepared in 58%, 86% and 26% yields, respectively. They were labelled by exchange with $Na^{125}I$ in acetone. All three esters showed marked adrenal uptake when injected in saline using Tween 80/ethanol for solubilization. Both I and II showed less predilection for the target organs when an isopropyl myristate vehicle was used.

Other compounds where the acyl portion carries the label rather than the steroid moiety are represented by structures IV and V. These esters were prepared in 65% yield by use of 1,1'-carbonyldiimidazole (2). Synthesis of the radioiodinated derivatives and analysis of their tissue distribution is now in progress.

(1) Sabine, John R., "Cholesterol", Marcel Dekker Inc., New York, 1977.

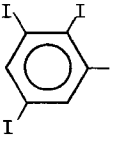
(2) Grover, A. K. and Cushler, R. J., J. Labelled Compd. Radiopharm., 16, 307 (1979).

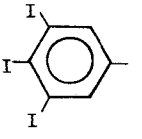


(I) $R_1 = I$ $R_2 = CH_3(CH_2)_{14}-$

(II) $R_1 = I$ $R_2 = CH_3(CH_2)_7CH{=}CH(CH_2)_7-$

(III) $R_1 = I$ $R_2 = CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7-$

(IV) $R_1 = H$ $R_2 =$ 

(V) $R_1 = H$ $R_2 =$ 

A7

A-RING BROMINATIONS OF ESTRADIOL. AN INVESTIGATION OF THE PRODUCT RATIOS OBTAINED.

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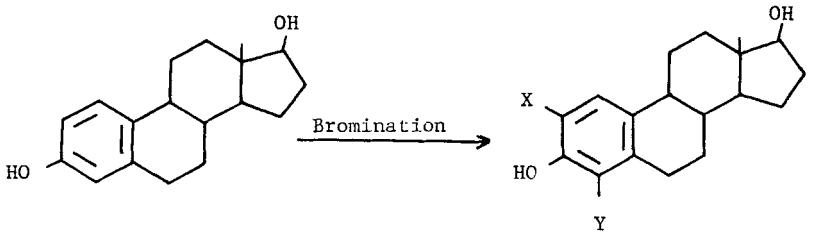
Utilization of Br-77-labelled steroids in the detection and/or selection of therapy for 'hormone-associated' cancers(1) is currently under investigation. Of particular interest is the A-ring (aromatic) brominated derivatives of estradiol. Bromination in the phenolic A-ring is attractive because a) the brominations are relatively easy to accomplish and b) the bromine atom should be less labile on the aromatic ring than it would be at other positions in the molecule.

Although there are several reports of A-ring brominations of estradiol in the literature(2-4), none of the investigations were directed at the quantitation of the components of the reaction mixtures obtained. The need for such an analysis is exemplified by the recent report on the A-ring iodination of estradiol(5), where the often cited procedure for preparing 2-iodoestradiol (mercuric acetate catalyzed iodination) was shown to yield '... mixtures of four or five components ...'. Brominations of estradiol can be expected to yield four components: 2-bromoestradiol (II), 4-bromoestradiol (III), 2,4-dibromoestradiol (IV), and unreacted estradiol (I). An investigation to determine the ratios of these reaction products obtained by direct bromination was undertaken.



I

II: X = Br, Y = H

III: X = H, Y = Br

IV: X = Br, Y = Br

Bromination of the phenolic A-ring of estradiol was accomplished using a variety of brominating reagents (e.g. bromine, N-bromoacetamide, N-bromosuccinimide, pyridinium bromide per bromide, etc.). The product ratios for the bromination reactions (I:II:III:IV) were determined by high performance liquid chromatography on a μ-porasil column, eluting with a 100:1 mixture of chloroform/acetonitrile, and using UV for detection. Variation of the product ratios with varing equivalents of brominating agents was also studied for the commonly used brominating agents: bromine, N-bromosuccinimide (NBS), and N-bromoacetamide (NBA). The results of this study are shown in the accompanying table. Particularly noteworthy is the observation that the ratio of 2-bromo (II) to 4-bromo (III) isomers of estradiol varies depending on whether one brominates with bromine in acetic acid or the N-bromoamides in ethanol. Perhaps equally important is the fact that the ratio of II:III does not appear to vary with variance of the amounts of a particular brominating agent.

Purification and isolation of the brominated isomers was accomplished via both column chromatography and preparative HPLC. Characterization of the individual components was obtained from melting points, IR spectra, UV spectra, mass spectra, and nuclear magnetic resonance spectra.

(1) Miller A. B., Cancer Res., 38, 3985 (1978).
(2) Woodward R. B., J. Amer. Chem. Soc., 62, 1625 (1940).
(3) Slaunwhite W. R. and Neely L., J. Org. Chem., 27, 1749 (1962).
(4) Utne T., Jobson R. B., and Landgraf F. W., J. Org. Chem., 33, 1654 (1968).
(5) Sweet F., Patrick T. B., and Mudd J. M., J. Org. Chem., 44, 2296 (1979).

BROMINATED REACTION PRODUCT RATIOS

Reagents/solvent	# equiv.	%II	%III	%IV	%Unk.*
Br_2/HOAc	0.5	41	59	trace	---
Br_2/HOAc	1.0	40	51	9	---
Br_2/HOAc	2.0	4	4	45	47
NBS/ETOH	0.5	24	67	9	---
NBS/ETOH	1.0	23	69	8	---
NBS/ETOH	2.2	--	trace	100	---
NBA/ETOH	0.5	29	64	trace	7
NBA/ETOH	1.0	25	69	6	---
NBA/ETOH	2.2	3	8	87	2

*%Unk. - refers to one or more unidentified brominated species.

A8

RADIOLABELED ENZYME INHIBITORS AS POTENTIAL ADRENAL IMAGING AGENTS

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Radiolabeled adrenal enzyme inhibitors have been proposed as adrenal imaging agents to replace the current radiopharmaceuticals, [^{131}I]19-iodocholesterol and [^{131}I]6β-iodomethyl-19-norcholesterol. Beierwaltes, et al, using iodinated derivatives of inhibitors of corticosteroid biosynthesis, have successfully imaged the adrenal glands of dogs (1,2). Our approach has focused on labeled analogs of the 11β-hydroxylase inhibitors metyrapone and SU-9055 in which the terminal pyridine ring is replaced by an isosteric group which could be radiolabeled. In our initial study a [^{75}Se]5-1,2,3-selenadiazole group was substituted for the pyridine, however, little adrenal selectivity was demonstrated (3,4). In our current series the pyridine group has been replaced with a 2-aminoselenazole or 2-aminothiazole which can be labeled with the ^{125}I-o-iodobenzoyl moiety.

The desired products have been prepared from the appropriate 1,3-diketone. Alkylation followed by bromination produces the bromomethyl ketones in good yields. The Hantzsch reaction with either thiourea or selenourea provides, in good yields, the 2-aminoazoles which are then acylated with iodobenzoyl chloride. Radioiodination in aqueous buffer generates the labeled products in good radiochemical yields and good specific activity. The biodistribution in normal rats indicates that the adrenals have the greatest uptake with maximal concentration at 5 minutes. Target to nontarget ratios which are high at 5 minutes fall markedly over the first 2 hours.

(1) Beierwaltes W.H., Wieland D.M., Ice R.D., et al, J. Nucl. Med., 17, 998 (1976).
(2) Beierwaltes W.H., Wieland D.M., Mosley S.T., et al, J. Nucl. Med., 19, 200 (1978).
(3) Hanson R.N. and Davis M.A., J. Heterocyclic Chem., in press.
(4) Hanson R.N. and Davis M.A., J. Pharm. Sci., in press.

A9

COMPARISON OF THE IODOGEN AND IODINE MONOCHLORIDE METHODS FOR RADIOIODINATION OF FIBRINOGEN

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The use of reaction vials coated with Iodogen, 1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril, has been claimed to be convenient for radioiodination of proteins, viruses and cells to high specific activities and with minimal damage(1,2). We have investigated the suitability of using Iodogen to prepare radioiodinated fibrinogen, which is used extensively in binding studies and assays as well as in clinical studies of thrombus uptake(3). Because of its labile nature, fibrinogen is a good model compound for testing the mildness of labeling procedures.

Fibrinogen was isolated from fresh human plasma by glycine precipitation(4). For each reaction, a mixture of 5 mg fibrinogen in 5 ml buffer (0.05M Tris, 0.10M NaCl, 0.0025M citrate, pH 7.9) with 250 uCi I-125-iodide and carrier NaI (1 atom I per molecule fibrinogen)were chilled in an ice bath, then added to a chilled glass reaction vial pre-coated with 50 ug Iodogen(Pierce Chemical Co.), according to Fraker, et al(1). The protein solution was decanted from the vial after stirring at 0°C for 1 hour. The reactions were performed in triplicate. For comparison with Iodogen, fibrinogen was labeled with I-125 or I-131 by the Iodine Monochloride technique(5). Efficiency of labeling was higher using the Iodogen technique under these conditions(mean 60% as compared with 36% for ICl in dilute solution).

Thrombin clottability tests by the method of Ratnoff and Menzie(6) indicated that the clottability of the Iodogen preparations (mean 85.3%) were slightly lower than the clottability of the ICl preparations (mean 88.2%)(done in the presence of Ca^{++}).

The binding of fibrinogen to Staphylococci is a very sensitive test of the binding function of fibrinogen(7). The extent of binding of the Iodogen preparations to Staph is only about 73% of the extent of binding of the ICl preparation.

