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RADIOIODO (Z)-1,2-BIS(4'-HYDROXYPHENYL)-1-IODO-1-PROPENE.

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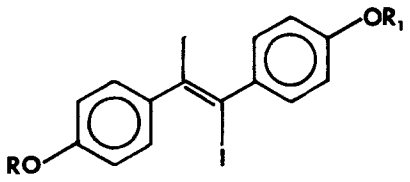
As part of a current project to develop radiolabelled estrogens, a series of compounds based on the DES (diethylstilbestrol) molecule in which one of the ethyl groups has been replaced by iodine was prepared. In accordance with the



I R = H R_1 = H
II R = H R_1 = CH_3
III R = CH_3 R_1 = CH_3

established (1) structure activity relationships, the remaining ethyl has been reduced to a methyl group in order to preserve the desired steric properties of the molecule. These derivatives have been prepared in a dihydroxy, dimethoxy and a mixed monohydroxy monomethoxy form. The ^{125}I and ^{131}I labelled molecules were generated using $Na^{125}I$ or $Na^{131}I$ and a suitable precursor, and the ^{128}I derivative by neutron activation in the U of A Slowpoke reactor.

The title compound was prepared as follows. 1,2-Bis(4'-methoxyphenyl)-2-methyl-ethanone IV was transformed into the hydrazide V with hydrazine hydrate. Treatment of the latter with a solution of iodine in ether at R.T.(2) afforded the cis isomer VI which was easily transformed into the trans III by isomerization with U.V. light. The removal of the methoxy groups was effected with trimethylsilyl-iodide (3) giving either I or II depending on the amount used.

Initial labelling experiments involved exchange labelling of I, II and III with $Na^{131}I$ or $Na^{125}I$ in DMF. This was found to proceed slowly even at 160°C. Eventually the reaction could be performed at 60°C in 4 hours using cuprous ion as a catalyst. Presumably this improvement was due to the formation of an "ate" complex. Recently in an attempt to prepare labelled I, II and III of much higher specific activity, the cyano and the fluoro derivatives VII and VIII were synthesized by treatment of III with CuCN and AgF. Initial experiments with VII have resulted in the production of almost carrier free III albeit in low yield. Initial biological experiments with I, II and III showed each compound to have an in vivo ½ life of approximately 24 hours with rapid deiodination and appreciable excretion of radioactivity via both biliary and urinary routes (4).

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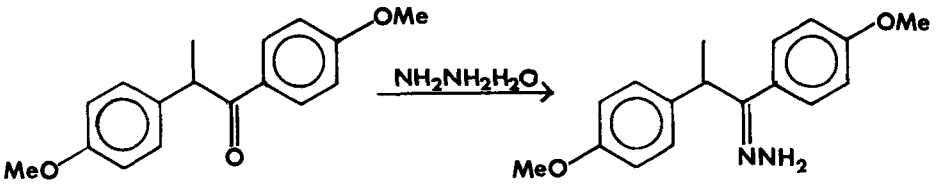
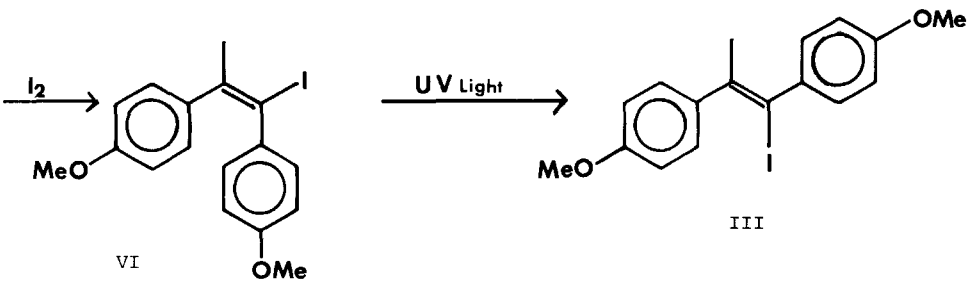
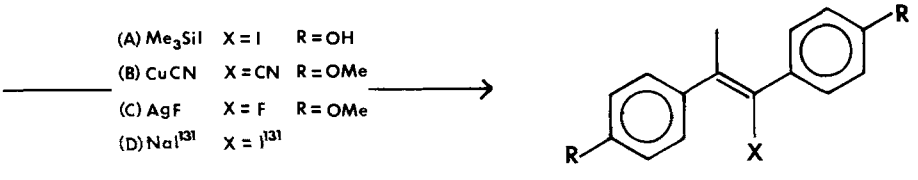
See also J.A. Katzenellenbogen, D.F. Heiman, R. Gosuami, et al. J. Nucl. Med. 20, 671 (1979) for a recent paper in which some similar tritiated derivatives were studied.



IV V



VI III



Substrate	Nucleophile	Catalyst	Temp.	Time	Yield
III	NaI^{131}	NONE	100°C	16 hrs	<20%
III	NaI^{131}	Cu^{+}	60°C	4 hrs	>95%
II	NaI^{131}	Cu^{+}	60°C	8 hrs	60%
I	NaI^{131}	Cu^{+}	60°C	8 hrs	50%
III	NaI^{125}	Cu^{+}	60°C	4 hrs	>95%
III	CuCN	-	160°C	16 hrs	>95%
III	AgF	-	160°C	16 hrs	80%
VII	NaI^{131}	Cu^{+}	160°C	16 hrs	0%

All reaction performed in DMF

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(^{77}Br)-16α-BROMOESTRADIOL-17β, A HIGH SPECIFIC ACTIVITY, GAMMA-EMITTING ESTROGEN THAT SHOWS SELECTIVE, RECEPTOR-MEDIATED UPTAKE BY UTERUS AND DMBA-INDUCED MAMMARY TUMORS IN RATS

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16α-Bromoestradiol-17β (16αBr-$E_2$17β) is synthesized from estrone 3-acetate-17-enol acetate ($E_1(OAc)_2$)(1) by treatment with bromine-acetic acid in buffered diethyl ether for 15 min at 0° C producing 16α-bromoestrone-3-acetate (16α-E_1OAc) (2) which is subsequently reduced with $LiAlH_4$ in THF at 0° C to give a 1.7 : 1 mixture of 16αBr-$E_2$17β and 16αBr-$E_2$17α.(3) These epimeric alcohols are readily separable by chromatography on silica gel.

Binding to the estrogen receptor, determined by competition with (^{3}H)-estradiol-17β (E_2-17β) (in lamb uterine cytosol) indicates affinities of 125% for 16αBr-$E_2$17β and 5.5% for 16αBr-$E_2$17α relative to that of estradiol-17β (100% represents K_a of 3 x 10^9 M^{-1}). Based on our empirical model for non-receptor binding (4), 16αBr-$E_2$17β is expected to have a binding selectivity (distribution between receptor versus non-receptor binding sites) comparable to that of E_2-17β.

The synthesis of (^{77}Br)16αBr-$E_2$17β followed that of the unlabeled material, as outlined in Figure 1. $E_1(OAc)_2$ in acetate-buffered THF-Et_2O at room temperature was mixed with Na^{77}Br (produced by spallation at the Los Alamos Meson Physics Facility (5)), 30% H_2O_2 and glacial acetic acid. Organic incorporation of the ^{77}Br was 65-80% complete within 3 hours. Analytical hplc after work-up shows a single radioactive peak corresponding to 16αBr-E_1OAc. This material was reduced with a clarified $LiAlH_4$ solution in THF for 10 min at -78° C, followed by quenching with THF-ethyl acetate (1:1) at -78° C, to minimize reductive and hydrogenolytic debromination. The resulting epimeric alcohols were separated by hplc on a Whatman PARTISIL M-9 column to yield 75% (^{77}Br)16αBr-$E_2$17β and 25% (^{77}Br)16αBr-$E_2$17α. The separation allows the isolation of (^{77}Br)16αBr-$E_2$17β with no impurities detectable by ultraviolet absorbance or radioactivity.

Tissue uptake studies were done in immature (25 day) Sprague-Dawley rats and mature (110-130 day) Sprague-Dawley rats bearing mammary adenocarcinoma (induced by intravenous injection of 5 mg 7,12-dimethylbenz(a) anthracene (DMBA) on day 50). The rats were injected intravenously and sacrificed after one hour. Five immature rats were injected with (^{77}Br) 16αBr-$E_2$17β; doses of 2-20 μCi were administered, with no differences in tissue distribution. The data in Table I show the average % injected dose per gram for each of ten tissue samples, and the ratios of counts per gram to counts per gram of blood. Five rats were injected with 15 μg estradiol (E_2-17β) in addition to 20 μCi (^{77}Br)16αBr-$E_2$17β. As shown in Table I, the average uterus-to-blood ratio decreases from 13.0 to 1.95 in the presence of estradiol, confirming that the uptake of the radiolabeled estrogen is receptor-mediated.

Four mature rats bearing mammary adenocarcinoma were injected with 20-150 μCi (^{77}Br) 16αBr-$E_2$17β, and four were administered 15 μg estradiol in addition to 20 μCi (^{77}Br)16αBr-$E_2$17β. As shown in Table II, the presence of 15 μg estradiol decreases the uterus-to-blood ratio from 13.1 to 1.29, and the mammary tumor-to-blood ratio from 6.27 to 1.00.

In summary, the synthesis of ^{77}Br-labeled 16αBr-$E_2$17β has been accomplished in high yield. Tissue distribution studies indicate selective, receptor-mediated uptake of this high specific activity gamma-emitting estrogen in uterus and DMBA-induced mammary tumors in rats. An estimate of 500 Ci/mmole for the minimum value of the specific activity of the (^{77}Br)16αBr-$E_2$17β was determined from the uptake of the radiolabeled estrogen in mammary tumors which contain ∿1 pmole estrogen receptor per gram (6), assuming a maximum of 33% receptor saturation.

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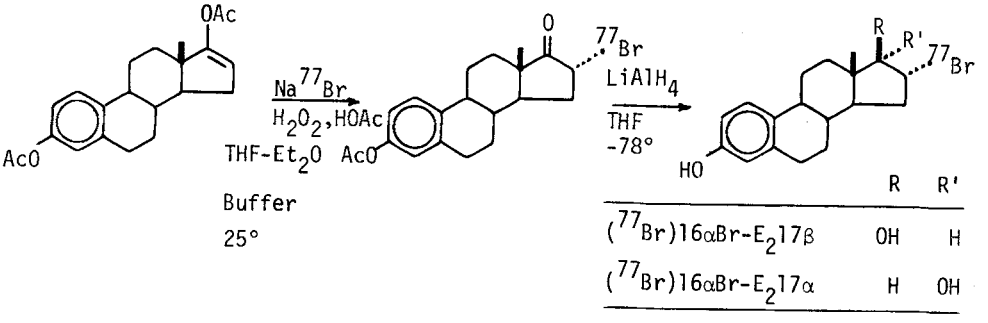

FIGURE 1. Reaction Scheme for the Synthesis of (^{77}Br)16αBr-$E_2$17β

TABLE I. Tissue Uptake Studies of (^{77}Br)16αBr-$E_2$17β in Immature Rats*

	% ID per gram	cts per gram / cts per gram of blood	
	(^{77}Br)16αBr-$E_2$17β	(^{77}Br)16αBr-$E_2$17β	(^{77}Br)16αBr-$E_2$17β + 15 μg E_2-17β
blood	0.49±0.12	1.00	1.00
uterus	7.83±2.20	13.0±3.4	1.95±1.24
ovaries	2.17±0.29	2.43±1.99	2.54±2.42
lung	0.58±0.08	1.15±0.19	1.20±0.46
kidney	1.23±0.28	2.18±1.42	1.39±0.79
spleen	0.31±0.03	0.59±0.23	0.91±0.35
liver	2.36±0.50	5.02±2.08	3.96±1.87
stomach	0.91±0.38	2.03±0.78	1.22±0.83
esophagus	0.85±0.87	1.19±0.51	2.33±1.51
muscle	0.67±0.22	1.19±0.51	1.07±1.14

*five rats were injected in each case

TABLE II. Tissue Uptake Studies of (^{77}Br)16αBr-$E_2$17β in Mature Rats Bearing Mammary Adenocarcinoma*

	cts per gram / cts per gram of blood	
	(^{77}Br)16αBr-$E_2$17β	(^{77}Br)16αBr-$E_2$17β + 15 μg E_2-17β
blood	1.00	1.00
tumor	6.27±2.66†	1.00±0.17§
uterus	13.1±2.7	1.29±0.20
ovaries	5.10±0.67	2.17±0.51
lung	1.27±0.12	1.25±0.11
kidney	2.48±0.46	1.43±0.12
spleen	1.23±0.12	0.99±0.11
liver	4.12±0.60	3.88±0.68
stomach	0.87±0.62	1.68±1.94
esophagus	2.37±1.22	0.93±0.34
muscle	1.10±0.28	1.00±0.17

*four rats were injected in each case

†eight tumors in four rats

§seven tumors in four rats

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ENHANCING RADIOCHROMATOGRAPHY SCANNING: A PRACTICAL APPROACH

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The most frequent way of measuring the radiochemical purity of a given labelled compound still is to subject the compound to one or another form of chromatography or electrophoresis with a subsequent scanning of the chromatogram/electrophoregram.