Electrophoresis on SDS polyacrylamide disc gels, followed by slicing and counting, gave the following information about the two labeling methods: 7% reduced gels, which separate the three polypeptide chains of fibrinogen, showed that there was no difference in the distribution of radiolabel among the chains; 3.5% nonreduced gels showed that there were no labeled impurities of higher or lower molecular weight than fibrinogen, resulting from either labeling method.

Plasma clearance experiments in rabbits, in which each rabbit received I-125-Iodogen-fibrinogen and I-131-ICl-fibrinogen, indicated that the clearance rates and y-intercepts for the two preparations were identical in five rabbits.

We conclude that fibrinogen labeled by the Iodogen method would not be suitable for use in binding studies, but would be excellent for structural studies of fibrinogen and fibrin degradation products, or for in vivo metabolic studies.

(1) Fraker P.J. and Speck J.C., Biochem.Biophys.Res.Commun. 80,849 (1978).
(2) Markwell M.A.K. and Fox C.F., Biochemistry 17,4807 (1978).
(3) Kakkar V.V., Arch.Surg. 104,152 (1972).
(4) Kazal L.A., Amsel S., Miller O.P., et al, Proc.Soc.Exp.Biol.Med. 113,989(1963).
(5) Mc Farlane A.S., J.Clin.Invest. 42,346 (1963).
(6) Ratnoff and Menzie, J.Lab.Clin.Med. 37, 319 (1951).
(7) Budzynski A.Z., et al, Fed.Proc. 38, 996 (1979).

Table I

Clottability of Labeled Fibrinogen

Labeling Method	Clottability (%) (with EDTA)	Clottability (%) (with Ca^{++})
ICl (n=3)	86.3 ± 0.4	88.2 ± 0.3
Iodogen (n=9)	80.6 ± 9.3	85.3 ± 4.0

Table II

Binding of Labeled Fibrinogen to Staphylococci

Labeling Method	Binding (ng/mg Staph)	Relative Binding, %
ICl	77 ± 3.9	100
Iodogen	56 ± 9.4	73

Table III

Plasma Clearance (Rabbits)

Rabbit #	$t_{\frac{1}{2}}$ of clearance (hr)		y-intercept (% of radioactivity in second component of curve)	
	ICl	Iodogen	ICl	Iodogen
I	33	33	94	94
II	34	34	98	100
III	44	44	96	96
IV	35	35	65*	65*
V	32	32	70*	70*

* Initial blood samples in these animals were drawn at earlier times, before equilibration into the extravascular fibrinogen space had been completed.

A THIOACETAL DERIVATIVE AS A CHELATING AGENT FOR Tc-99m BIOCHEMICAL ANALOGS: APPLICATION TO FATTY ACIDS.

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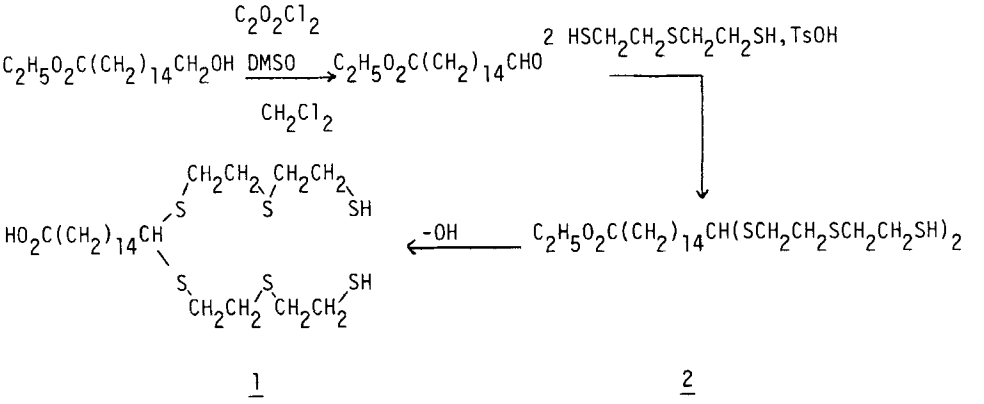
The development of effective Tc-99m labeled biochemical analogs has been hampered by alterations of charge and size due to complex formation. These alterations can be minimized by the use of chelating groups which result in a complex with lipophilic properties and a 1:1 ligand to metal ratio. This report describes the first of a series of compounds designed to fulfill these objectives. The compound prepared, the thiodithioethane thioacetal of 15-carboxaldehydepentadecanoic acid (1), is expected to form a technetium complex with lipophilic character due to the use of sulfur donor atoms and to result in 1:1 ligand to metal complex since the chelating group contains six sulfur atoms.

Commercially available 16-hydroxyhexadecanoic acid was converted to the ethyl ester and then oxidized to ethyl 15-carboxaldehydepentadecanoate by oxalyl chloride activated dimethyl sulfoxide (1). Addition of thiodiethanethiol to the aldehyde ethyl ester (2,3) and column chromatographic purification on silica gel gave a 2:1 sulfur group to fatty ester adduct. Hydrolysis under mild base conditions gave the fatty acid thioacetal (nmr: δ 1.37 (broad



1 2

singlet,-CH_2-,24H), 1.63 (complex, $CH_2CH(-SR)_2$,2H), 2.35 (t,-CCH_2-,2H), 2.90 (broad singlet,-SCH_2-,16H), and 3.9(complex, $CH(-SR)_2$,1H). The infrared spectrum was consistent with the functional groups present.

Technetium-99m complexes of 1 and the ethyl ester (2) were prepared with stannous chloride as the reducing agent. The compounds (10 mg) were dissolved in tetrahydrofuran (1 ml) and 0.2 mg $SnCl_2 \cdot 2H_2O$ in 0.05 ml ethanol added under nitrogen. High concentration Tc-99m-pertechnetate was added. The preparation was solubilized for biological studies by addition of 0.3 ml of the THF solution to 1.0 ml of 10% Tween 80.

Chemical properties of the complexes of both the thioacetal acid and ester were compared chromatographically, electrophoretically, and in ethylenedichloride-water mixtures for lipophilicity (Table 1). The data show both to be relatively lipophilic with R_f values in methyl ethyl ketone of 0.7 and 0.9 for 1 and 2 respectively for the major bands. The percentage in the organic layer was 69% for the ester (2) and 51% for the acid complex (1). Under these conditions less than 1% of Tc-99m-pertechnetate was found in the organic

layer. The electrophoresis results indicate no net charge on the complex. The complex of 1 moved as an anion while that of the ester with the carboxyl group masked did not migrate.

Preliminary biological studies of Tc-99m-1 in mice at 30 min indicate some myocardial uptake (Table 2). Thus 0.68 + 0.28% of the dose was found in the heart which corresponds to 5.82 + 1.19% dose/g heart tissue. The ratio of activity in the heart to that in the blood on a dose/g basis was 0.57 for the complex and 0.09 for I-125-albumin.

(1) Muncuso, A.J., Huang, S.L., and Swern, D., J. Org. Chem., 43, 2480 (1978).
(2) Coates, R.L., and Jones, M.M., J. inorg. nucl. Chem., 39, 943 (1977).
(3) Jones, M.M., Pratt, T.H., Mitchell, W. G., et.al., J. inorg. nucl. Chem., 38, 613 (1976),

TABLE 1. CHEMICAL PROPERTY COMPARISONS OF Tc-99m COMPLEXES OF THIOACETAL FATTY ACID AND ESTER

Complex	R_f(MEK)[a]	R_f(Saline)[a]	$\%C_2H_4Cl_2/\%H_2O$	Electrophoresis migration in cm[b]
^{99m}Tc-1	0.0(23%),0.7(77%)	0.2(100%)	1.04	2.1
^{99m}Tc-2	0.0(20%),0.0(80%)	0.1-0(major)	2.22	0.0

[a]Chromatography was run on silica gel (ITLC-SG, Gelman). [b]Electrophoresis was carried out on paper for 30 min at 250 volts. Under these conditions bromecresol green migrated 6.4 cm and Tc-99m-pertechnetate migrated 10 cm.

TABLE 2. BIODISTRIBUTION OF Tc-99m COMPLEX OF FATTY ACID THIOACETAL AND I-125 HSA IN MICE AT 30 MIN[a]

Radiochemical	Heart	Blood	Liver	Intestines	Stomach	Kidneys
^{99m}Tc-1	0.68 ±0.28	15.58 ±1.00	23.58 ±2.66	6.94 ±1.28	0.73 ±0.36	2.46 ±0.32
^{125}I-HSA	0.49 ±0.11	72.14 ±2.69	7.81 ±1.01	4.58 ±1.03	0.91 ±0.22	3.38 ±0.71

[a]Values are mean and standard deviation for 5 mice.

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SYNTHESIS OF TETRADENTATE AMINOTHIOL LIGANDS AND THEIR TECHNETIUM COMPLEXES

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Recently, several investigators have suggested that lipid soluble complexes of technetium-99m (Tc-99m) might be useful for measuring brain blood flow as a consequence of the ability of the complexes to diffuse across the blood brain barrier (1,2,3). It has also been proposed that such complexes may be suitable for incorporation into bifunctional Tc-99m-radiotracers designed to localize at intracellular receptors such as estrogen receptors (4). Loberg reported that octanol: saline partition coefficients of 0.5:1 are necessary to permit passive diffusion across cell membranes (2).

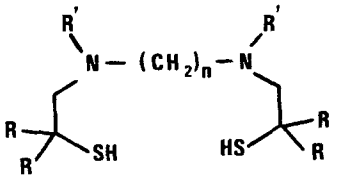
As part of a project aimed towards identifying ligands which form stable, neutral, lipid soluble complexes with Tc-99m, an investigation of the chemistry of Tc-99m and Tc-99 complexes of aminothiol ligands was undertaken (4,5). Mixed ligand experiments demonstrated that simple aminothiols form neutral, bis-complexes with Tc-99m (4). Although these compounds were found to be lipophilic with octanol: saline partition coefficients as high as 266:1, lipid soluble complexes of greater stability were sought.

A series of tetradentate diamino-dithiol ligands (Table 1) were synthesized by methods which allow for the incorporation of a variety of substituents at several positions on the tetradentate ligand backbone. All of these ligands readily form complexes with Tc-99m when pertechnetate ion is reduced by either stannous chloride or sodium borohydride in an aqueous solution of the ligand. Electrophoresis demonstrated that these complexes are not charged at physiological pH. Octanol:saline partition coefficients (Table 1) were determined which showed that lipid solubility is closely related to the degree of substitution on the ligand. All of the complexes except those of compounds 1 and 2 have partition coefficients greater than 0.5:1 indicating that these complexes are sufficiently lipid soluble to diffuse across membrane barriers.

Attempts to further characterize the complexes, and biodistribution studies are now in progress. The simple bidentate aminothiols form intensely green solids with Tc-99. Elemental analysis of the Tc-99 complex of benzylaminoethanethiol (Table 2) is consistent with an empirical formula of $C_{18}H_{24}N_2S_2TcOCl$, suggesting that in addition to the two amino-thiol ligands, oxo and chloro ligands are also present in these complexes. The tetradentate ligands form orange to red complexes with Tc-99 which to date have been obtained only as oils.

1. Oldendorf, W.H., J Nucl Med 19, 1182 (1978).
2. Loberg, M.D., Corder, E.H., Fields, A.T. and Callery, P.S., J Nucl Med 20, 1181 (1979).
3. Kung, H.F., Blau, M., J Nucl Med 21, 147 (1980).
4. Burns, H.D., Dannals, R.F., Marzilli, L.G. and Wagner, H.N., J Nucl Med 20, 641 (1979).
5. Burns, H.D., Manspeaker, H., Miller, R., Risch, V., Emrich, J. and Heindel, N.D. J Nucl Med 20, 654 (1979).

Table 1. Tetradentate Diamino-dithiol Ligands



Compound	R	R′	R″	n	*Partition Coefficient of Tc-99m-Complex
1	H	H	H	2	1:12
2	H	CH_3	CH_3	2	1: 4
3	H	C_2H_5	C_2H_5	3	5: 1
4	CH_3	H	H	2	14: 1
5	CH_3	Ø-CH_2-	H	2	10: 1

* Octanol/Saline

Table 2. Elemental Analyses of ^{99}Tc-Complex of Benzylaminoethanthiol

	Percent			
	C	H	N	Cl
calculated	44.77	5.01	5.80	7.34
found	44.47	4.89	6.10	8.39

A12

Properties of various IDA derivatives

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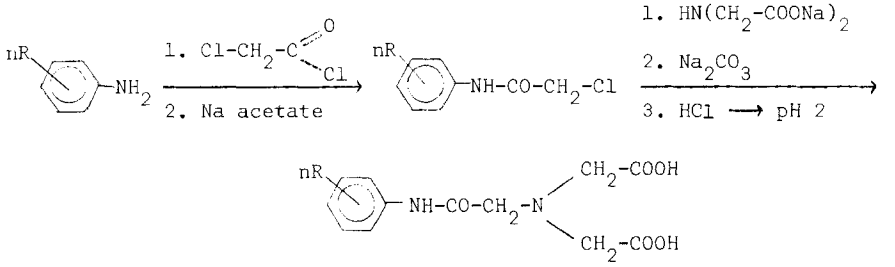
A series of derivatives of acetanilidoiminodiacetic acid with various substituents on the aromatic ring were synthesized and then tested for their suitability as diagnostic agents for liver function. The synthesis was carried out as indicated in the following diagram:



The synthesized compounds were then used to produce labelling kits, each of which contained 20 mg of the corresponding IDA derivative and 0.2 mg $SnCl_2 \cdot 2H_2O$ in freeze-dried form. The pH of the stock solution was adjusted to pH 6 for all compounds (the pH of the stock solution has a marked influence on the urinary elimination rate). Labelling was performed by the addition of $^{99m}TcO_4^-$ in isotonic saline (generator eluate). Radiochemical purity was tested after a delay of about 30 min.; the purpose of this delay was to allow time for the formation of the 1:2 complex. The determination of TcO_4^- was carried out by means of paper chromatography (Whatman 1; methyl ethyl ketone) and that of reduced, unbound technetium by thin layer chromatography (silica gel; methanol/water ◘ 8/2). Pertechnetate was not detected in any instance; the proportion of reduced, unbound technetium ranged from 1 to 5 %, depending on the derivative.

Organ distribution studies in Wistar rats were conducted as follows: The penis of male rats was ligatured under narcosis and the diagnostic agent was injected into the thigh vein. The penis was ligatured in order to record the amount of radioactivity eliminated with the urine into the bladder without error. At the times 5, 10, 20 and 30 min. after injection the animals were sacrificed with ether and the distribution in the organs was determined. The values for 30 min. P.I. are shown in the table. Differences are apparent above all in renal cumulation and in the radioactivity in the bladder. The values for the 4-n-pentyl derivative are conspicuously low; the amount of radioactivity eliminated in the urine is 0.13 % (!).

The following animal model was used to determine certain of the kinetic parameters: Narcotized rabbits were injected with the diagnostic agent in the ear vein and the time depending distribution of the radioactivity was followed with a scintillation camera. Scintigrams were recorded at 15-second intervals for from 1 to 2.5 hours, depending on the preparation. By means of a suitable computer program the coarse of radioactivity was determined over certain areas such as the liver or one of the kidneys. The curves thus obtained could be used to determine the time of maximum radioactivity cumulation (t_{max}) and the half-life ($t_{1/2}$) in the liver; these two parameters convey information about liver kinetics. The individual values are shown in the table. They show that 2,4,5-trimethyl-IDA has the fastest liver kinetics and that all preparations with a low renal elimination rate have comparatively slow liver kinetics (e.g. 4-n-butyl-IDA, 4-n-pentyl-IDA and pentafluoro-IDA).

To try to explain the relatively large differences in pharmacokinetics of the various derivatives, the distribution coefficients between octanol and water and the protein-binding rates were determined; the findings are shown in the table. The method used to measure the protein-binding rate was as follows: The labelled diagnostic agent was incubated with human serum for 3 hours at 37 °C. Then o.1-o.2 ml of the incubation solution was analyzed by gel chromatography on Sephadex G 25; the solution emerging from the column was examined by means of UV and radioactivity detectors. The protein-binding rate was determined by integration of the radioactivity peak(s).

As the values in the table show, there is no direct correlation between distribution in the organs of the rat and lipophily or the protein-binding rate, but it is striking that 4-n-pentyl-IDA, which is characterized by its extremely low urinary elimination rate, also exhibits the highest distribution coefficient in octanol/water and the highest protein-binding rate.

It can be said in summary that, provided the data found here are applicable to humans, 2,4,5-trimethyl-IDA should be the derivative of choice where the bilirubin plasma level is low because of its fast liver kinetics, whereas the 4-n-pentyl derivative should preferably be used where the bilirubin level is over 5 mg/100 ml because it is practically not at all eliminated via the kidneys.

Parameters of the animal experiments, the distribution coefficients and the protein-binding rate of the IDA derivatives studied

Derivative	Distribution in organs of rat (n=3) in % of the applied dose 3o min. p.i.							Liver kinetics (rabbit)		Distribution coefficient octanol/water	Protein binding rate after 3 h incubation in %	Not yet described in the literature
	liver	intestines	kidneys	bladder	thyroid gland	1 ml blood	rest of body	t_{max} (min)	$t_{1/2}$ (min)			
2,6-Dimethyl-IDA	o.83	84.1	5.14	7.74	o.ol	o.13	6.94	5.7	9.5	o.o38	1o	
2,6-Diethyl-IDA	2.53	78.8	4.47	5.62	o.ol	o.18	8.14	6.8	15.7	o.14	1o	
2,6-Diisopropyl-IDA	9.3o	61.6	5.8o	6.2o	o.ol	o.32	17.2o	4.6	15.3	o.42	12	
4-Methyl-IDA	2.4o	62.3	4.69	16.31	o.oo8	o.15	1o.5o	3.7	12.7	o.38	14	x
4-Ethyl-IDA	1.6	78.5	2.8o	9.7o	o.oo5	o.o9	6.4o	6.2	14.7	o.94	11	x
4-Isopropyl-IDA	1.97	86.8	2.83	8.44	o.oo6	o.13	6.56	3.9	11.7	1.74	11	
4-n-Butyl-IDA	4.ol	83.3	2.97	2.76	o.oo9	o.32	9.43	6.3	27.6	4.35	5o	
4-n-Pentyl-IDA	12.47	85.4	o.96	o.13	o.oo4	o.o5	3.11	7.4	41.o	>32	>9o	x
4-t-Butyl-IDA	2.8o	8o.4	2.16	4.89	o.ol	o.2o	9.o	4.2	18.4	2.17	1o	x
4-Phenyl-IDA[+)]	4.66	85.5	1.96	o.79	o.oo9	o.19	7.17	6.8	38.7	4.66	48	x
4-Methoxy-IDA	1.6o	53.o	6.4o	2o.1o	o.oo8	o.14	1o.7o	3.6	12.8	o.oo3	8	x
3,5-Dimethyl-IDA	1.3o	62.1	5.9o	8.4o	o.ol	o.13	9.9o	5.o	9.9	o.19	5	
2,4,6-Trimethyl-IDA	1.6o	81.o	3.3o	2.9o	o.ol	o.13	6.4o	4.2	1o.4	o.o75	<1	
2,4,5-Trimethyl-IDA	1.7o	8o.6	2.28	5.74	o.ol	o.17	9.7o	2.6	7.7	o.o37	1	x
4-Fluoro-IDA	1.9o	64.o	4.4o	15.6o	o.oo7	o.1o	8.1o	5.5	15.3	o.o96	18	x
2,4-Difluoro-IDA	2.7o	53.8	4.8o	21.5o	o.ol	o.16	12.4	7.5	31.5	o	5	x
2,5-Difluoro-IDA	3.1o	53.3	8.3o	18.4o	o.ol	o.15	11.1	7.6	25.4	o	3	x
2,3,4,5,6-Pentafluoro-IDA	3.8o	81.8	1.57	1.79	o.oo5	o.o7	4.7o	14.3	48.7	o	o	x

[+)] Here the pH of the stock solution was 6.5; the labelled injection solution was distinctly turbid.