The different methods for running the chromatograms/electrophoregrams are usually described in some detail; often a strict adherence to the described method is necessary in order to obtain reliable, reproducible results.

The actual scanning procedure, however, has so far received only little attention. Apart from some papers dealing with the theoretical aspects of f.ex. the importance of a correct background subtraction (1) only little has been published in this field. In practice, the individual laboratories have been left to do their own instrument optimization on an empirical basis. We felt there was a need for a Pharmacopoea standard, describing the requirements to be fulfilled by a scanning instrument. In particular, parameters like resolution, efficiency, stray background interference, and collimation seemed relevant.

As most chromatogram scanners today are of the moving chromatogram/fixed detector type, an approach based on a chromatogram phantom seemed the best solution. The requirements of such a phantom should be

1. Possibility to estimate collimator efficiency and counter resolution with a free choice of isotopes, scanning speed, and slit width
2. Possibility to discover any lon-linear response throughout the length of a chromatogram
3. Small sample volume(s)
4. Easeness of operation and decontamination.

With these considerations in mind we have constructed a phantom (based on an idea from a HEW publication (2)) consisting of a perspex plate with parallel, 1 mm wide and 1 mm deep grooves. The grooves are ground with varying distances symmetrically around a median groove close to the middle of the phantom. The grooves are fitted with a thin silicon rubber tubing, which fits snugly into the grooves. The open ends of the tubing are fitted with standard 21G tuer injection needles (cut short) and teflon stopcocks. The tubing may be in one length (wound in zig-zag through the grooves) or in several lengths wound in patterns suitable for one's purpose.

When filled with a radioactive solution the pattern of tubing represents a "chromatogram" with "bands" of activity. With the tubing in one length the bands contain equal amounts of activity as viewed by the detector.

We have used the phantom on 3 different scanners with different isotopes (Tc-99m, I-131 and Na-22). The results showed great differences; in particular the resolution and stray background interference showed large variations. The results showed large differences in scanner performance with different types of detectors and/or collimators. The results together with a detailed description of the scanning phantom will be presented.

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17.1

IN VITRO BINDING ASSAYS AS A GUIDE FOR OPTIMIZATION OF THE STRUCTURE OF STEROID RECEPTOR-BINDING RADIOPHARMACEUTICALS: ESTIMATION OF RECEPTOR VS NON-RECEPTOR BINDING

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The achievement of adequate target to background activity ratios with radiopharmaceuticals whose uptake is mediated by steroid receptors requires careful consideration of the receptor concentration in the target site and the binding affinity that the radiopharmaceutical has for the receptor and non-receptor binding proteins. Eckelman (1) has provided a model which can be used to estimate the maximum receptor-bound to free ratio that can be attained with a radiopharmaceutical, based on its receptor binding affinity and the concentration of receptor. While it is useful to know this ratio, it is more important to understand that the majority of background activity produced by a steroid radiopharmaceutical will be due to agent bound to non-receptor proteins rather than to free reagent. We have developed an approach that provides a quantitative estimate of the "binding selectivity" of a radiopharmaceutical, i.e., its distribution between receptor vs non-receptor binding sites. This approach will be illustrated in connection with the development of estrogen receptor-based imaging agents for human breast tumors.

Non-receptor binding for estrogens is of two types: high affinity (specific) and low affinity (non-specific). The high affinity (serum) binders, such as sex-steroid binding protein (SBP), show a pronounced specificity for structural and stereochemical features of ligands, but a specificity that is different from that of the estrogen receptor. Therefore, the binding of hormone receptor-based radiopharmaceuticals to these proteins can be minimized by introducing structural features (e.g., steroidal 11β-methoxy or 17α-ethynyl groups) or employing non-steroidal skeletons (e.g., hexestrol) that greatly reduce binding to SBP, but do not affect binding to the estrogen receptor. The binding to low affinity, non-receptor proteins, such as serum albumin, shows little selectivity towards ligand structure or stereochemistry, and studies by Hansch (2) have shown that serum albumin binding of neutral compounds depends mainly on lipophilicity.

To extend this analysis to the type of low affinity binding likely to be encountered with steroid radiopharmaceuticals, we have measured the non-specific binding affinity of several radiolabeled steroids and steroid analogs in uterine cytosol and have examined the correlation between their non-specific binding index (nk) and various estimates of their lipophilicity (measured or calculated octanol-water partition coefficients (P); reverse phase chromatographic capacity factors). The most satisfactory relationship (equation 1) was obtained using P calculated according to the fragment method of Rekker (3).

$$\log nk^{\text{cytosol}} = 0.438 \log P(f)_{\text{calc}} - 2.11 \qquad \ldots.1$$

It is particularly convenient to express the non-specific binding of a compound (NSB) relative to that of the parent estrogen estradiol (equation 2). This equation can be used to calculate the NSB of any new estrogen derivative.

$$\begin{aligned} \log \text{NSB} &= 0.438 \left[\log P(f)^{\text{compound}} - \log P(f)^{\text{estradiol}}\right] \\ &= 0.438 \left[\log P(f)^{\text{compound}} - 4.63\right] \end{aligned} \qquad \ldots.2$$

In addition, we propose to use the ratio of the receptor binding affinity of an estrogen radiopharmaceutical ($\text{RBA} = K_a^{\text{compound}}/K_a^{\text{estradiol}}$) to its non-specific binding (NSB) as an index of its binding selectivity (BSI).

$$\text{BSI} = \frac{\text{RBA}}{\text{NSB}} \times 100\% \qquad \ldots.3$$

The receptor binding affinity (RBA), the non-specific binding (NSB), and the binding selectivity indices (BSI) of several halogenated estrogens are given in Table 1.

It is apparent that among the estrogens bearing halogens in aromatic positions, only those containing fluorine have respectable BSI values; even though bromo and iodohexestrols have moderate receptor binding affinities, the aromatic halogens are sufficiently lipophilizing to raise the NSB values of these compounds to unacceptable levels. The situation is different for estrogens bearing halogens at aliphatic positions: 1-Substituted hexestrols all have quite high receptor binding affinities, although the NSB values for the bromo and iodo analogs remain quite high. The D-ring bromo and iodo estradiols have some of the highest Binding Selectivity Indices (BSI): their affinities for receptor are very good and introduction of the halogen at an aliphatic ring position causes only a moderate increase in NSB. While comprehensive, controlled, in vivo tissue distribution data on these compounds are not yet available, the data that do exist (4-7) support the use of the binding selectivity index as an appropriate means for estimating target to background ratios for steroid radiopharmaceuticals based on the estrogen receptor.

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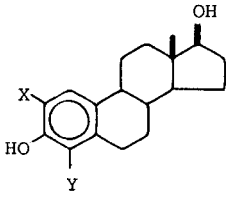
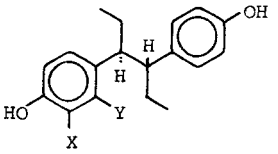
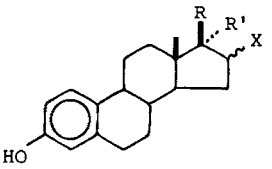
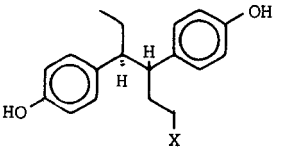
Table 1. Receptor Binding Affinity (RBA), Non-Specific Binding (NSB), and Binding Selectivity Indices (BSI) of Halogenated Estrogen Derivatives.

Aromatic Ring Halogenated Estrogens	X	Y	RAC[a] x100% (E_2=100)	NSB[b] (E_2=1)	BSI[c] (E_2=100)
	F	H	101	1.24	81
	H	F	128	1.24	103
	Br	H	1.2	2.71	0.44
	H	Br	10	2.71	3.7
	X	**Y**			
	F	H	240	3.30	73
	Br	H	19	7.21	2.6
	I	H	14	9.72	1.4
	H	F	75	3.30	23
	H	I	29	9.72	3.0
Aliphatic Halogenated Estrogens	**R**	**R' X**			
	=O	(α)Br	3.5	0.92	3.8
	=O	(β)Br		0.92	
	OH	H (α)Br	125	1.52	82
	OH	H (β)Br	5.5	1.52	3.6
	OH	H (α)I	150	2.19	68
	OH	H (β)I	57	2.19	26
		F	127	1.43	89
		Br	65	3.10	21
		I	60	4.45	13

[a] RAC = ratio of association constants: $K_a^{compound}/K_a^{estradiol}$ x 100.

[b] NSB = non-specific binding, calculated according to equation 2.

[c] BSI = binding selectivity index = RAC x 100%/NSB; by definition, for estradiol, BSI=100.

17.2

IDENTIFIABILITY OF KINETIC MODELS FOR IN VIVO RECEPTOR SYSTEMS

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Among the many mechanisms for radiopharmaceutical localization, receptor mediation currently has an exciting potential. Receptors transfer blood-borne radioindicators into the molecular biological realm of metabolism where many disease processes begin. Many researchers are now asking how external measurement of the time-dependent distribution of gamma-emitting receptor ligands can be used to estimate such important physiological properties as receptor concentrations, ligand-receptor binding constants and metabolic rates in addition to hemodynamic parameters. The latter govern delivery of the tracer to the receptor site and are thus prerequisite to localization. A successful design strategy for receptor-based radiopharmaceuticals must allow the experimenter to differentiate affects of blood flow from those of receptor binding, for without proper design, the tracer's fate could be determined solely by organ blood flow.

Consider the kinetic scheme illustrated in figure 1. The ligand dose ($L_0^!$ moles) injected into the blood may reversibly communicate via blood flow (Q ml/min) with the blood of the target organ. Once at the reaction site the ligand may reversibly combine with a fixed but unknown number of available receptors to form a ligand-receptor complex (C moles). The reaction sequence may continue in the forward direction to produce a metabolic product (P moles). The kinetics of this model can be described by the set of differential equations. Because of the second-order nature of the ligand-receptor interaction, the set will be nonlinear. The degree of non-linearity will depend upon the ratio of ligand dose ($L_0^!$ moles) to the total number of receptors (R moles). When this ratio is below 1%, the initial condition used in most radiotracer studies, pseudo-first-order kinetics results: dR/dt is zero and $k_b^!R$ can be replaced by $k_b^!$. The binding rate constant and the receptor concentration are now mathematically inseparable. Thus, receptor mapping at tracer doses can be achieved only if the forward binding rate constant is regionally and pathologically invariant.

How can ligand-receptor binding at tracer doses be used to map receptors without blood flow being the rate-limiting step? The range of $k_b^!R$ relative to Q/V' should be chosen to allow kinetic parameters to be uniquely estimated from a set of external observations, namely the measured radiopharmaceutical distribution kinetics. This condition is referred to in the systems analysis literature as identifiability (1) and depends upon the means by which the system response is observed. In an *in vivo* receptor system the target organ is viewed by a single detector (D_2) that will measure both the ligand-receptor complex (C) and the free ligand (L) in the blood within the organ. A second detector (D_1) would be used to monitor the labeled components (L') of blood outside of the target-organ. Together they make up a set of observers by which the response of the system is recorded and its parameters numerically estimated. An estimate of the magnitude of a parameter can be made only if an alteration of its value elicits a change in the observed response. This condition is referred to as system observability. To investigate the range of forward binding rate constants that allow observation of altered ligand uptake produced by changes in receptor concentration, we performed a number of kinetic simulations of a ligand-receptor system (2) similar to that shown in figure 1. The first set of simulations given in figure 2 reveal an observable system, because different observer responses are produced by progressive decreases in receptor concentration.

In addition to the property of observability, the model must also be identifiable; that is the computer program must be able to a priori select a set of model parameters that produce a unique minimum of chi square. Standard linear algebraic methods are used to examine an n-dimensional surface of error versus each parameter. The best fit occurs at the bottom of an error parabola on this surface. If the error parabola has steep edges it will be easier to find than if it is a

broad trough. We have quantitatively evaluated the error parabola of several models by calculating an "identifiability index" which should ideally approach unity for a steep or easily identifiable system.

The receptor system (Figure 1) using the rate constants listed on figure 2 is judged to be identifiable with an identifiability index of 10^{-3}. We have assumed the collection of 10^7 counts and the requirement of 95% confidence level for a 25% change in any parameter value. Given these criteria, a value of 10^{-3} is the minimum for practical identification. Figure 3 illustrates a system within which a 50% decrease in receptor population fails to produce an observable change in either detector. The only difference between the two systems is a 1000-fold increase in the forward binding rate constant, k_b. It should be obvious that it would be difficult to estimate the magnitude of a parameter which has no influence upon the system's behavior. As a result, we would expect an unobservable system to also be unidentifiable. This is confirmed by the computed identifiability index of 10^{-6} and is numerically unidentifiable regardless of observer precision. A value of 10^{-6} is within the range of computational noise. Note the expected rate limiting kinetics indicated by the altered response to a 25% decrease in organ blood flow (Q).

In order to more generally apply the above results we have found it helpful to relate the identifiability index of the ligand-receptor model to the ratio of the pseudo-first order rate constant (k_bR) to the forward first order hemodynamic transfer constant (Q/V'),

Model	k_b $(sec \cdot mole)^{-1}$	$\frac{k_bR}{Q/V'}$	Identifiability index
HBP (2)	5.0×10^3	5.1×10^{-1}	10^{-3}
	5.0×10^6	5.1×10^3	10^{-6}
MQNB (3)	8.3×10^8	2.8×10^3	10^{-5}
Spiperone (4)	3.2×10^8	1.2×10^4	10^{-7}
Haloperidol (4)	9.8×10^5	1.8×10^1	10^{-3}

In conclusion, given the classical ideal radiopharmaceutical requirements of high target to background, low radiation dose and low extratarget uptake, a tracer radioligand can be used to map receptor sites providing the forward rate constants governing the transfer and binding of the ligand are of the same order of magnitude and that the binding rate constant is regionally and pathologically invariant.

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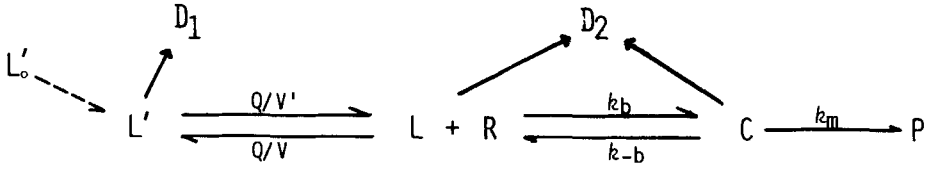
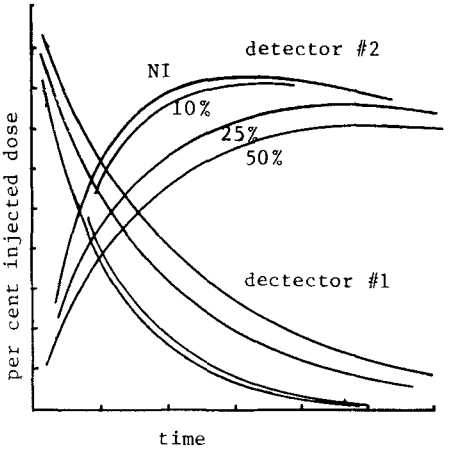
Figure 1



L' = moles of ligand in extra target organ blood
L = moles of ligand in target organ blood
R = moles of target organ receptor
C = moles of ligand receptor complex
P = moles of metabolic product
L'_o = moles of injected ligand
Q/V= blood transfer rate constant (sec^{-1})
k_b = forward binding rate constant (sec^{-1} $mole^{-1}$)
k_{-b}= reverse binding rate constant (sec^{-1})
k_m = metabolic rate constant (sec^{-1})
D_1 α L' = precordial
D_2 α L + C = target organ detector

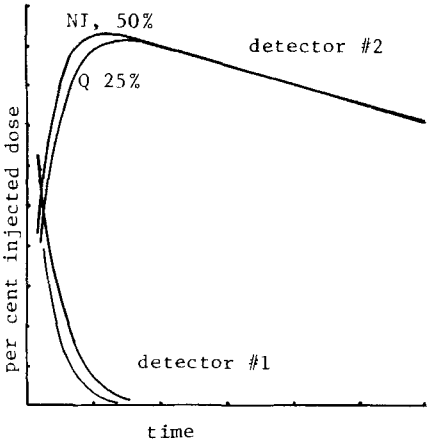
FIGURE 2



Simulation Parameters

R_o = 3 x 10^{-6} moles = NI
L'_o = 3 x 10^{-8} moles
k_b = 5 x 10^3 $mole^{-1}sec^{-1}$
k_m = 1 x 10^{-4} sec^{-1}
Identifiability Condition
3 x 10^{-3}

FIGURE 3



Simulation Parameters

R_o = 3 x 10^{-6} moles = NI
L'_o = 3 x 10^{-8} moles
k_b = 5 x 10^6 $mole^{-1}sec^{-1}$
k_m = 1 x 10^{-4} sec^{-1}
Identifiability Condition
1 x 10^{-6}

17.3

(^{99m}Tc)-NEOGALACTOALBUMIN: A GENERAL MODEL FOR SOME BIFUNCTIONAL CARBOHYDRATES

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Carbohydrates covalently attached to polypeptide chains form a class of molecules, glycoproteins, that is widely distributed in nature. Because glycosidic bonds allow extensive structural branching that is unimportant in peptide bonding, the number of conceivable oligosaccharide configurations is large compared to that for polypeptides. This class of molecules is important in nature because cell-surface glycoproteins, with their highly variable arrangements of sugar structures, serve as identifying markers that allow cells to uniquely distinguish individual chemical structures via an electrostatic "lock-and-key" interaction with matching glycoproteins. We are convinced that the chemical specificity encoded in carbohydrate structures can be exploited to develop new receptor binding tracers that will be useful tissue-specific radiodiagnostic agents. The general developmental scheme involves identifying receptors on the cell surface that are unique to the tissue of interest and then labeling the matching receptor ligand. In this paper we will describe our application of this concept to ligands for hepatic binding protein (HBP), specifically (^{99m}Tc)-neogalactoalbumin, and offer some general comments on the applicability of synthetic bifunctional carbohydrates to other cell receptors.

In recent years researchers have identified a hepatic pathway for the accelerated elimination of desialylated glycoproteins. Their removal from circulation was mediated by membrane receptors found only on hepatocytes (1). The common structural feature for all glycoprotein derivatives which were susceptible to rapid hepatic clearance was the presence of non-reducing unmodified terminal galactose. When asialoproteins were subjected to β-galactosidase digestion to cleave the galactosyl residue, or galactose oxidase to convert C6 from an alcohol to an aldehyde, the resulting glycoproteins showed circulatory survival times similar to their native counterparts (1). Trypsinized asialoglycoproteins showed the same clearance kinetics as the intact asialoglycoprotein (2), indicating the relative unimportance of the peptide backbone in accelerated clearance. The HBP receptor is highly specific for ligands containing exposed unmodified galactose in the appropriate anomeric form.

Although we have successfully used Tc-99m-asialoceruloplasmin for hepatic imaging in rabbits (3-4), its routine use in humans was inevitably limited due to a variety of practical considerations. Instead of enzymically removing terminal sialic acid groups to expose the molecular ligand, galactose, a more clinically acceptable analog was produced by adding the galactose unit to a human serum albumin backbone. The coupled carbohydrate has been named neogalactoalbumin (NGA).

Of the many methods available for attaching sugars to proteins, we chose amidination using 2-imino-2-methoxyethyl-1-thioglycosides (5). It has the practical advantages of convenience, low cost, high chemical stability, and coupling at mild pH. Amidination is specific for primary NH_2, the amidine bond and thioesters are stable under acidic conditions (6,7) that are used for technetium labeling and the thio-C_1 ester is resistant to plasma and membrane glycosidases (7). Details of our synthetic procedure are presented in figure 1.

The extent of sugar attachment was controlled by the molar ratio of reacting IME-thiogalactose and albumin. We can reproducibly achieve any desired average ratio from 3 to 40 gal/HSA. The HBP receptor binding affinity of NGA depends on the number of galactose residues per albumin molecule and may be adjusted over several orders of magnitude (8).

Technetium labeling was done by the electrolytic method of Benjamin (9) as modified by Dworkin (10). Immediately after electrolysis (100 mA, 42 sec), 100μl of 25% NGA was added, incubated 30 min at room temperature, neutralized, and then purified by polyacrylamide P-2 gel chromatography. Electrophoresis of the unpurified reaction product at various times after labeling indicated that the product lost 5% of its initially bound activity (90-95%) within 20 min but the remaining 90% was completely stable for several hours.

One concern related to the use of Tc-NGA as a liver radiopharmaceutical was the possibility of aggregation which could convert a hepatocyte agent into a reticuloendothelial agent. The molecular weight distribution was measured by P-300 polyacrylamide gel chromatography. Just prior to elution of the first peak, the column was temporarily removed from the chromatographic system and scanned. Activity at the top of the column would indicate an aggregate which had not penetrated the gel and any material at the void volume would suggest a high MW product which could localize via phagocytic cells rather than by HBP binding. The elution profile reflected only the small molecular weight shift produced by multiple galactose residues per HSA molecule. There was no radiopharmaceutical in the void volume or at the top of the column, indicating that the Tc-NGA was consistently free of colloids.

With the confidence of high HBP ligand purity, Tc-NGA was injected into rabbits. A time-activity curve generated over a precordial ROI showed rapid blood clearance, 5% remaining in circulation at 15 min. A curve over a hepatic ROI showed a complementary rapid increase involving two phases, the first related primarily to hepatic blood flow and the slower second phase to receptor binding. The liver curve peaked at about 15 min and then gradually decreased as lysosomal enzymes began to break down the Tc-NGA. From 4-6% of the injected Tc appeared in the urinary bladder shortly after injection, but within 15 min this concentration started to slowly rise as labeled lysosomal products (ionic Tc-99m) returned to the blood and were excreted by glomerular filtration. The urine at 30 min contained approximately 15% of the injected dose. In all rabbits we saw a small amount of GI activity, but gallbladders removed at 2 hrs contained $\leq 0.1\%$ of the injected Tc. Ligation of the common bile duct did not prevent gut localization, suggesting that it resulted from ionic Tc in the stomach secondary to lysosomal breakdown.

We believe that Tc-99-neogalactoalbumin has the features needed for successful cell-receptor imaging of the liver. It can be synthesized with the number of gal/HSA required to produce the desired receptor affinity, its labeling is rapid and efficient, it produces a tracer ligand of high purity and stability, and it binds only to hepatocytes. Tc-NGA is different from all other liver agents in that it accumulates in hepatocytes and stays there for sufficient time to allow multiple images of that organ. It does not compete with bilirubin, thus its value is independent of jaundice, and the tracer does not enter the biliary tract.

In addition to neogalactoalbumin for hepatic scintigraphy, other synthetic lectins might be useful radiopharmaceuticals. For example, gal-β-gal-β-albumin could be useful for imaging the myocardium (11) and mannose-β-albumin for the renal tubules (12,13). Lectins have been identified that bind to pancreatic membrane and to some tumor cells but the exact carbohydrate groups causing binding have not been identified. Each of these receptor systems suggest areas for developing new bifunctional carbohydrate radiopharmaceuticals.

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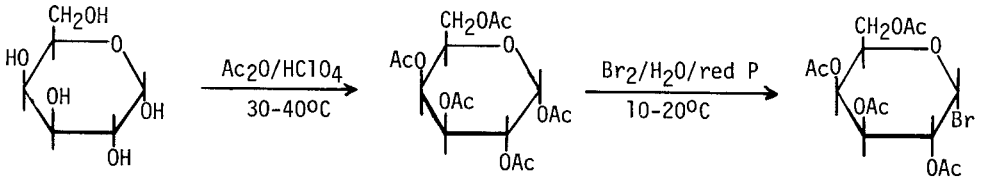
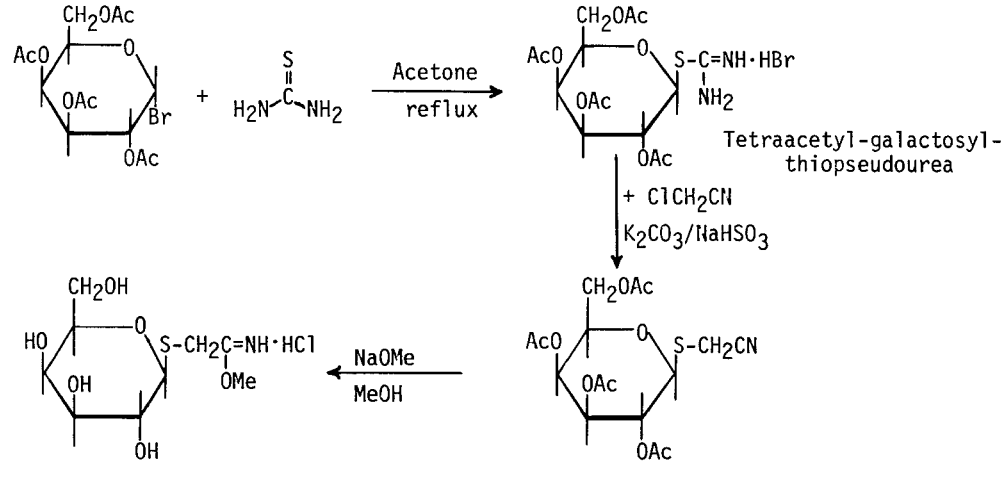
Figure 1. Synthesis of IME-thiogalactose and neogalactoalbumin.



Galactose | Galactose pentaacetate | Acetobromo-galactose

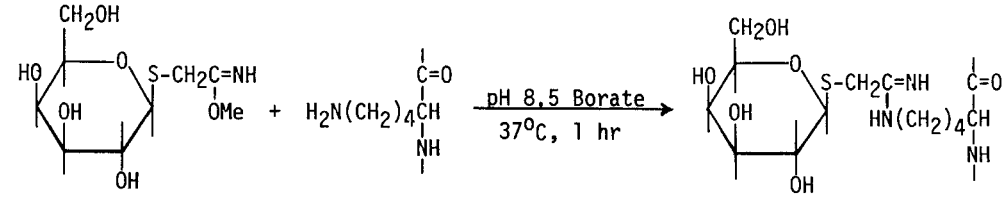
Acetobromo-galactose is extracted with chloroform, evaporated to a syrup and recrystallized with ether. (14) The yield is 70%.