A13

AN IN VITRO INVESTIGATION OF THE MECHANISM OF INTERACTION OF TECHNETIUM PYROPHOSPHATE WITH HYDROXYAPATITE AND COLLAGEN

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Technetium 99m pyrophosphate has become an established radiopharmaceutical for bone imaging in Nuclear Medicine. While it is generally agreed that the uptake on the hydroxyapatite of bone is at least in part responsible for the localization of this radiopharmaceutical, the degree of involvement of the collagen in its localization has not been determined beyond dispute. Furthermore the exact mechansim by which the technetium becomes localized on either entity has received little study. There are two reports (1, 2) in the literature on in vitro studies using hydroxyapatite with another similar bone localizing radiopharmaceutical, technetium 99m hydroxyethylene diphosphonate, in which some aspects of the interaction of hydroxyapatite with this similar radiopharmaceutical are explored. This investigation was undertaken to determine the mechanism by which technetium pyrophosphate interacts with both collagen and hydroxyapatite.

(A) Technetium Pyrophosphate and Hydroxyapatite.

Technetium pyrophosphate was prepared by our routine technique of electrolytic generation of stannous ion in the presence of sodium pyrophosphate and pertechnetate. 1.5 ml of this preparation diluted to 2.0 ml with saline was incubated with various quantities of hydorxyapatite for 1/2 hour before being separated and the distribution of the radioactivity between the supernant and the hydroxyapatite determined. This was done using tracer quantities of Sn-113 and P-32 pyrophosphate in the initial preparation as well as technetium 99m. The results, shown in Table 2, indicate that, while the hydroxyapatite is saturated by the pyrophosphate at approximately 1.6×10^{-7} moles of pyrophosphate per mg of hydroxyapatite, the stannous ion is not found in the supernant until the pyrophosphate content exceeds 4.3×10^{-7} moles per mg of hydroxyapatite. The technetium 99m is found in the supernant along with the pyrophosphate above 1.6×10^{-7} moles of pyrophosphate per mg. However its distribution does not parallel that of the pyrophosphate but rather a greater percentage of the technetium is associated with the hydroxyapatite than the pyrophosphate. Variations in the ratio of pyrophosphate to stannous ion in the preparation indicated that it was the pyrophosphate which controlled the percentage of technetium which was absorbed onto the hydroxyapatite.

(B) Technetium Pyrophosphate and Collagen.

Various volumes of technetium pyrophosphate prepared as before were diluted to 2 ml with saline and incubated for 1/2 hour with 10 mg. of collagen. The collagen and supernant were then separated and counted. The results, shown in table 2, indicate the distribution of technetium-99m, tin-113 and P-32 pyrophosphate at the various concentrations of the pyrophosphate preparation. It is clear that the pyrophosphate does not associate with the collagen while the stannous ion, once over a minimum concentration, is distributed between the collagen and the supernant at a constant value of approximately 30% on the collagen. The maximum binding of technetium occurs between 3 and 6×10^{-8} equivalents of stannous ion, i.e. between 1.35 and 2.7×0^{-8} moles of pyrophosphate per mg. of collagen. Studies in which the ratio of pyrophosphate to stannous ion were varied showed that the uptake was dependent on the quantity of stannous ion rather than the amount of pyrophosphate.

In summary then, it has been shown that while the uptake of technetium onto hydroxyapatite is controlled by the pyrophosphate concentration with the hydroxyapatite becoming saturated with pyrophosphate and thus reducing the binding of technetium to the hydroxyapatite at 1.6×10^{-7} moles per mg., the binding of technetium to collagen is controlled by the stannous ion content with a clear maximum binding occurring at between 3 and 6×10^{-8} equivalents of

stannous ion. Thus it would appear that the relative importance of the binding to hydroxyapatite and the binding to collagen of the technetium 99m from technetium stannous pyrophosphate may be dependent on the proportions of stannous ion to pyrophosphate in the original preparation as well as the relative proportions of available collagen and hydroxyapatite in the individual patient.

TABLE 1

BINDING OF THE VARIOUS CONSTITUENTS OF TECHNETIUM STANNOUS PYROPHOSPHATE TO HYDROXYAPATITE

QUANTITY OF HYDROXYAPATITE B (mg)	% BOUND TO HYDROXYAPATITE Tc	Sn	PYRO
2	7	19	
5	14	49	9
7	18	62	
10	23	74	19
15	37	86	
20	45	95	34
30	59		53
40	73		69
50	89		90
60	96	99	93

TABLE 2

BINDING OF THE VARIOUS CONSTITUENTS OF TECHNETIUM STANNOUS PYROPHOSPHATE TO COLLAGEN

STANNOUS ION CONTENT (Equiv.)	% BOUND TO COLLAGEN Tc	Sn	PYRO
1.28×10^{-8}	23	23	0
2.24×10^{-8}	28	24	0
3.20×10^{-8}	40	30	0
6.40×10^{-8}	43	30	0
1.28×10^{-7}	40	31	0
2.24×10^{-7}	35	30	0
3.20×10^{-7}	28	29	0
4.80×10^{-7}	24	28	0
6.40×10^{-7}	22	28	0

(1) Van Langevelde A., Dreissen O.J.J., Pauwels E.K.J., Thesingh C.W., Eur. J. Nucl. Med. 2 47 1977
(2) Tafe A.J., Francis M.D., J. Mucl. Med 15 69 1974

A14

A HEAVY RARE-EARTH NUCLIDE GENERATOR

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Recent work at Los Alamos has resulted in a practical method for the synthesis, quantitative recovery, and purification of Ci quantities of NCA(1) ^{172}Hf. This long-lived (1.87 y half-life) nuclide decays to 6.70-d ^{172}Lu, a neutron-deficient isotope of the heaviest rare-earth element. Heavy lanthanides, particularly ^{169}Yb, have found increasing utilization in nuclear medicine for cisternography, tumor localization, and numerous other diagnostic procedures. Although ^{172}Lu decays to stable ^{172}Yb via electron capture, it produces a number of abundant, high-energy gamma rays that would prohibit its use in patient imaging with present instrumentation. It has been proposed(2), however, that ^{172}Lu would be useful for compound-labelling investigations and animal biodistribution studies, and its availability would spur increased preclinical research with rare-earth compounds. Following successful preclinical studies, another radiolanthanide with better nuclear properties for diagnostic imaging could be interchanged for ^{172}Lu. The convenience of generator availability and the very long shelf-life of the ^{172}Hf-^{172}Lu system could make it an attractive research tool for medical investigators.

The production of ^{172}Hf is accomplished by irradiating metallic Ta targets with medium-energy protons at the Los Alamos Meson Physics Facility (LAMPF). The targets are then remotely processed in a hot cell to radiochemically isolate a pure radiohafnium fraction, and significant quantities of ^{172}Hf are thus made available for use in isotopic generators.

A preliminary literature search uncovered a number of potentially useful analytical procedures for the chemical separation of Lu from Hf, of which three were selected for evaluation as ^{172}Hf-^{172}Lu generators. These were an anion exchange column eluted with 12M HCl(3), solvent extraction utilizing TTA/2M HCl(4,5), and solvent extraction with HDEHP/9M HCl(6). Initial experiments employed 10–50 μCi of ^{172}Hf per run, and each separation system was studied in duplicate. Experimental solutions were analyzed for ^{172}Lu yield and (after the reattainment of equilibrium) ^{172}Hf breakthrough by Ge(Li) gamma-ray spectrometry. Comparisons with a primary external standard were made for activity-balance determinations, and agreements to within ±5–10% were obtained.

The anion columns loaded with ^{172}Hf were milked with conc. HCl, and average ^{172}Lu yields of 0.92 and ^{172}Hf breakthroughs of 9.6×10^{-5} were measured. The average separation factor for this system was therefore (0.92/9.6E-5) = 9600. The TTA extraction systems proved to be decidedly inferior and resulted in an average separation factor of 39, primarily due to increased ^{172}Hf breakthroughs. Moreover, appreciable radiation damage to the TTA with but μCi quantities of ^{172}Hf was observed over the three-month duration of this study. Results with the HDEHP generators were 0.95 ^{172}Lu yield and a separation factor of 8300. The HDEHP/9M HCl extraction system and the anion exchange column were therefore comparable in generator performance at μCi activity levels.

For reasons of convenience and resistance to radiolysis effects, it was decided to implement the HDEHP generator in LASL's radiochemistry hot cells for the processing of LAMPF-irradiated targets. A test of this system under actual hot cell conditions was then performed. A total of 430 mCi of ^{172}Hf was extracted into a 50% HDEHP(by volume) in toluene solution, and ^{172}Lu was then back-extracted from the organic phase with four sequential washings of 9M HCl [multiple extractions are required for quantitative Lu recovery since the yield from a single step is only approximately 0.5(6)]. The HCl fractions were combined, and aliquots of the organic and inorganic phases were assayed for ^{172}Lu and ^{172}Hf. The separation factor obtained in this experiment was 1.7×10^{4}, in reasonable agreement with the results of the laboratory-scale work.