2-Imino-2-methoxyethyl-1-thio-β-D-galactose (IME-thiogalactose)

Cyanomethyl-tetraacetyl-thiogalactose

This reaction sequence is described in references 5 and 15. The yield of each step is about 75%. The cyanomethyl is the best derivative to store.



IME-thiogalactose | Albumin (lys) | Neogalactoalbumin

The neogalactoalbumin is purified by hollow-fiber dialysis against sterile saline. It is then membrane filtered, dispensed into multidose vials and lyophilized.

17.4

(R,S)-3-QUINUCLIDINYL-4-IODOBENZILATE (4-IQNB). THE TRIAZINE APPROACH

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The development of receptor binding radiotracers for myocardial imaging depends on a high concentration of receptor in the heart and a radioligand with a high affinity for that receptor. The binding of QNB to the muscarinic cholinergic receptor in the heart is an example of such as system. In animals heart to blood ratios of as high as 30 are obtained using tritiated QNB (1). Based on these results the development of a gamma emitting derivative of QNB would allow the external detection of the change in blood flow or receptors as a function of disease.

Our early attempts to synthesize 4-IQNB through a direct thallation of QNB were unsuccessful. The second synthetic variant - the nitration of either benzilic acid or benzil - was similarly abortive. The only successful synthetic route to date is the one described below:

4-Aminobenzil (I) was synthesized according to a modified method of Augl and Duffy (2) requiring six synthetic steps. The overall yield was poor (< 10%) but the availability and inexpensiveness of the starting materials renders this approach practicable. The 4-aminobenzil was rearranged to yield 4-aminobenzilic acid (II) which in turn was esterified to its ethyl ester (III). The ethyl ester of 4-aminobenzilic acid was reacted with quinuclidin-3-ol to give the expected 4-amino-QNB (IV). The purified 4-amino-QNB was converted to the 4-triazino-QNB (V) according to the procedure reported by Tewson and Welch (3). Due to the numerous by products the purification of 4-triazino QNB is tedious and requires several chromatographic techniques.

The pure 4-triazino-QNB in a catalyzed reaction with iodide gives the expected 4-iodo-QNB (VI). The structure and purity of the 4-IQNB was ascertained by elemental analysis and mass spectroscopy.

Preliminary studies using muscarinic cholinergic receptor isolated from rat heart show that the 4-IQNB has a high affinity for the receptor.

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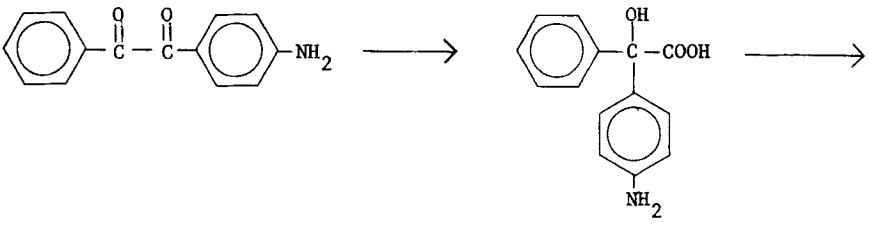
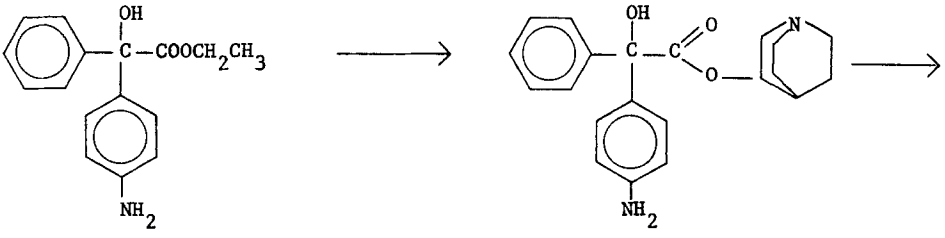
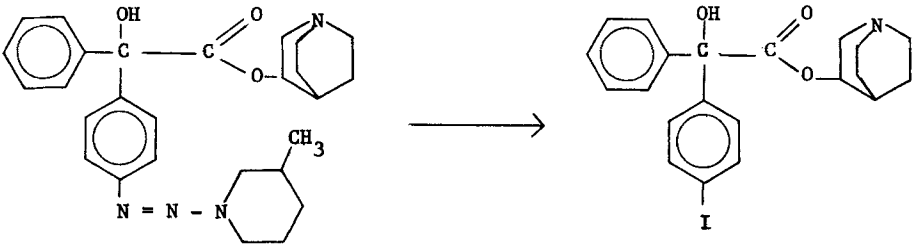


I II



III IV



V VI

17.5

RADIOLABELED ADRENERGIC AND MUSCARINIC BLOCKERS FOR IN VIVO STUDIES

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At present there are two approaches to imaging acute myocardial infarcts: (1) the use of compounds such as Tl-201 that distribute as a function of blood flow and (2) the use of compounds that concentrate in areas of active ischemia or necrosis such as the Tc-99m bone imaging agents. Another process that may be altered as a function of pathologic state is the receptor binding of alpha and beta adrenoceptor antagonists and muscarinic cholinergic receptor antagonists. From literature data it appears that both the adrenoceptors and the muscarinic receptors are present in the myocardium in detectable quantities, although this is no guarantee that these receptors can be used as a mechanism of localization of radiotracers. Because of the large number of pharmaca available and the low target to blood ratio of most therapeutic drugs, we chose a single biomolecular model to evaluate possible radiotracers (1). A combination of literature values and in vitro experiments gives the theoretical heart to blood ratios (H/B). Two tritium labeled alpha adrenoceptor blockers dihydroergocryptine and WB 4101 gave theoretical heart to blood ratios of greater than one. Upon injection in rats and guinea pigs, H/B ratios of greater than one were obtained but in vivo displacement studies showed that the ratio was not a result of alpha adrenoceptor binding. The two classes of compounds that these represent do not appear to be reasonable candidates for in vivo studies because of the combination of low receptor concentration and high nonreceptor binding. A series of beta adrenoceptor blockers were also evaluated but only one, tritium abeled carazolol (CAR) showed beta adrenoceptor binding in the heart based on in vivo displacement studies. A series of eight radioiodinated compounds showed high H/B ratios but none of these concentrated because of beta adrenoceptor binding. CAR is the first example in vivo of detectable beta adrenoceptor binding in the heart. Because no present day beta adrenoceptor blocking agent has an affinity constant greater than that of CAR, this class of compounds likewise seems unsuitable for use as myocardial receptor radiotracers. The most interesting class of compounds is the muscarinic cholinergic blockers. We have evaluated 10 tritium and halogen labeled compounds of which tritiated quinuclidinylbenzilate (QNB) gave H/B ratios of 30 (2). Specific binding to the desired receptor was proved by in vivo displacement studies. 3-Iodo-4-hydroxy-QNB did not achieve high H/B ratios in spite of theoretical values of 8 because of nonreceptor binding. Various isomers of monoiodo QNB have high theoretical H/B ratios based on in vitro determinations of their affinity constants and should have lower nonreceptor binding than iodohydroxy-QNB. These iodinated compounds have been prepared and are presently being evaluated in animals. The muscarinic receptor blockers seem to be the class of choice because of the high receptor concentration, the high affinity constants, and the low nonreceptor binding.

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TABLE 1: In Vivo Distribution of Alpha Adrenoceptor Blocking Agents in Rats.

Compound	Time (hr)	%Dose/g in Heart
WB 4101	1/4	.19 ± .03
	2	.03 ± .01
	2	.05 ± .01
	2*	.04 ± .00
DHE	1/4	.26 ± .09
	2	.07 ± .02
	2	.09 ± .05
	2*	.07 ± .01

*100 µg Regitine

TABLE 2: Maximal B/F Ratios of Alpha Adrenoceptor Blockers in Rats.

Compound	R_o	K_A (M^{-1})	B/F
DHE	3 to 4 nM	4.2×10^8	1.5
WB 4101	3 to 4 nM	2.0×10^9	7

TABLE 3: Maximal B/F Ratios for Beta Adrenoceptor Ligands (Tritium Labeled).

Compound	K_A (M^{-1})	B/F
PLP	9.1×10^7	<1
ALP	$1.2 \quad 10^9$	<1
IPL	$2.9 \quad 10^6$	<1
EPI	$6.6 \quad 10^5$	<1
NE	$6.6 \quad 10^5$	<1
NH_2ALP	$1.4 \quad 10^6$	<1
DHA	$.13 \quad 10^9$	<1
CAR	$1.6 \quad 10^9$	11.2

TABLE 4: In Vivo Distribution of Tritium Labeled Beta Adrenoceptor Ligands in Rats.

Compound	H/B 1/4 hr	H/B 2 hr
^{3}H PLP	.7	.3
^{3}H ALP	.3	.3
^{3}H IPL	.4	.4
^{3}H EPI	1.3	1.3
^{3}H NE	8.2	8.7
^{3}H NH_2ALP	0.3	0.4
^{3}H DHA	3.7	1.4
^{3}H CAR	5.7	5.1

TABLE 5: Maximal B/F Ratios for Beta Adrenoceptor Ligands (Radioiodinated).

Compound	K_A (M^{-1})	B/F
TYR-ALP	5×10^6	<1
TC-PRAC	2.6×10^5	<1
TCC-PRAC	5×10^5	<1
TYR-PRAC	5×10^5	<1
PD-3	4.3×10^6	<1
HYP	8.6×10^7	<1

TABLE 6:
In Vivo Distribution of Radioiodinated Beta Adrenoceptor Antagonists in Rats.

	Heart to Blood Ratios						
^{125}I-TYR-PRAC	^{125}I-TYR-BUN	^{125}I-TYR-ALP	^{125}I-TCC-PRAC	^{125}I-TC-PRAC	^{125}I-PD-3	^{125}I-TYR	^{125}I-HYP
11.7*	2.0	3.5	5.0	11.3	2.2	.4	.8
18.7+	3.2	1.8	4.0	6.2	0.8	.4	1.0

*1/4 hour

+2 hour

TABLE 7: In Vivo Displacement of Radiolabeled Beta Adrenoceptor Blockers by Propranolol (PLP) in Guinea Pigs.

Radiotracer	%Dose/g ± SD at 2 hr Heart
^{125}I TYR-PRAC & PLP	.10 ± .02
Control	.09 ± .01
^{125}I TYR-BUN & PLP	.08 ± .02
Control	.06 ± .02
^{125}I TYR-ALP & PLP	.30 ± .06
Control	.22 ± .07
^{3}H DHA & PLP	.13 ± .03
Control	.12 ± .01
^{3}H CAR & PLP	.07 ± .01
Control	.21 ± .05
^{125}I HYP & PLP	.13 ± .04
Control	.21 ± .09

TABLE 10: In Vivo Displacement of Radiolabeled Muscarinic Cholinergic Blockers by Atropine (ATR) at 2 hr.

	%Dose/g ± SD	
Radiotracer	Heart	Species
^{3}H MQNB & ATR	.10 ± .01	Rat
Control	.91 ± .19	Rat
^{3}H MATR & ATR	.12 ± .02	Guinea Pig
Control	.04 ± .03	Guinea Pig

TABLE 8: Maximal B/F Ratios for Muscarinic Cholinergic Blockers.

Compound	K_A	B/F
QNB	7.0×10^{9}	115
MQNB	2.0×10^{9}	33
ATROPINE (ATR)	2.5×10^{8}	4
MATR	1.0×10^{8}	1.6
pBr ATR	4×10^{7}	<1
pBr MATR	4×10^{7}	<1
BATR	1×10^{7}	<1
BrB QNB	3×10^{7}	<1
I OH QNB	5×10^{8}	8
I OH MQNB	5×10^{8}	8

TABLE 9: In Vivo Distribution of Radiolabeled Muscarinic Cholinergic Blockers in Guinea Pigs.

Compound	Time (hr)	H/B
^{3}H MQNB	1/4	32.2
	2	31.6
^{3}H ATR	1/4	1.7
	2	1.8
^{3}H MATR	1/4	3.3
	2	4.9
^{125}I OH QNB	1/4	.3
	2	.4
^{125}I OH MQNB	1/4	.5
	2	.3

17.6

PREPARATION AND BIODISTRIBUTION OF THE ANTIESTROGEN [^{125}I]IODOTAMOXIFEN

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Radioligands for the estrogen receptor have been proposed as radiotracers for the detection and external visualization of estrogen-dependent breast neoplasms. Although most investigations have utilized radioiodinated derivatives of estradiol or hexestrol no satisfactory agents have yet emerged from these studies. Because of the extensive literature concerning the clinical usefulness of tamoxifen in the treatment of breast tumors (1,2) we chose to examine a radioiodinated derivative of this compound as a potential imaging agent. In addition, its chemical structure was amenable to a novel radioiodination method which had been developed in our laboratories (3).

The preparation of [^{125}I]iodotamoxifen was achieved in two steps. The tributyltin intermediate was obtained in high yield and high purity from the reaction of tamoxifen free base with sec-butyllithium followed by tributyltin chloride. Radioiodination with [^{125}I]-I_2 gave the desired product (4.5 Ci/mmol) which was chromatographically indistinguishable from authentic 4-iodotamoxifen.

The radiopharmaceutical was evaluated in both adult female rats and immature female rats (19-21 days old) the uteri of which are a rich source of estrogen receptors. In the adult female rats, the uterus to blood concentration ratios varied from 11:1 to 14:1 with a maximum at 6 hours. Ratios of 11:1 to 23:1 were observed in the immature female rats.

Ligand displacement studies to determine specific *in vivo* estrogen receptor binding are currently in progress.

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17.7

CARBON-11 LABELLED MOXESTROL AND 17α-METHYLESTRADIOL AS RECEPTOR BINDING RADIOPHARMACEUTICALS

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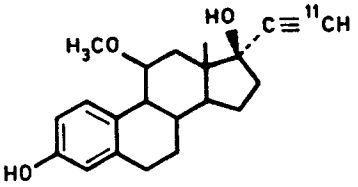
In the last decade it has been shown that the response of a mammary cancer to endocrine therapy is correlated with the concentration of estrogen receptors in the tumor and the binding of steroids to these receptors. Our efforts to develop radiopharmaceuticals, which can be used for the localisation of hormone-sensitive tumors and for the in vivo measurement of tumor response to alternations in the hormonal environment are based on these findings.

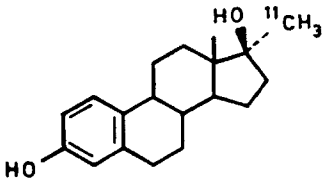
From a comparative study by Raynaud et al. (1,2) of the estrogen action of estradiol derivatives, emerged that introduction of an 17α-ethynyl group into estradiol, decreased metabolic degradation of the hormone and increased the affinity for the estrogen receptor, without destroying the specificity for this receptor. They also found that the 11β-methoxy derivative showed a total absence of specific or non-specific binding to plasma proteins. This information prompted us to investigate the potential of carbon-11 labelled 11β-methoxy-17α-ethynylestradiol (moxestrol) as estrogen receptor binding radiopharmaceutical.

Loading dose studies in mature female rats with 3H-analogue (specific activities 7 and 77 Ci/mmole) showed, 30 minutes after intravenous administration, uterus to blood radio's of 22,0 ± 7 and 19,0 ± 7 respectively and an uterus uptake of 2,5 ± 0,8 percent of the injected dose per gram wet tissue.

To investigate the biodistribution of 17α-methylestradiol the tritiated derivative was prepared by methylation of 3H-estrone following a method described previously (3). With a specific activity of 5 Ci/mmole, 30 minutes after injection an uterus to blood ratio of 17,6 ± 5,2 was found. The organ uptake was 1,2 ± 0,3% dose/g. wet tissue. From these data we concluded that ^{11}C-moxestrol (I) as well as ^{11}C-17α-methylestradiol (II) have potential as receptor binding radiopharmaceuticals.



I



II

Compound (I) was prepared by ethynylation of 11β-methoxy estrone (4) with ^{11}C-acetylene (5). Due to the production method of H^{11}C≡CH, rather low specific activities could be achieved. Improvement of the specific activity is expected after production of ^{11}C-acetylene as reported by Crouzel et al. (6). Compound (II) was prepared with a specific activity of 6 Ci/mmole by ^{11}C-methylation of estrone with $^{11}CH_3Li$.

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Group, Oss, The Netherlands.
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17.8

SYNTHESIS OF HIGH SPECIFIC ACTIVITY ^{18}F-SPIROPERIDOL FOR DOPAMINE RECEPTOR STUDIES

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There is great interest in the development of ligands labeled with shortlived positron-emitting radionuclides to map brain receptors in vivo using positron emission tomography. The studies of most receptors necessitates the synthesis of material with a very high degree of specific radioactivity.

We have recently developed the preparation of no carrier added ^{18}F-aryl fluorides using piperidyl triazenes as a source of diazonium salt (1), and further applied to the synthesis of high specific activity ^{18}F-haloperidol which is one of the most potent antagonists of the dopamine receptor (2). We have also utilized this approach to ^{18}F-labeling of the dopamine antogonist spiroperidol (II) which has been proposed as the ligand of choice for postsynaptic dopamine receptors in view of its low rate of dissociation and the high percentage of specific binding as compared to haloperidol when measured in vitro (3,4).

Our approach to the preparation of ^{18}F-spiroperidol (II) which utilized the triazene (I) is shown in the Scheme. ^{18}F-Cesium fluoride was prepared at the Washington University Medical School Cyclotron by the ^{20}Ne(d,α) reaction; as has been previously reported (5). 15% Hydrogen was added to the target gas and the nucleogenic fluorine atom reacts with the hydrogen to produce H^{18}F, then passed over a silver wool plug containing cesium carbonate to give high specific activity ^{18}F-cesium fluoride.

Initial experiments with ^{18}F-cesium fluoride were performed by adding the silver wool plug containing the activity to a solution of the triazene (I) in various solvents with methanesulfonic acid or 2-mesitylenesulfonic acid and heating at 120° for 4∿6 min. The reactions were also done using methanesulfonic acid and carrier free H^{18}F which was trapped in a Nickel tube. The fluorine-18 labeled spiroperidol was separated from the reaction mixture by HPLC. Among solvents used, bromobenzene proved to be the best solvent, but only 0.6∿7% yield of no carrier added ^{18}F-spiroperidol was obtained. Reasons for the low yield and methods of improvement are being investigated and will be discussed.

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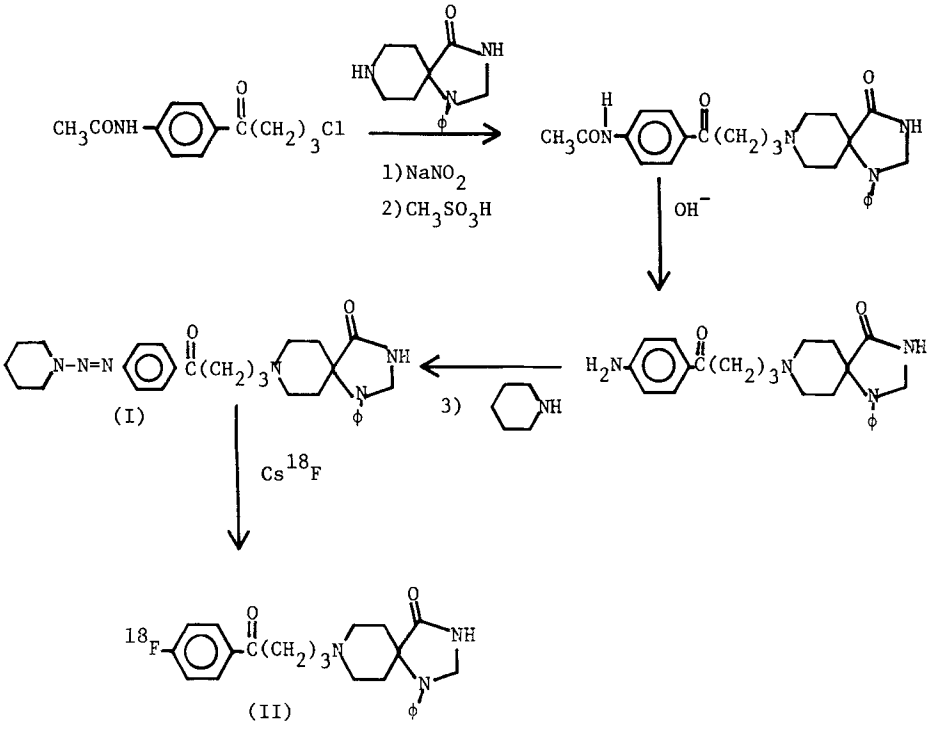
The Formation of ^{18}F-spiroperidol (II) from Triazene (I)[a]

Acid[b]	Solvent	Radiochemical Yield (%)[c]
MESA	C_6H_5Br	0.5
MA[d]	C_6H_5Br	0.7
MA	CF_3CH_2OH	--
MA	$CHCl_3$	--
MA	DMA	--
MA	C_6H_5Br	0.6
MA	EGME	--
MA	AcOH	0.025

a) Weight of triazene was 40–80 mg, total preparation time 60–80 min.
b) MESA = 2-mesitylenesulfonic acid, MA = methanesulfonic acid.
c) Not decay corrected.
d) Containing H^{18}F.



17.9

(^{77}Br)-*p*-BROMO-SPIROPERIDOL AS A DOPAMINE RECEPTOR MARKER

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Neuroleptic drugs act by blocking the dopamine receptors in the brain. Spiroperidol, one of the most potent neuroleptics in use, shows a very strong affinity for dopamine receptors both *in vivo* (1,2) and *in vitro* (3). Since the receptor binding interaction is a specific, saturable and reversible process, a high specific activity of labeled ligand is essential. Because radio-labeling with ^{18}F in an aromatic ring usually results in a low specific activity, we have decided to investigate the use of radiobromines. Modification of the spiroperidol structure by substituion of bromine for the *para* hydrogen may cause minimal change of the biological activities.

p-Bromospiroperidol was tested for its receptor binding activity *in vitro*, and its ability to stimulate prolactin secretion *in vivo*, as compared with its parent compound. The results indicate no significant difference in the receptor binding ability between the two compounds.

^{77}Br-bromospiroperidol was prepared by reaction of $^{77}Br_2$ with spiroperidol in a mixture of CH_2Cl_2 and CCl_4 at 0°, and was purified by reversed-phase TLC (Whatman KC_{18}). A specific activity of approximately 4 Ci/mol was obtained.

Distribution of the radiobrominated spiroperidol in rats showed a ratio of striatum-to-cerebellum of 1.83 at 2 hr. A better striatum-to-cerebellum ratio would be expected if Br-75 is used (a specific activity of 160 Ci/mol would be obtained if the same concentration of Br-75 were present).

(Work performed under the auspices of the Division of Biological and Environmental Research of the U. S. Department of Energy).

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17.10

SYNTHESIS AND BRAIN UPTAKE OF RADIOIODINATED ANALOGS OF CHOLINE

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The level of acetylcholine in the brain varies with the physiological activity of the brain (1) and is affected by a variety of drugs (2,3). Since the brain does not synthesize choline to any significant extent (4,5), acetylcholine synthesis in cholinergic nerve terminals depends upon their ability to transport choline from extracellular sources into the nerve ending. It has been proposed that choline acetyltransferase, the enzyme which catalyzes the synthesis of acetylcholine from choline and acetyl-CoA, is coupled to the high affinity choline transport system (6). This transport system has properties which suggest that it may be the regulating step in the synthetic pathway (7).

The intravenous administration of tritium-labeled choline has been shown to be useful in studying the *in vivo* acetylcholine turnover in the mouse brain (8). The technique involved serial sacrifices of laboratory animals at various time intervals and quantification of results by liquid scintillation counting methods. Examination of this process in the intact animal could also yield useful information but would require that the choline molecule be labeled with an appropriate gamma emitting isotope. We have synthesized several iodine-containing analogs of choline which may satisfy this requirement.

The iodomethyl ammonium analogs of choline shown in Table I were prepared by quaternization of the appropriate amine with diiodomethane and were characterized by IR, PMR, and elemental analysis. The radioactively labeled analogs were prepared by exchange labeling diiodomethane (9) with sodium iodide-125 in methylethylketone prior to quaternization. The progress of the iodomethylation was followed by chloroform:water extraction of small aliquots of each reaction mixture at 0, 30, 60, 120, and 240 minutes (see graph). The radioiodinated products were characterized by ion exchange chromatography and paper electrophoresis.

The results of brain uptake studies with these compounds in normal ICR mice at various time intervals post injection will be discussed.

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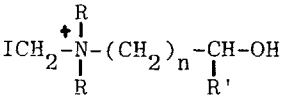
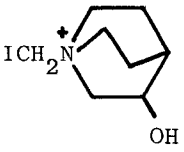
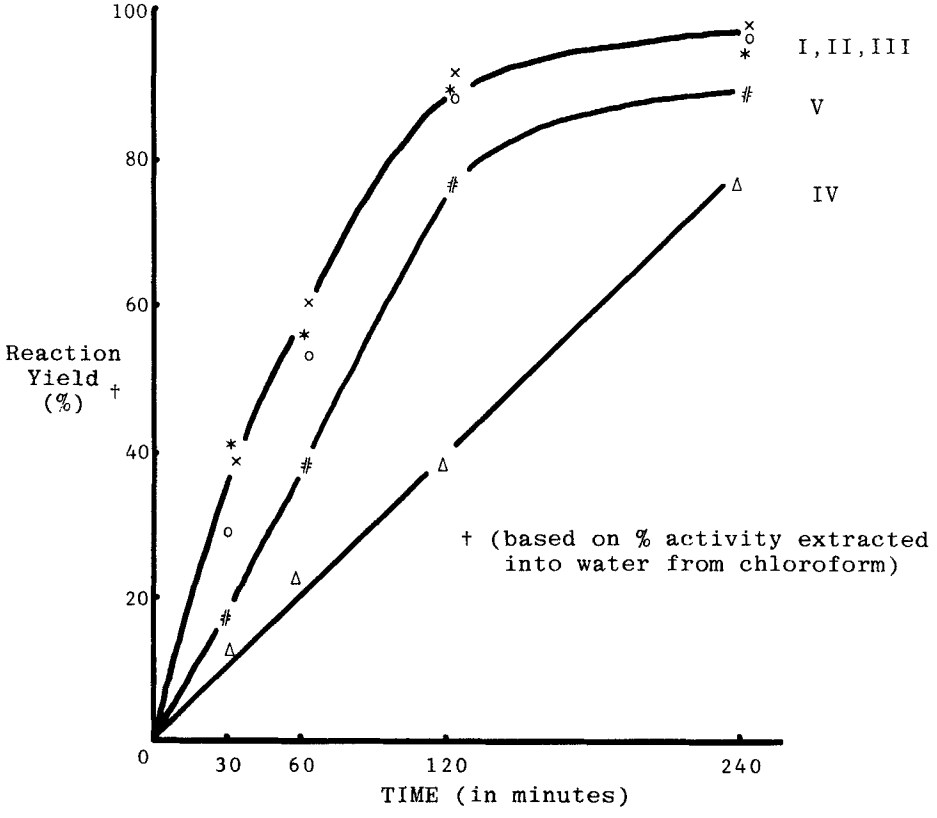
Table I - Iodomethyl Choline Analogs

General form:



Compound	R	R'	n
I	CH_3	H	1
II	CH_3	CH_3	1
III	CH_3	H	2
IV	CH_3CH_2	H	1
V			

Graph: Reaction Yield† vs. Time



17.11

SELENIUM AND IODINE LABELED pH SHIFT BRAIN IMAGING AGENTS.