A generator for the separation of ^{172}Lu from ^{172}Hf has thus been developed. Quantitative ^{172}Lu yields are obtainable with a separation factor of 10^4, and the generator has been found to be effective at loadings up to about 0.5 Ci. The HDEHP medium in the hot cell has been very resistant to radiolytic perturbations for nearly two years, with no novel chemical phenomena observed over that period.

The performance parameters of the HDEHP generator should be satisfactory for the proposed preclinical uses of ^{172}Lu. Investigators that are interested in obtaining research quantities of ^{172}Hf-^{172}Lu (or the separated daughter alone) should contact this laboratory.

(1) Wolf A. P. and Fowler J. S., "Radiopharmaceuticals II", Society of Nuclear Medicine, New York, 1979, p.73.
(2) Daniels R. J., Grant P. M., and O'Brien H. A., Int. J. Nucl. Med. Biol. 5, 11 and 207 (1978).
(3) Kraus K. A. and Nelson F., Proc. Int. Conf. on Peaceful Uses of Atomic Energy, Geneva 7, 113 (1955).
(4) Morrison G. H. and Freiser H., "Solvent Extraction in Analytical Chemistry", Wiley and Sons, New York, 1957, p.160.
(5) Hala J., J. Inorg. Nucl. Chem. 29, 187 (1967).
(6) Qureshi I. H., McClendon L. T., and LaFleur P. D., "Modern Trends in Activation Analysis", NBS Special Publication 312, Vol. I, 1969, p.666.

A15

A NEW CD-115/IN-115M RADIOISOTOPE GENERATOR

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Recent interest has focused on indium radioisotopes, primarily In-111, because of that element's ability to label cellular blood components when complexed with 8-hydroxyquinoline (oxine). However, In-111 has a relatively long half-life (2.83 days) and is not generator-produced, while the previously available, generator-produced In-113m is somewhat short-lived (99.5 min) and has a high gamma energy (393 keV).

We have developed a column Cd-115/In-115m radionuclide generator system providing 4.49 hr In-115m from 53.4 hr., reactor-produced Cd-115. The Cd-115 is loaded quantitatively on an anion-exchange resin as CdI_4^{-2} and the carrier-free In-115m is eluted off with .05 M HCl in 90% yield. Breakthrough of parent Cd-115 is less than .0003%. Generators of up to 30 mCi have been produced with no degradation of performance.

Labeling of equine platelets with multi-millicurie amounts of In-115m oxine was accomplished using a modified version of the method of Thakur and Welch (1) in which chloroform extraction of the In-oxine complex is not necessary (2), demonstrating a labeling yield of 70%.

(1) Thakur, M. L., Welch, M. J., Joise, J. H., et. al., Thromb. Res., 9, 345-357 (1976).
(2) Heaton, W. A., Davis H. H., Welch, M. J., et. al., Brit. J. of Haematology, 42, 613-622 (1979).

Cd-115/In-115m Generator System

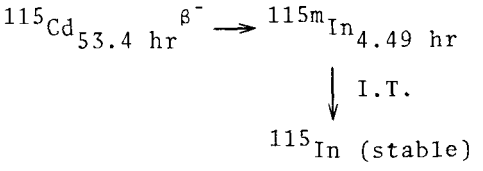

Table of In-115m Decay Characteristics*

Radiation	Energy	% Abundance
Gamma	336 keV	45.9%
Conversion electron	∿ 300 keV	49.1%
Beta particle	840 keV	5.0%

*Lederer + Shirley, Table of Isotopes, Seventh Edition, John Wiley & Sons, Inc. 1978.

A16

A SIMPLE AND INEXPENSIVE Rb-81→Kr-81m GENERATOR.

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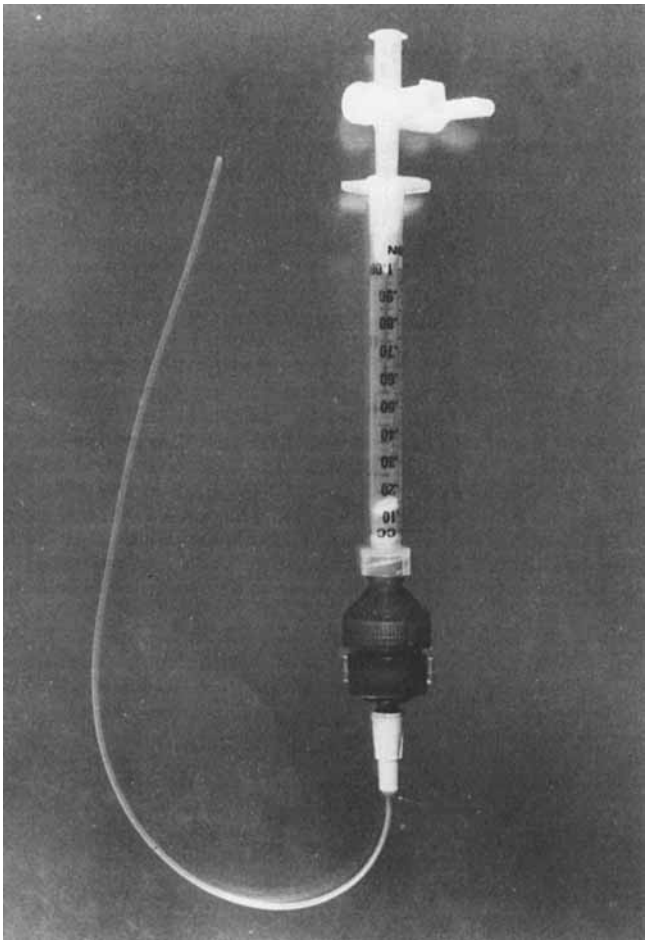
In the past decade several investigators (1,2,3) have developed sterile Rb-81→Kr-81m generators. All these generators can not be fabricated readily due to: (a) the limited availability materials used in the column design (1) (b) complexity of design (1,2) and (c) cost of fabrication. (1,2,3) In this work we have designed a least expensive generator using a 1c.c. capacity sterile plastic syringe containing 0.1 c.c. Dowex-50-x8, 100-200 mesh resin (washed with 9M; 0.1M-HCl and deionized water) sandwiched between two porous (1.5 micron) polypropylene discs. The Rb-81 was produced through Kr-82(d,3n)Rb-81 and was flushed with condensed steam on to the resin. The column was eluted with dry nitrogen gas at 25-290cc/min flow rates and the Kr-81m washout was monitered using a computer coupled Ge(Li)-4096 system by measuring the peak areas under 190- and 550-keV photopeaks. The extraction efficiency varied between 80-85% depending on flow rate of N_2. Thus we have designed a simple, clinically useful Kr-81m generator with a tuberculin syringe and Kr-81m can be administered orally by eluting with N_2 as well as intravenously by eluting with 5% dextrose solution aseptically into patient's vein while the patient is positioned on line with a gamma camera.

(1) Colombetti, L.G., Mayron, L.W., Kaplan, E., Barnes, W.E., Friedman, A.M., Gindler, J.E., J. Nucl. Med. 15 868 (1974)
(2) Yano, Y., McRaye,J., Anger, H.O., J. Nucl. Med. 11, 674 (1970)
(3) Fazio, F., Jones, T., Br. Med. J. 3 673 (1975)



A17

USE OF PERTURBED ANGULAR CORRELATION STUDIES IN THE ELUCIDATION OF THE MECHANISM OF INDIUM-111 LABELING OF HUMAN PLATELETS

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The mechanism of labeling and the intracellular localization of indium-111 in cellular blood components have been studied by several groups. Thakur et al. (1) used subcellular fractionation and gel filtration to investigate the sites of labeling in human neutrophils; Mathias et al. (2) using chromatographic and electrophoretic techniques, have studied the localization of the indium-111 in human platelets. Eakins et al. (3) have investigated the location of the indium-111 in rabbit platelets using electron microscope autoradiography. The fractionation studies were inconclusive, probably due to the low stability of the In-111 bond to the subcellular protein(s).

Perturbed Angular Correlation (P.A.C.) studies have been used to further investigate the localization and mechanism of indium-111 labeling. The Perturbed Angular Correlation technique has the advantage that it is highly sensitive and non-destructive, and can be used to investigate the indium-111 protein bond. Relative time-integrated perturbation factors have been obtained for labeled platelets at various steps in the labeling procedure (Figure 1) and for intact and disrupted labeled platelets in various media (Table 1). These values were obtained using counting equipment similar to that described by others (4).

As discussed by Shirley (5), the time-integrated perturbation factors can be related to the rotational correlation times of the environment of the indium-111 nucleus. A lower value of $[G_{22}(\infty)]$ is indicative of a slower rotational correlation time. The unincorporated indium-111 activity remaining in the supernatant after labeling has a rotational correlation time similar to that of the indium-111 oxine, however indium-111 incorporated by the platelets has a much lower correlation time. This suggests that upon uptake in platelets the environment of In-111 is altered and the remaining In-111 in the supernatant after labeling is probably still bound to a relatively small molecule. The determining factor in the labeling yield has been assumed to be the presence of transferrin in the supernatant, however, our data is not consistent with this and some other factor, possibly the citrate concentration (6), must affect the amount of activity in the supernatant. It is noted that when the platelets are lysed in plasma, the value for $[G_{22}(\infty)]$ does not change (Table 1). A possible explanation would be the indium undergoing exchange with transferrin. When the platelets are lysed, either by sonication or osmotically, the rotational correlation time increases. Whether this effect is due to exchange from the intracellular labeling site or simply due to the exchange from weak complex at the lower concentration of the protein following lysis should be further investigated.