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Dept. of Nuclear Medicine, SUNY/Buffalo, VA Medical Center, Buffalo, NY 14215.

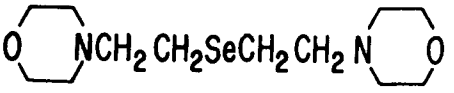
We have synthesized and studied a new group of brain imaging agents which take advantage of the pH gradient that exists between blood (pH∿7.4) and brain (intracellular pH∿7.0). At high pH these compounds are neutral and lipid soluble and can freely diffuse into cells but at low pH they become charged and can no longer diffuse out. The concentration gradient is a function of the equilibria established by the local pH shift.

A series of Se-75 and I-125 labeled tertiary diamines was studied. The Se-75 compounds were prepared by reducing H_2SeO_3 with $NaBH_4$ and reacting the intermediate with the N,N-disubstituted aminoethyl chlorides. To minimize in vivo deiodination, the radioiodine was covalently bound to an aromatic ring. A series α,α'-substituted iodo-xylenediamines was synthesized by treating the appropriate iodo-xylene with N-bromosuccinimide and reacting the product with disubstituted amines. Iodine-125 labeling was by exchange at elevated temperature.

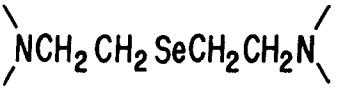
Brain uptake and wash-out curves were determined in rats as a function of inherent lipid solubility and the change in lipid solubility with pH for each compound. Regional distribution in brain tissue was studied by radioautographs of 20μ frozen sections. Partition coefficient-pH profiles and pK's were measured as well as binding to serum proteins.

Brain uptake was as high as 3-4% of the injected dose and retention half-times as long as 10-20 hours. Generally high initial uptake was related to high inherent lipid solubility and retention time depended on the slope of the partition coefficient-pH profile. Radioautographs showed the high cortical uptake pattern characteristic of brain blood flow or metabolism agents. Most compounds had low protein binding.

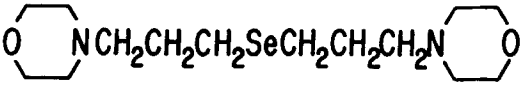
Using the Henderson-Hasselbalch equation, partition coefficient-pH profiles were calculated from the pK's and the inherent lipid solubility. These curves matched the experimental data closely. By changing the groups bonded to N and through other modifications, one can design agents with any desired partition coefficient-pH profile. Since this profile determines biological behavior, it is possible to design agents with optimal uptake and wash-out curves.



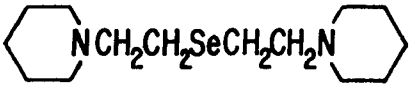
MOSE



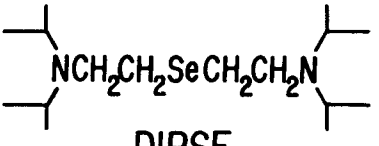
DMSE



MOSE-P



PIPSE



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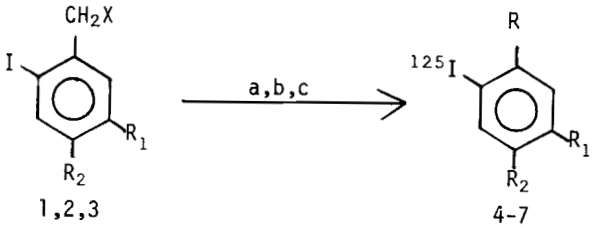
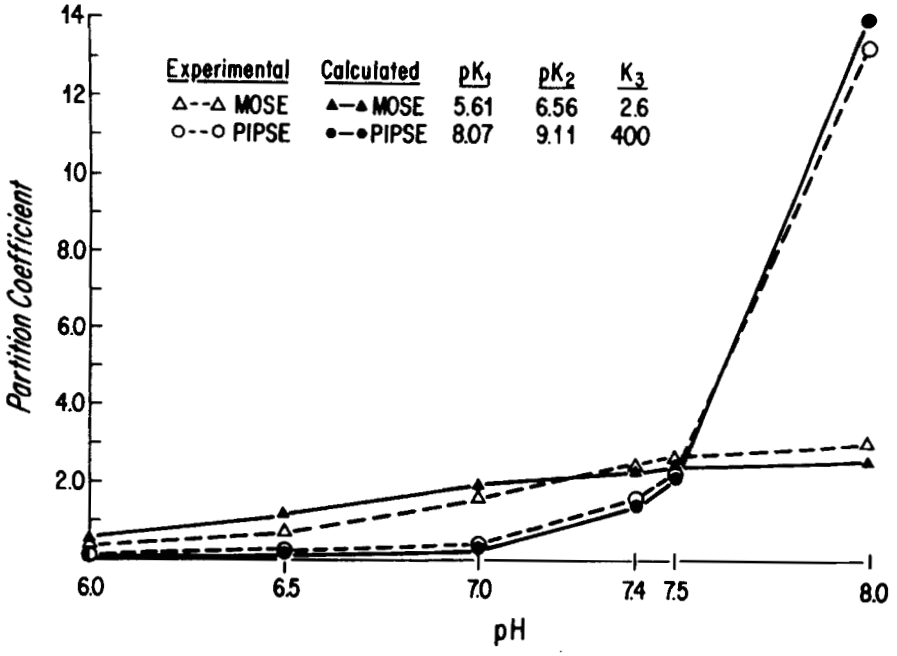


1,2,3 4-7

compound		X	R	R_1	R_2
	1	Cl	-	H	H
	2	Br	-	$-CH_2-Br$	H
	3	Br	-	H	$-CH_2-Br$
(m-IDM)	4	-	$-CH_2-N(CH_3)_2$	$-CH_2-N(CH_3)_2$	H
(p-IDM)	5	-	$-CH_2-N(CH_3)_2$	H	$-CH_2-N(CH_3)_2$
(m-IPIP)	6	-	$-CH_2-N$	CH_2N	H
(m-IMO)	7	-	$-CH_2-N$ O	CH_2N O	H

a.) NBS/CCl_4, b.) HNR_2, c.) $Na^{125}I/H_2O$, 204°.

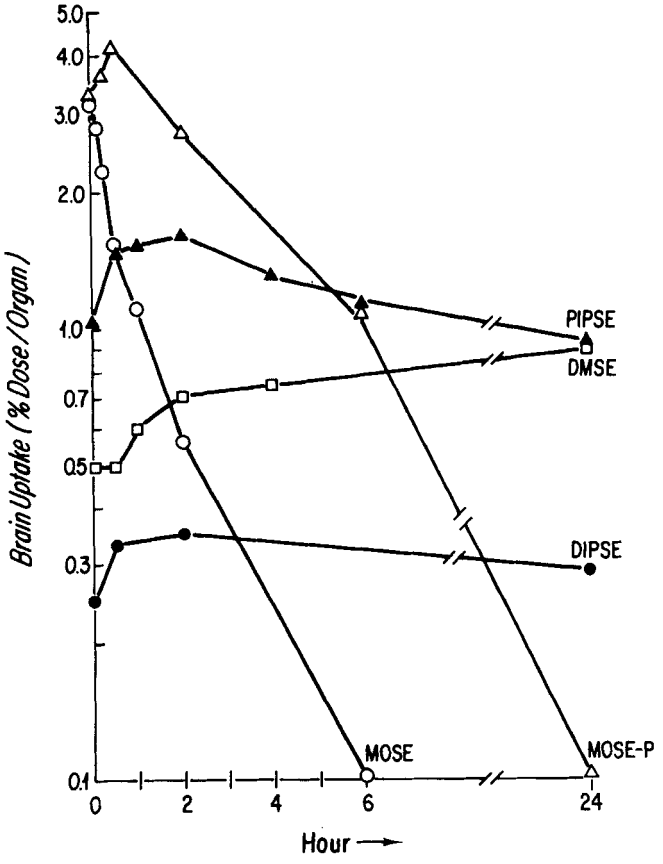


Partition Coefficient-pH Profile

Brain Uptake of I-125 Tertiary Amines
Avg. of 3 Rats, % Dose/Organ

	2 min	30 min	1 h	2 h	6 h
m-IDM	2.40	2.52	2.36	2.36	1.51
p-IDM	1.86	1.37	1.39	1.49	0.97
m-IPIP	1.70	1.41	1.47	1.22	0.70
m-IMO	2.12	0.29	0.171	0.15	0.05

Brain Uptake of Se-75 Tertiary Amines
Avg. of 3 Rats, % Dose/Organ



17.12

STUDIES WITH ^{203}Hg-ESTRADIOL FOR THE DETECTION OF MAMMARY TUMORS

J.Shani[1], L.M. Lieberman[2], M.Cais[3], Y. Josephi[3] and M. Shimoni[3],

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Studies on the antigenicity of metallohaptens in metalloimmunoassay (1) indicated that immunorecognition by appropriate anti-estrogen antisera for mercurated estrogens was retained to a reasonable degree, despite the stereoelectronic effects of the substituents (M. Cais, Y. Josephi and M. Shimoni, to be published). Similarly, recent glycerol gradient experiments (M.R. Sherman, M. Shimoni, F.B. Tuazon and M. Cais, to be published) demonstrated that, along with a high-level of non-specific binding, there was a distinct specific binding of mercurated estradiol by estrogen receptors in human breast tumor cytosols. In view of these observations, the γ-emitters ^{203}Hg-2-chloromercury-estradiol-17β and ^{203}Hg-4-chloromercury-estradiol-17β were prepared as potential radiopharmaceuticals for the selective detection of estrogen-receptor-containing mammary adenocarcinoma, and as model substrates for similar studies with other metallohaptens (2).

The mercurated estradiols were synthesized, purified and characterized in our laboratories. They were injected intravenously into either Sprague-Dawley female albino rats bearing spontaneous mammary tumors, or into Fisher female albino rats transplanted with 13762 solid mammary adenocarcinoma. The rats were divided into groups of six each, and were decapitated 1h, 4h, 12h, 1d, 2d and 4d after injection of either radiochemical, at a dose of about 2 μCi/0.2 ml/rat for the first three groups and about 4 μCi/0.2 ml/rat for the three longer time intervals. Blood was sampled by heart puncture under light ether anaesthesia, and the following organs were completely removed : adrenals, brain, heart, kidneys, liver, lungs, inguinal mammary glands, ovaries, anterior pituitary, spleen, stomach (emptied by pressure), tumor and uterus. A representative sample of the liver, as well as a major part of one tibia and some muscle were also counted in a gamma-spectrometer. In a further experiment four groups of male albino rats of the "Sabra" strain (descendant of the Wistar strain) were injected with either 2 μCi/0.2 ml/rat (three groups) or 5 μCi/0.2 ml/rat for the fourth group, and decapitated 1h, 4h, 1d and 17 days after their iv injection. In addition to blood samples, the liver, spleen, testes, seminal vesicle, prostate, kidneys, adrenals, inguinal mammary glands and epididimis were excised and counted. CPM/g organ, % inj/g and % inj/organ were calculated for all sampled organs, and organ/blood ratios were expressed graphically as a function of time (see figure 1 for spontaneous mammary tumors).

In addition, three groups of 10 male rats each were injected with ^{203}Hg-4-chloro=mercury-estradiol-17β, 5 μCi/0.2 ml/rat, with various (0, 10 or 20 mg/kg) doses of 4-chloromercury-estradiol-17β. The higher doses are non-physiological, and the rational behind their mixing with the labelled drug was to evaluate the clearance of the label as a function of the "loading" dose. Those doses were far below the LD_{50} value for 4-chloromercury-estradiol-17β in the rat.

Our results show that binding of the labelled drugs to the mammary tumors was significantly higher than their binding to healthy mammary glands : while the organ/blood ratio for the healthy mammary gland was about unity, a ratio of 4 (at 2 days post injection) and 14 (at 4 days post injection) was obtained for the mammary tumors. Not unexpectedly, kidneys, liver and spleen concentrated the labels in much higher levels than the tumors. No apparent differences could be observed between the 2- and 4-chloromercury-estradiol-17β derivatives.