This preliminary study suggests that the P.A.C. studies have potential for the further elucidation of the localization of In-111 in platelets and other cells labeled similarly.

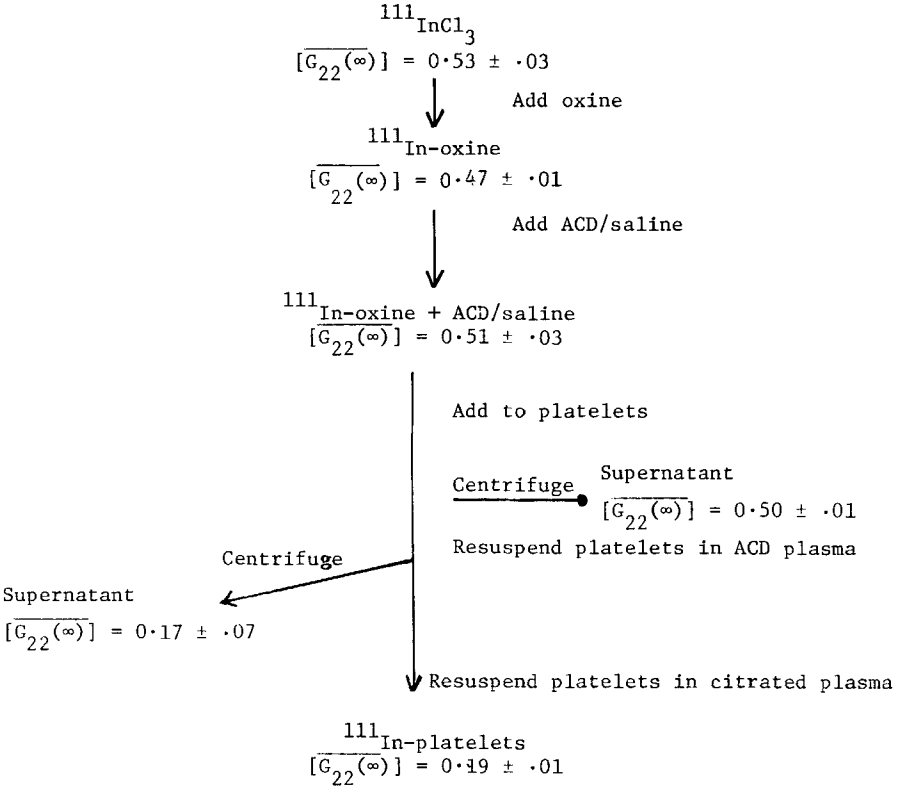
(1) Thakur, M.L., Segal, A.W., Louis, L., Welch, M.J., Hopkins, J. and Peters, T.J., J. Nucl. Med. 18, 1020-1024 (1977).
(2) Mathias, C.J. and Welch, M.J., J. Nucl. Med. 20, 659 (1979).
(3) Eakins, M.N., Baker, J.R.J., Butler, K.D., Pay, G.F., Wallis, R.B. and White, A.M., Thrombosis and Haemostasis 42, 469 (1979).
(4) Goodwin, D.A., Meares, C.F. and Song, C.H., Radiology 105, 699-702 (1972).
(5) Shirley, D.A., J. Chem. Phys. 53, 465-466 (1970).
(6) Scheffel, U., Tsan, M.F. and McIntyre, P.A., J. Nucl. Med. 20, 524-531 (1979).

TABLE 1

P.A.C. Studies on In-111 Labeled Platelets

	$[\overline{G_{22}(\infty)}]$
^{111}In-platelets suspended in plasma	0·17 ± ·02
Lysed ^{111}In-platelets (sonicated) in plasma	0·19 ± ·01
^{111}In-platelets suspended in ACD (not sonicated)	0·16 ± ·02
Lysed ^{111}In-platelets (sonicated) in ACD	0·40 ± ·02
Lysed ^{111}In-platelets in 9% saline (after being frozen in liq. N_2, thawed)	0·32 ± ·01

Figure 1. Relative time-integrated perturbation factors at various steps of the platelet labeling procedure.



A18

METHODOLOGICAL STUDY OF IN111-OXINE HUMAN PLATELET AND LEUCOCYTE LABELING

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Several groups have reported on In111-oxine labeling of platelets and leucocytes (1,2,3). The published methods show some disagreement with regard to the suspension medium (plasma environment or plasma-free) and use fairly long incubation times (30 and up to 90 minutes). We therefore studied the labeling at short incubation times with regard to temperature, cell and oxine concentration and in vitro function.

The In111-oxine was prepared by methylene chloride extraction and assayed for final oxine concentration by spectrophotometry at 240 nm and for free In^{3+} by a back-extraction procedure. Human platelets were isolated by differential centrifugation and washed, suspended and incubated with In111-oxine in tyrodes-albumin buffer. Starting from citrated blood prostacyclin (PGI_2, 25 ng/ml) was added to the suspensions in order to stabilize the platelet membrane (4).

Before and after labeling platelets were observed in a scanning electron microscope (SEM) and their function tested by 3 µM ADP induced aggregation in a Born aggregometer.

For leucocyte preparation red blood cells were first sedimented from citrated blood and then thrombocytes separated by centrifugation. The leucocyte pellet was suspended in a culture medium (RPMI 1640) and incubated with In111-oxine. Leucocyte in vitro function was tested by phagocytosis (latex particles), migration, light microscopy and SEM.

When platelets were labeled at 37°C In111-uptake rose to about 90 % during the first 60 seconds and was not improved up to 30 min incubation. PGI_2 had no effect on uptake but reduced the occurence of aggregates and adhesion to surfaces (Table 1).

Table 1

Platelets: 2.2 – 2.6 x 10^8 ml. Incubation at 37°C with In111-oxine: 9 – 12.5 µCi/ml, 2.6 – 5.5 µg oxine/ml

time	In111-uptake (%)		time	uptake (with PGI_2)
	without	with PGI_2	1 min	87.2
			2	95.5
10 sec	28.8	32.2	5	90.9
20	41.3	36.6	10	92.7
40	71.4	69.6	15	85.7
60	95.2	90.2	20	96.3
			30	87.7

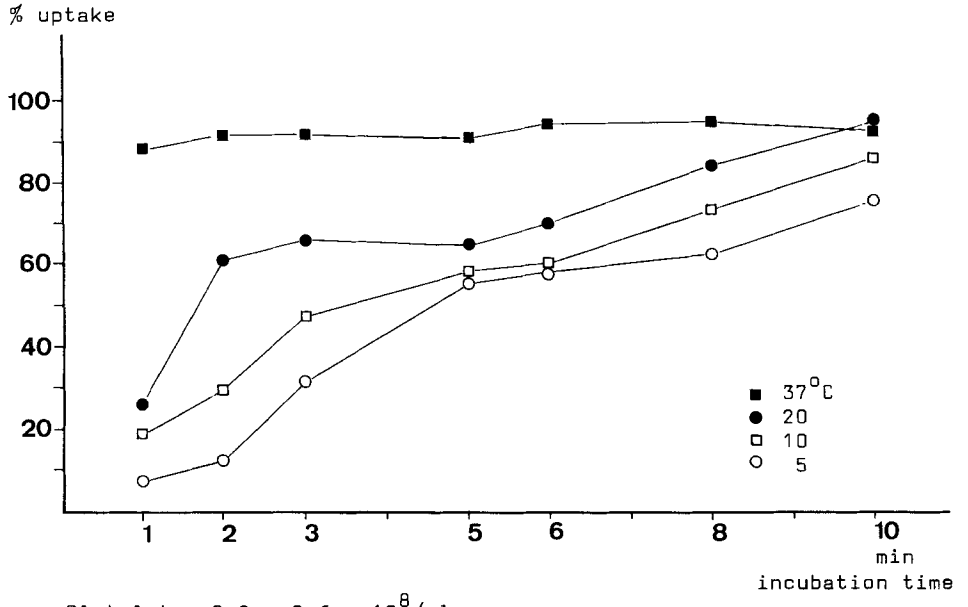
(mean values from duplicate runs)

Incubation temperature strongly influenced the labeling: below 37°C longer incubation times (10 minutes or more) were necessary to achieve comparable results (Fig. 1). Platelet concentration in the range 2 x 10^8 – 5 x 10^9/ml and oxine concentration in the range 3 – 30 µg/ml had no apparent influence on In111-uptake. The isolation and labeling procedure (total time <1 hour) caused only a slight decrease in aggregability.

Leucocytes also picked up In111-oxine rapidly at 37°C: plateau values of more than 85 % uptake were reached within 3 minutes and not significantly surpassed up to 30 minutes incubation. Lower temperatures slowed down the initial fast uptake and also decreased the plateau values (Fig. 2). Varying the leucocyte concentration from 10^7/ml to 2 x 10^6/ml slightly decreased the uptake at 37°C and had a stronger influence at lower temperatures. Leucocyte in vitro function was not affected by the isolation and labeling procedure.