Preliminary distribution studies in healthy male rats demonstrated a gradual uptake in their testes, seminal vesicle and prostate, but so far the peak concentration of the label in those organs was not pinpointed. Here too, the uptake of the label

in the kidneys, liver and spleen predominated. The LD_{50} of the "cold" 4-chloro=mercury-estradiol was apparently over 20 mg/kg iv, as none of the rats injected that dose died during the 17-day experiments. No higher doses could be administered, as higher concentration than 10 mg/kg (in ethanol:propyleneglycol = 1:2) could not be reached.

For clearance studies the retention of the label in the injected male rats was followed up with the aid of an home-made whole-body-counter, with 6" - plastic crystals shielded behind heavy lead bricks. The rats were counted for 17 consecutive days, background substracted and the results calculated for decay.Our results(Fig 2) indicate that there is a significant binding of the label in the body, and that the binding is dependent on the loading-dose : $T_{\frac{1}{2}}$ values were 4.86 & 16.78h when no "cold" 4-chloromercury-estradiol-17β was injected, and 1.37 & 10.45h respectively when 20 mg/kg were added to the labelled drug. The specificity of the binding to the mammary tissue is being studied now in our laboratories.

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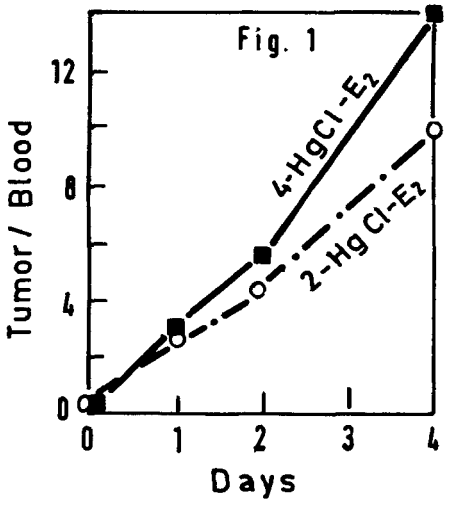

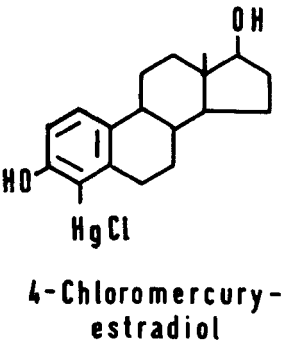

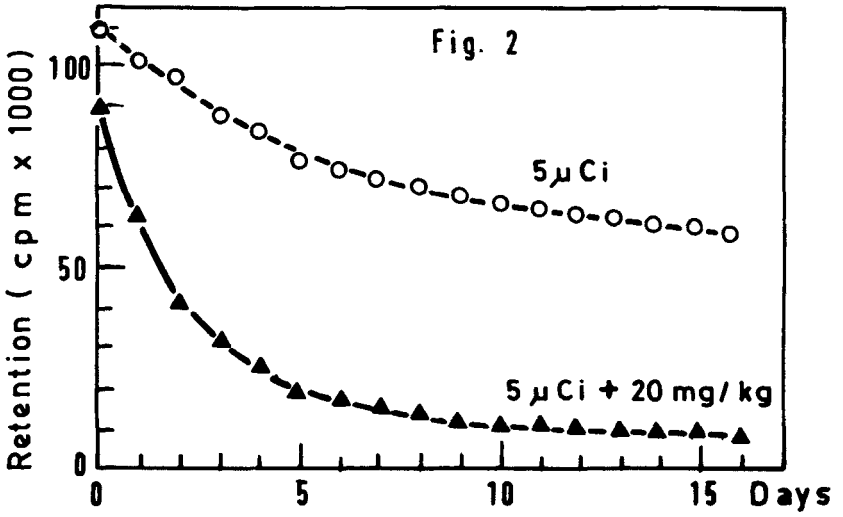

17.13

LABELING OF 6-IODOCHOLESTEROL WITH I-131

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In 1970, R. E. Counsell et al. (1) succeeded in imaging the adrenal with 19-^{131}I-cholesterol, but recently considerable attention has been focused on 6-substituted 19-norcholest-5 (10)-en steroids (2) (3). Considering the notable stability of 6-^{131}I-cholesterol reported by S.C. Wang (4), the authors synthesized 6-^{131}I-cholesterol from natural cholesterol. Its mercuration and iodination were carried out in a similar manner as described by W. Merz (5) and R. T. Levin (6). The 6-^{131}I-cholesterol obtained was subjected to trial uses in pharmacological studies and clinical diagnoses (7). Ease of preparation, simplicity of labeling procedure, high yield, constant quality, notable stability and easy storage are some advantages of 6-^{131}I-cholesterol is also a good and safe adrenal scanning agent.

In our work, radioiodination was achieved by the nomogeneous isotope exchange method in n-propanol. The kinetics of simple isotope exchange reaction followed the relation $-\ln(1-F) = \frac{(A) + (B)}{(A)(B)} Rt$ in our system, (A) and (B) indicate the concentrations of 6-I-cholesterol and KI respectively, R is the rate of isotope exchange, and F is the exchange fraction at time t, our results show the linear relation between ln(1-F) and t with the carrier amounts of 6-I-cholesterol and and KI of 20 mg and 0.4 mg respectively, the bath temperature being about 160°C, the half-time of exchange 1 hour.

Effects of carrier amounts on the percentage yield of labeling and effects of temperature (bath) on the reaction rate were studied. The optimal labeling conditions were ascertained. Labeling procedure: A solution of 6-I-cholesterol (20mg) in n-propanol (40ml) was placed in a 250 ml round bottom flask fitted with a condenser. Then, 0.13 ml of 1 mg/ml KI solution was added; after thorough mixing, 1 ml Na^{131}I solution (50 mCi) was added. The mixture was refluxed at a bath cool, and 40 ml of water added when the temperature was below 110°C, n-propanol was removed by distillation, and the water phase extracted three times with ether. On evaporating 6-^{131}I-cholesterol with a specific activity of 2.4 mCi/mg was obtained.

Thin layer chromatography was used to identify the radiochemical purity; it gave a single spot, the Rf value of which checked very well with the single radioactive peak. 6-^{131}I-cholesterol kept at room temperature for three months gave the same TLC result as that of the newly prepared product. There was no radiolytic and thermal dehalogenation.

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17.14

HEAVY HALOGENS AND RADIOPHARMACEUTICALS

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Short-lived neutron-deficient radioisotopes of bromine and iodine play an increasingly important role in diagnostic nuclear medicine. Several new production and labelling methods have been developed during the past years. Bromine and iodine radiopharmaceuticals represent an alternative approach to probing metabolism in-vivo with short-lived "organic" positron emitters such as ^{11}C, ^{13}N and ^{18}F since radiohalogen tagged biomolecules or metabolites can in principle also be used for tracing metabolic functions. When compared with the short-lived organic positron emitters, in particular with fluorine-18, the chemistry of bromine and iodine is considerably simpler and some of their isotopes have more convenient intermediate half-lives.

Among the neutron deficient bromine isotopes there are three which are potentially useful for positron emission tomography ($^{74m,75,76}Br$). From these the most promising is $^{75}Br(T_{1/2}$ = 98 min) which has recently been produced carrier-free with yields of 8 mCi/μAh via the $^{75}As(^{3}He,3n)^{75}Br$-reaction at a compact cyclotron using 36 MeV ^{3}He-particles. The 16 hr bromine-76 is less suitable for positron emission tomography due to its relatively small positron emission rate (57.1 %) and high positron energy (3.7 MeV). The longer lived γ-emitter $^{77}Br(T_{1/2}$ = 56 hrs) is a useful isotope for studying slow processes with a γ-camera equipped with a high energy collimator. Both the radioisotopes have recently been produced in good yields by various medium and high energy reactions. Excitation functions and production methods for $^{75-77}Br$ are critically reviewed. New procedures of labelling starting from radiobromide have also been developed both for nucleophilic aliphatic and electrophilic aromatic substitution. Modified succinimide-, hypohalite- and chloramine-T-methods have been successfully applied for introducing bromine practically carrier-free into aromatic systems while in aliphatic compounds bromine-for-iodine exchange can be used to obtain theoretically carrier-free products. Reactions can be carried out in solution, in the melt or via phase transfer catalysts. Recent progress in radiobromination procedures is reviewed and new potentially useful bromine radiopharmaceuticals are evaluated.

In the case of iodine-123 progress has been somewhat slower both in new production and labelling procedures, mainly due to the fact that a wealth of data and methods is already available for some time. The $^{124}Te(p,2n)^{123}I$-reaction at compact cyclotrons and the $^{127}I(p,5n)^{123}I$-process at high energy machines are being used as major production methods. Other reactions, including spallation still play a minor role. The different processes are critically evaluated with respect to radionuclidic purity and production efforts. While the number of iodine-123 radiopharmaceuticals is only slowly increasing their importance is gaining general recognition. The development of some new ^{123}I-products is outlined.

Application of heavy halogen labelled products to the measurement of metabolic functions requires a detailed knowledge of the catabolic and anabolic fate of the label in order to allow quantification of the turnover. The problem is discussed in the case of radiohalogen tagged fatty acids applied to the measurement of aerobic myocardial metabolism (1).

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17.15

FURTHER STUDIES ON PRACTICALLY CARRIER-FREE ^{123}I-IODINATION AND ^{75,77}Br-BROMINATION OF AROMATIC SUBSTRATES

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For tagging aromatic substrates with radiohalogens with high specific activities oxidation reagents are required which produce isotopically pure i.e. carrier-free electrophilic radiohalogen species. We have recently demonstrated that N-chlorotetrafluorosuccinimide (NCTFS) can be used effectively for radiobrominations of aromatic molecules starting with carrier-free bromide (1). This method was now extended to radioiodination with iodine-123. Model experiments have been carried out in monosubstituted benzenes. As in bromination, addition of halide carrier in the order of 10^{-5} to 10^{-1} mg/ml causes an increase of the radiochemical yield of about 50% e.g. in the toluene system. The velocity of the iodination reaction, on the other hand, is slower than that of the corresponding bromination, and a maximum yield of about 60% is only reached after 10 hrs using trifluoroacetic anhydride as solvent, compared to 0.5 hrs in the case of the bromination. The reactivity and selectivity within a series of benzene derivatives (C_6H_5X,X = CH_3,H,F and Br) (cf. Table 1) clearly indicate a pure electrophilic reaction with somewhat higher substitution yields and an enhanced para-selectivity when compared with the bromination reaction.

Further experiments have been carried out applying organic hypohalites to carrier-free radiohalogenation. Organic hypohalites have recently been used for electrophilic aromatic halogenation (2), and we have now successfully carried out radioiodination with hypohalites of trifluoroacetate or trifluoromethane sulfonate [CF_3CO_2Cl(Br) and CF_3SO_3Cl(Br)] in nitrobenzene. The absolute radiochemical yield and isomeric distribution are strongly dependent on the nature of the hypohalite, the reaction time and temperature. In the case of CF_3CO_2Cl iodination of phenol is completed within 10 min at temperatures ranging from 100 to 210° C. With increasing temperature, absolute ortho substitution yield rises from 32% (100° C) to 52% (210° C) while the para yield remains constant at 10% over the whole temperature range. Iodination of phenol (120° C, 40 min) e.g. gives rise to 43% para and 30% ortho product when using CF_3SO_3Br; with CF_3SO_3Cl 54% ortho and 35% para-iodophenol are obtained. These effects are more enhanced in aniline. The ortho/para selectivity and the reactivity within the series of benzene derivatives (cf. Table 2) also clearly indicate an electrophilic substitution mechanism.

The application of these reagents using solvents other than nitrobenzene seem to be more promising for simple one-pot syntheses. Corresponding bromination of biomolecules such as ω-phenylfatty acids have also been carried out.

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Table 1:
Iodination of benzene derivatives by NCTFS/^{123}I-iodide
(2 mg NCTFS, 0.5 ml TFA, 4 hrs, room temperature)

Substrate	Radiochemical Yield (H-Substitution)	Isomer Distribution* ortho	meta	para
Toluene	54.0 ± 2.4	36.0	1.0	63.0
Benzene	62.3 ± 2.3	-	-	-
F-Benzene	53.6 ± 2.3	2.0	0.02	98.0
Br-Benzene	39.1 ± 3.7	8.0	0.04	92.0

Table 2:
Iodination of benzene derivatives by CF_3SO_3Cl/^{123}I-iodide
(5 mg CF_3SO_3Cl, 1 ml $C_6H_5NO_2$, 1 hr, 100° C)

Substrate	Radiochemical Yield (H-Substitution)	Isomer Distribution* ortho	meta	para
Aniline	94.0 ± 6.0	13	-	87
Phenol	91.0 ± 3.0	62	-	38
Anisole	56.0 ± 5.6	5	-	95
Benzene	60.0 ± 4.7	-	-	-
F-Benzene	19.0 ± 4.0	6	3	91
Br-Benzene	45.0 ± 4.5	18	-	82

*o + m + p = 100%, standard deviation ± 5%

17.16

A DIRECT METHOD FOR CARRIER-FREE RADIOHALOGENATION

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While there are a number of well-documented methods for the radiohalogenation of systems containing aromatic functions, most of these have some limitations when applied to labelling with carrier-free radiohalogens. For example. the enzymatic method (1) of introducing ^{77}Br must be carried out at low pH, and is therefore unsuitable for labelling acid-sensitive compounds.