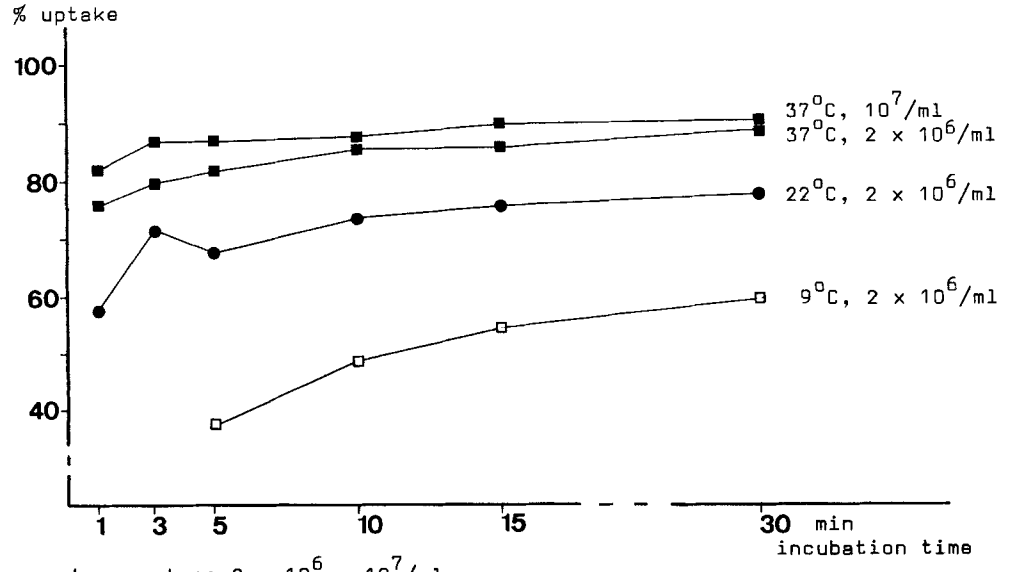
(1) Joist I.H., Baker R.K., Thakur M.L. and Welch M.I.,
J. Lab. Clin. Med. 92, 829 (1978).
(2) Goodwin D.A., Bushberg I.T., Doherty P.W., Lipton M.I., Conley F.K., Diamanti C.I. and Meares C.F.,
J. Nucl. Med. 19, 626 (1978).
(3) Scheffel U., Tsan M.F. and McIntyre P.A.,
J. Nucl. Med. 20, 524 (1979).
(4) Silberbauer K., Sinzinger H. and Winter M.,
Lancet 1978, 1356.

Fig. 1: In111-oxine uptake by platelets



Platelets: 2.2 - 2.6 x 10^8/ml
In111-oxine: 10 - 12 µCi/ml, 3.5 - 5.5 µg/ml
(data from a single incubation experiment)

Fig 2: In111-oxine uptake by leucocytes



Leucocytes: 2 x 10^6 - 10^7/ml
In111-oxine: 13 µCi/ml, 9.5 µg/ml
(data from a single incubation experiment)

A19
THE LABELING OF STAPHYLOCOCCUS AUREUS WITH IN-111 OXINE

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The In-111 oxine method of cell labeling, originally developed by Thakur and McAfee (1,2) has found wide application for tagging leukocytes (3,4), lymphocytes (5), platelets (6), and tumor cells (5). In this report we describe the further application of the method to the labeling of bacteria. Our eventual purpose in these experiments is to study the effect of blood borne distribution of bacteria on the development of osteomyelitis. The studies described herein are primarily concerned with the labeling procedure and the stability of the label.

Methods:
S. Aureus was provided by the Culture Media Laboratory of the Yale School of Medicine and grown overnight in trypticase soy broth. At the start of the experiment an aliquot was removed from the bacterial suspension and was plated using the agar pour method to determine the number of viable bacteria, about 10^9 organisms/ml. This procedure was repeated at various stages of the experiment (vide infra). The bacteria were centrifuged, washed once and resuspended in 2 mls of a modified Hank's buffered salt solution (HBSS) pH 7.4 where phosphate has been replaced by 0.005M N-2-hydroxyethyl-piperazine-N'-2-ethanesulfonic acid. The bacterial suspension was divided into a number of duplicate aliquots, 10-50 µl of In-111 oxine yielding 10-40 µCi was added; 20 µl of the suspension was removed in duplicate and the radioactivity counted. The suspension was then incubated in a water bath at 37°C with gentle agitation. After incubation the nonspecifically bound In-111 was separated from the S. Aureus by three washes with cold HBSS. The bound activity was measured by counting an aliquot from the bacterial suspensions in an automatic well-type counter (1 µCi/1.0210^{6}CPM). This identical protocol was followed for the controls with the exception that the controls contained only activity and buffer. Any significant activity in controls was subtracted from the samples (usually ≤1% of added activity). The results are given as the mean ± SEM of duplicates and the student t-test was used to test for statistical significance.

Result:
The binding efficiency of In-111 oxine for bacteria as a function of time is shown in Table 1A. These data show that In-111 oxine can label S. Aureus with high efficiency in a relatively short time. Since the 30 min incubation tended to give better overall labeling than either 10 or 60 min incubations ($.1<p<.2$) this incubation time was used subsequently. The In-111 oxine concentration in the incubation solution was varied between .986-77.2 µCi/10^6 cells to examine its influence on labeling efficiency. The data was plotted as the In-111 concentration in the incubation solution per 10^6 cells (graph not shown) vs In-111 bound/10^6 cells and there was a high positive linear correlation (R=.95). This suggests the number of organisms in the incubation solution is important in determining the amount In-111 eventually bound per bacteria.

Since we eventually wished to examine the distribution of live bacteria in an animal it was necessary to determine if this protocol for labeling was deleterious to the organism. The results showed that first step in the experiment, the removal of the growth media and subsequent resuspension in HBSS pH 7.4, caused a considerable decrease in the surviving fraction of S. Aureus, while the incubation with In-111 oxine and subsequent washings produced only a moderate, if any, additional loss of bacteria (Table 1B). To provide an estimate of in vivo generation of "free" In-111 oxine, the labeled bacteria were incubated at 37°C in a water bath. At appropriate times the preparation was centrifuged, the supernatant assayed for radioactivity, the bacterial pellet resuspended and replaced in the water bath. An appropriate control was run in parallel to sample. Relatively little unbound In-111 was generated by this procedure; at 3.75 hours only 11.35±3.2% of the activity originally in the bacterial pellet was in the supernatant. Incubation of the sample longer, for 17.75 hours total, only modestly increased the activity in supernatant (14.0±2.9%).

To investigate the mechanisms of the labeling, the influence of incubation temperature on the efficiency was determined. Temperature had no significant effect on labeling (Table 2A). This suggests that bacterial metabolism, reduced at lower temperatures, was not involved. To further examine the influence of bacterial metabolism, duplicate samples of normal and heat "killed" (80°C for 5 min) S. Aureus were labeled. There was no significant difference in cell labeling between normal and heat "killed" S. Aureus even though the heat reduced the number of live organisms, five orders of magnitude (Table 2B).

Discussion:

Thus In-111 oxine can label S. Aureus in a relatively short time with a very high efficiency. The efficiency compares favorably to the standard labeling technique in which the organisms are grown overnight with ^{14}C labeled amino acids (7). The specific activity of the ^{14}C labeled bacteria is usually very low, 200 CPM/10^6 cells. In this study a maximum of 56,000 CPM/10^6 cells was obtained. However, since it appeared the cell labeling was linearly related to In-111 incubation concentration/10^6 cells, a higher specific activity could be attained, if necessary. The effect of In-111 concentration, temperature and heat killing the S. Aureus on labeling efficiency, suggests the In-111 oxine binding is a passive phenomena i.e., requires only the cell's surface membrane and is non-energetic. This is consistent with the work of Hwang (8) and others (3,6) which suggests that the In-111 oxine complex is intercalated in the lipid bilayers of the surface membrane of cells. This must be fairly tight binding since long incubation at 37°C causes only a small amount of spontaneous dissociation.

(1) McAfee J.B. and Thakur M.L., J. Nucl. Med. 17, 488 (1976).
(2) McAfee J.B. and Thakur M.L., J. Nucl. Med. 17, 480 (1976).
(3) Thakur M.L., Segal A.W., Louis L., Welch M.J., Hopkins J. and Peters T.F., J. Nucl. Med. 18, 1022 (1977).
(4) Thakur M.L., Coleman R.E. and Welch M.J., J. Lab. Clin. Med. 89, 217 (1977).
(5) Chisholm P.M., Danpure H.J., Healey G., Osman S., J. Nucl. Med. 20, 1308 (1979).
(6) Joist J.H., Baker R.K., Thakur M.L. and Welch M.J., J. Lab. Clin. Med. 92, 829 (1978).
(7) Wang-Ivenson P., Pryzwansky K.B., Spitznagel J.K. and Cooney M.H., Infec. Immun. 22, 945 (1978).
(8) Hwang K.J., J. Nucl. Med. 19, 1162 (1978).

TABLE 1

A. S. Aureus Labeling with In-lll oxine as function of incubation time

Incubation Time [a]	Cell Bound Activity		
	µCi†	µCi/10^{6}†S x10^{-3}	%†
10	17.3±0.9	5.51±0.29	68.0±10.0
30	19.4±0.1	6.18±0.03	73.6±02.1
60	18.2±0.5	5.8 ±0.16	56.2±02.6

B. Influence of In-lll oxine labeling protocol on viability of S. Aureus

	Exp 1		Exp 2	
	Organisms/ml[S]	viable (%)	Organisms/ml[S]	viable (%)
Before Protocol	1.57x10^{9}	100.0	12.0x10^{8}	100.0
Before addition of In-lll oxine	1.20x10^{9}	76.4	5.7x10^{8}	47.5
After washing and centrifugation	1.20x10^{9}	76.4	4.8x10^{8}	40.0

a incubation temperature 37°C and 28.2±2.8µCi average activity added
† mean of duplicates ±SEM
S viable organisms as determined by pour plate technique

TABLE 2

A. Labeling efficiency of S. Aureus with In-lll oxine as influenced by incubation temperature

Incubation Temperature[a]	Cell Bound		
	µCi	µCi/10^{6}†S x10^{-3}	%†
0°C	16.9±1.2	5.44±0.44	68.8±0.80
25°C	16.6±1.8	5.38±0.57	71.8±4.7
37°C	19.1±0.0	6.20±0.001	73.1±2.3

B. Influence of S. Aureus viability on cell labeling efficiency

	Cell Bound		Viability	
	µCi/ml†	%†	Cells/ml[S]	%‡
Normal	.724±.154	57.6±9.3	4.8x10^{8}	40
Heat Killed	.690±.044	63.3±4.7	3 x 10^{3}	.00025

a 30 min incubation time and 26.6±1.4 µCi, average activity added
† mean of duplicates ±SEM
‡ percentage of viable cells at the start of experiment, 12x10^{8} cells/ml
S viable organisms as determined by pour plate technique

A20

RADIOIODINATION OF ARACHIDONIC ACID: PREPARATION AND EVALUATION

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As a precursor to the prostaglandin axis (1), radiolabeled arachidonic acid was considered to have potential for following associated physiological processes in vivo (2). Since at least one of the four double bonds of arachidonic acid (AA) is not essential for prostaglandin biosynthesis (3), partial iodination of the parent compound was investigated with the aim of producing a mixture of isomers, some of which might retain biological activity associated with the prostaglandin axis.

Iodination was carried out by a method involving the dissolution of AA in polar aqueous solution (50% propylene glycol in water mildly buffered to pH 8.5), in situ generation of iodine, and lastly the addition of acid catalyst (HCl or HAc), required for both iodine generation and electrophilic attack on the double bonds of AA. Under these conditions, a rapid and controlled partial iodination of variable I/AA molar ratio was possible at room temperature (Table 1). The gross degree of iodination was determined by thiosulfate titration and agreed with the weight of the final product. The nature and degree of iodination was also characterized by proton NMR spectroscopy (4). The decreased proton integration for the vinyl protons was associated with an equivalent increase of a new prominent doublet in the methylene region (1.2ppm), and a new triplet on the shoulder of the major methylene peak for AA (1.3ppm). Additional new peaks and their splitting patterns, in the region from 3 to 4ppm were associated with protons bound to iodinated carbon atoms. These were used to verify the partial iodination of AA.

AA is known to aggregate human platelets, the response of which is uniquely inhibited by drugs such as indomethacin and aspirin (4). With partially iodinated AA, the platelet aggregation was delayed and diminished (60%) compared to AA (75%), but the response was inhibited by indomethacin. The radioiodination of AA was carried out by the method described. Product analysis was done by paper chromatography using hexane/ether/acetic acid (70/30/1 vols) as a solvent. 75–95% radioactivity was incorporated with AA. Incubation of either H-3AA or I-131 labeled H-3AA with cyclooxygenase, the central enzyme of the prostaglandin axis, followed by the silica gel TLC of the reaction mixture gave a new peak in either case. This peak was not observed when cyclooxygenase was pre-incubated with its inhibitor, indomethacin. When radioiodinated AA was injected into 3 dogs, the in vivo deiodination was minimal, up to 8% of the injected radioactivity was taken up by the normal myocardium, and its blood clearance resembled that of F-18 long-chain fatty acid (5), clearing more than 90% of the radioactivity within 15 minutes after injection (Figure 1).

The results suggest that the partial iodination of AA preserves at least some biological activity of the compound. This may permit the use of the compound for in vivo physiological studies.

(1) Flower F.J., "Oxygen Free Radicals and Tissue Damage", Ciba Symposium 65 (new series), Casparia, Netherlands, 1979, p. 123.
(2) Berger H., Addabbo M., Wolk S., Thakur M., Zacharis H., Caride V., Gottschalk A., and Zaret B., Abstract 35, 52nd Meeting of the Am. Heart Assoc., Nov. 12, 1979, Anaheim, California.
(3) Marcus J.J., Lipid Research 19, 793 (1978).
(4) Zanger M. and Rabinowitz J.L., J. Org. Chem. 40, 248 (1975).
(5) Knust E.J., Kupfernagel C.H. and Stocklin G., J. Nucl. Med. 20, 1170 (1979).

TABLE 1

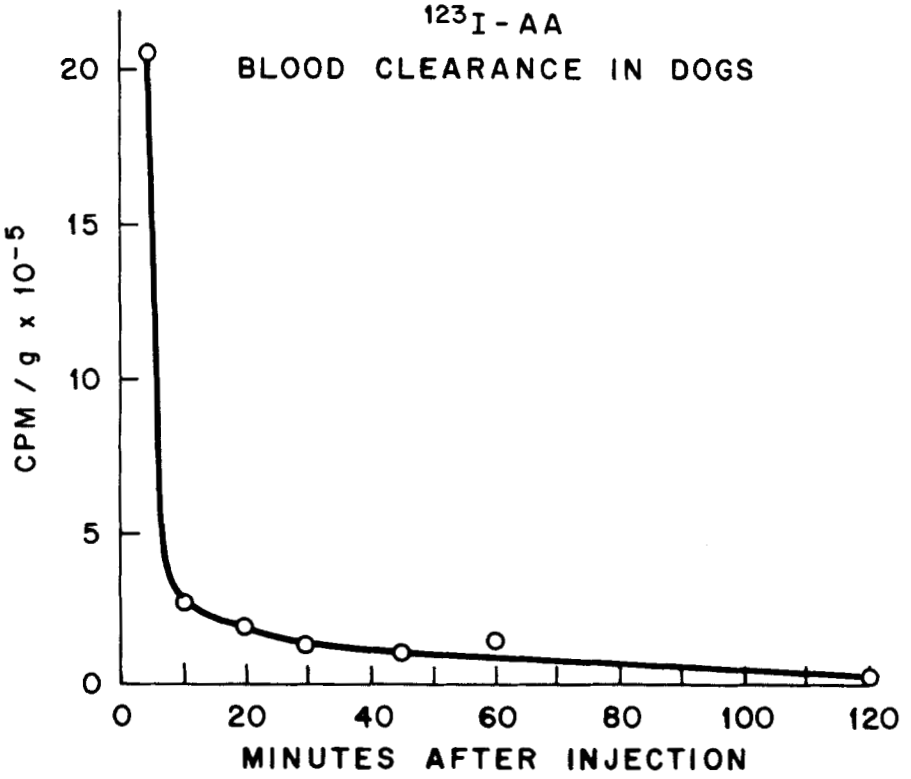
Partial Iodination of AA

RUN	H^+	AA	I_2		I/AA
	Molarity	M Moles x 10^3	M Moles x 10^3		Molar Ratio
			Generated	Reacted	
1	0.04	82	11	3	0.07
2	0.15	82	30	22	0.54
3	0.36	82	60	51	1.24

TABLE 2

Distribution of Radioiodinated AA in Dogs

Organ	Two Hours Post Injection (% of administered dose)	
	% dose/g	% dose/total organ
Liver	0.044 ± 0.001	46.95
Spleen	0.037 ± 0.005	6.48
Kidneys	0.044 ± 0.01	4.66
Thyroid	0.24 ± 0.03	0.56
Myocardium	0.029 ± 0.021	8.03
Lungs	0.021 ± 0.005	7.64


^{123}I - AA
BLOOD CLEARANCE IN DOGS
CPM / g x 10^{-5}
20
15
10
5
0
0
20
40
60
80
100
120
MINUTES AFTER INJECTION

A21

YTTERBIUM-169-PHYTATE, A NEW LIVER IMAGING AGENT

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Ytterbium-169(^{169}Yb) was introduced in the preparation of ^{169}Yb-DTPA as a new brain-scanning radiopharmaceutical by Hosain et al. (1). Later on, studies on the application of ^{169}Yb-DTPA in cisternography have been investigated (2-4). ^{99m}Tc-Sn-phytate, a new in vivo colloid for imaging the reticuloendothelial system was introduced by Subramanian et al. (5). The colloidal property of phytate with ytterbium has been investigated in our laboratory. The predominant gamma rays energy of 198 Kev, makes ytterbium-169 a suitable radionuclide for external radiation detection. The fast liver uptake and short biological half-time of ^{169}Yb-phytate encourage us to develope a new radiopharmaceutical for liver imaging.

Experimental studies involve the effect of the concentration of phytate and trivalente ytterbium-169 on the labelling yield of the compound. Paper chromatography was applied for determination the unbound ytterbium-169 in the preparation. 98-99% of labelling yield was obtained at optimal conditions.

In vivo study, about 80% of the total administered activity was accumulated in the liver after 5 min of i.v. administration of the radioactivity into mice. Since, negligable amount of radioactivity was found in spleen and lung. Despite the releatively long physical half-time of ytterbium-169 (32 days), a rapid execretion of the activity from the liver in rabbit was observed under gamma camera. However, more than 90% of the liver activity was cleared within 96 hr post-injection.

(1) Hosain F., Richard C.R. and Wagner H.N.,Jr., Radiology, 91, 1199 (1968).
(2) Wagner H.N., Hosain F., Deland F.H. and Som P., Radiology, 95, 121 (1970).
(3) Alazraki N., Hurwitz S., Halpern S.E. and Ashburn W.L., J. nucl. Med., 15, 643 (1974).
(4) Richard L.M. and Brookeman V.A., J. nucl. Med., 15, 786 (1974).
(5) Subramanian G., McAfee J.G., Mehter A., Blair R.J. and Thomas F.D., J. nucl. Med., 14, 459 (1973).