We report on a novel method of radiohalogenation which appears to proceed rapidly and efficiently with carrier-free radiobromine and radioiodine, occurs under very mild conditions and leaves the labelled product in a solution from which it can be readily isolated. Radiochemical yields obtained using a number of target compounds will be reported; a few examples are shown in the table below.

The direct radiohalogenation method (2) is based on the passage of the halogenating species (in the gas phase) into a solution of the target compound. While the technique has many advantages over conventional methods of radiohalogenation (3), probably its greatest potential arises through the absence of other chemical reagents in the reaction vessel.

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Radiochemical Yields obtained following direct ^{77}Br-bromination of selected compounds.

Target compound	Radiochemical Yield % (approximate)
Uracil	95
Tyrosine	70
Tyramine	80
2'deoxyuridine	90
Fibrinogen	80
Human Serum Albumin	90

17.17

A RAPID AND MILD METHOD FOR LABELING FUNCTIONALLY SUBSTITUTED MOLECULES WITH IODINE RADIONUCLIDES

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T. T. Sun and L. C. Washburn.
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Iodine radioisotopes have been used extensively in nuclear medicine. The utility of radioiodine is based on both the accessibility of a variety of useful isotopes and the availability of reliable incorporation routes (1-3). In certain instances, however, the classical methods for iodine incorporation are ineffective due to the absence of appropriate starting materials or inefficient utilization of radioiodine.

The utility of organoborane reagents in organic synthesis has become apparent in recent years due to the availability of a variety of functionally substituted organoboranes (4) and the diverse transformations which they undergo (5). The value of the organoboranes in radiopharmaceutical research lies in the fact that the boron atom is rapidly replaced by a variety of nuclides under mild conditions. Recently, organoborane reactions have been utilized for the incorporation of carbon-14 (6), carbon-13 (7), and carbon-11 (8).

The rapid, regiospecific conversion of alkenes to alkyl iodides via organoboranes has been reported using molecular iodine (9,10). The reaction proceeds via a hydroboration - iodination sequence resulting in high yields of the product iodide. The reaction exhibits two characteristics which reduce its utility in the

$$3RCH{=}CH_2 \xrightarrow{BH_3} (RCH_2CH_2)_3B \xrightarrow[NaOCH_3]{3I_2} 3RCH_2CH_2I + 3\ NaI$$

syntheses of radioiodine containing pharmaceuticals. The first is the fact that the maximum radiochemical yield is 50% (based on iodine) since one-half of the iodine molecule is lost as iodide. The second is the required presence of strong bases which can react with sensitive functional groups in complex molecules and initiate dehydrohalogenation reactions.

We wish to report that the incorporation of iodine-125 (as well as other isotopes of iodine) can be accomplished rapidly under extremely mild conditions utilizing labeled iodine monochloride. The reaction is complete in a matter of minutes at temperatures as low as -78°C. The reaction is carried out using two equivalents of the iodine monochloride which react quantitatively (NMR analysis) with two of the three alkyl groups attached to boron.

$$RCH{=}CH_2 \xrightarrow[THF]{BH_3} (RCH_2CH_2)_3B \xrightarrow[NaOAc]{2ICl} 2\ RCH_2CH_2I$$

The products are isolated via chromatography on alumina using mixed hexanes as eluting solvents.

We have synthesized a variety of functionally substituted alkyl iodides which were isolated in high radiochemical yields (Table I).

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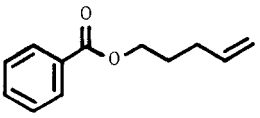
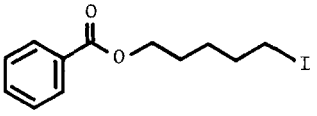
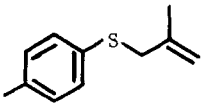
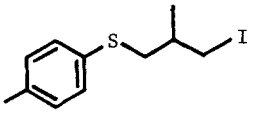
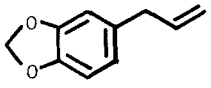
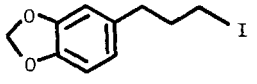
Table I. Synthesis of Iodine-125-Labeled Alkyl Iodides[a,b]

Substrate	Product[c]	Radiochemical[d] yield (isolated)
		82%
		94%
		74%
		78%

[a]Reactions were run by hydroborating 30 mmol of the alkene with 10 mmol of BH_3•THF at 0°C in 10 mL of THF. Sodium acetate in methanol (30 mL of a 1.0 M solution) was added to the mixture, followed by the dropwise addition of ICl (20 mmol). [b]Activity of the ICl was 2.5 mCi/mole. [c]Products were isolated by column chromatography. [d]Percentage yields are based on ICl added.

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17.18

ADRENAL AFFINITY AND CHOLESTEROL ESTER METABOLISM OF 6β-IODOMETHYL-19-NORCHOLEST-5(10)-EN-3β-OL, 3-ACETATE, AND 3-PALMITATE

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Earlier studies on the structure-activity relationships of cholesterol derivatives, have made various modifications of 3β-hydroxyl group of cholesterol. They are 3α-OH, 3β-acetate, 3β-palmitate, 3β-halide, and 3β-methyl ether of iodo, bromo, fluoro, seleno, and telluro derivatives of cholesterol. Counsell et al. reported that the acetate and palmitate of 19-iodocholesterol failed to show appreciable adrenal uptake following i.v. administration to rats (1). However, recent studies on 19-iodocholesterol, 6β-iodomethyl-19-norcholest-5(10)-en-3β-ol, and 6β-methyl-seleno-methyl-19-norcholest-5(10)-en-3β-ol, revealed that 3β-OH and 3β-acetate had almost the same adrenal uptake in rats (2,3). 19-Iodocholesterol is supposed to be subject to in vivo esterification in dogs (4,5). In order to evaluate 3β-hydroxyl group of iodocholesterol derivatives as tracers for cholesterol, we have prepared the title compounds (Fig 1,I,II,III), and studied the adrenal affinity and the cholesterol ester metabolism of them in rats and mice.

Compound (I) and (II) were prepared according to Maeda et al. (6). Reaction of (I) with palmitoyl chloride in pyridine gave (III). ^{125}I-labelled compounds were prepared by the isotopic exchange reaction with Na ^{125}I in acetonitrile, and were purified by the preparative TLC on silica gel.

Kinetics of mice adrenal uptake of the above compounds is shown in Fig 2. The free compound (I) was accumulated in the mouse adrenals more rapidly and actively than the esters (II,III) up to one day after i.v. administration. There was about one day lag time for 3-palmitate to be incorporated in the adrenals. But after five days, the adrenal uptakes of free and ester compounds were almost the same.

In order to make clear the mechanism of the adrenal uptake and in situ metabolism of the ester compound, double labelled compound, ^{123}I-6β-iodomethyl-19-norcholest-5(10)-en-3β-ol acetate-[1-^{14}C] was prepared. Mice tissue distribution data are shown in Table 1. Adrenal I-123 radioactivity was 27 times higher than C-14 activity at 24hr after i.v. injection. Liver and plasma C-14 activity were, also, much lower than I-123 activity.

Following i.v. injection of the compound (I-III) to rats, lipids of the liver, blood, and the whole adrenal glands were extracted by the Folch procedure, and were separated into fractions by silica gel preparative TLC in the presence of a few mg of the carrier compounds. Using the system, hexane, ether, acetic acid (75:25:2; vols), radioactivity was detected exclusively in the following bands: origin, free sterol fraction (Rf=0.20), acetate fraction (Rf=0.60, if present), and the palmitate ester fraction (Rf=0.80). Radioactive palmitate fraction of iodo cholesterol (Rf=0.80) was clearly separated from non radioactive, endogenous cholesterol ester band (Rf=0.90). Results are summarised in Table 2. In the liver, hydrolysis of the ester compounds occurred, and about 80% of the radioactivity was present in the free sterol fraction after 24 hr following i.v.injection. On the contrary, in the adrenal glands at 24 hr, more than 90% of the radioactivity was recovered in the palmitate ester fraction. In the plasma, esterification of the free compound was two or three times more active than in the liver.

From these experimental results, we conclude that both acetate and palmitate of iodo cholesterol derivatives are transformed into the free compound by the liver cholesterol ester hydroxylase. The free sterol is released into the blood, and is accumulated in the adrenal glands, where it is rapidly reesterified and stored. One of the important characteristics of the iodo cholesterol derivatives, compared with cholesterol, is, that the main fraction of iodo compound in the plasma is in the free form, which has the higher adrenal concentration than the ester form.

The predominant free form in the plasma of the iodo cholesterol may be attributed to the palsma lipoprotein binding of it. Our previous study (2) showed that iodo cholesterol was bound mainly to LDL and VLDL, and little to HDL. It is established (7) that the plasma cholesterol is present mainly in the ester form, and that lecithin cholesterol acyltransferase (LCAT) esterifies more rapidly HDL bound cholesterol than LDL bound cholesterol in vitro.

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Table 1. Mouse Tissue Distribution of ^{123}I and ^{14}C from ^{123}I-6β-iodomethyl-19-norcholest-5(10)-en-3β-ol acetete-[1-^{14}C]

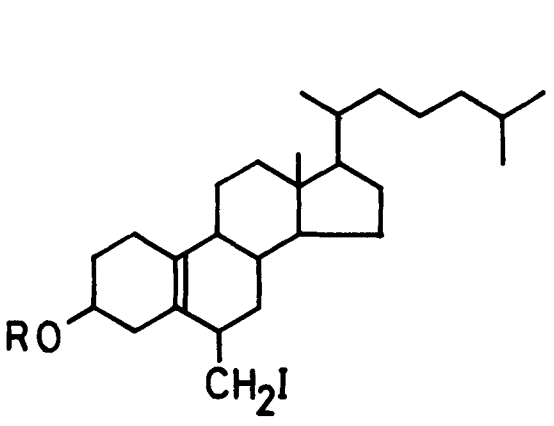
	^{123}I (D.A.R.)*			^{14}C (D.A.R.)		
Tissues	2 hr	6 hr	1 day	2 hr	6 hr	1 day
Adrenals	9.28	9.85	17.3	2.96	0.94	0.63
Thyroids	2.82	3.64	9.39	0.58	0.24	0.27
Liver	6.87	6.18	2.56	3.36	2.57	0.61
Kidneys	1.24	1.62	1.60	0.82	0.40	0.27
Small Int.	1.28	1.39	0.86	0.85	0.61	0.31
Blood	4.11	2.41	1.25	3.05	0.52	0.11

* D.A.R. is % dose/g x body weight (g)/100

Table 2. Biotransformation of 6β-iodomethyl-19-norcholest-5(10)-en-3β-ol, 3-acetate, and 3-palmitate in Wistar Rats.

		3-OH		3-Acetate			3-Palmitate	
Time after dose	Tissues	Free (%)	Ester (%)	Free (%)	Acetate (%)	Ester (%)	Free (%)	Ester (%)
2 hr	Adrenals	44	56	41	15	44		
	Liver	93	7	78	19	3		
	Plasma	89	11	27	72	1		
6.5 hr	Adrenals	14	86				12	88
	Liver	94	6				64	36
	Plasma	87	13				6	94
24 hr	Adrenals	9	91	2	0	98	5	95
	Liver	92	8	77	1	21	79	21
	Plasma	78	22	79	3	17	26	74

Fig. 1



R

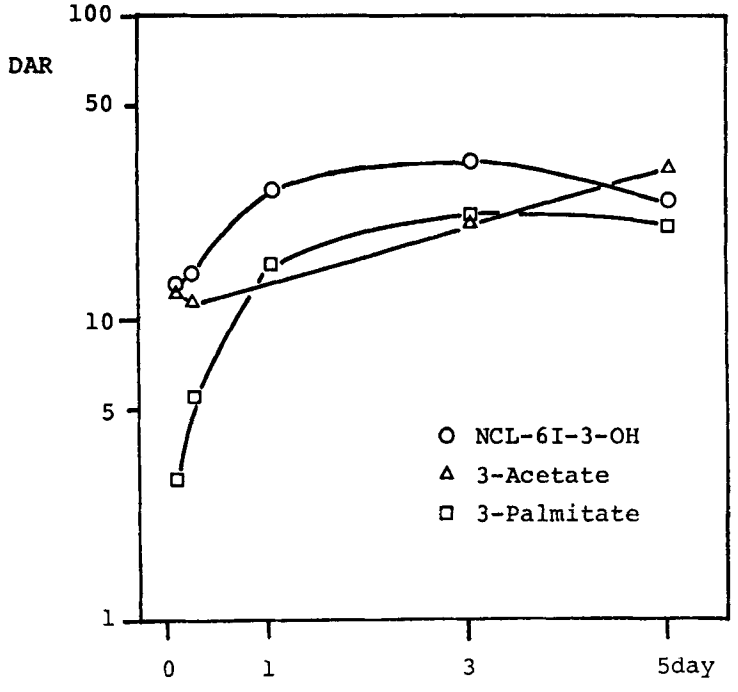
(I) H

(II) CH_3CO

(III) $CH_3(CH_2)_{14}CO$

Fig. 2

MOUSE ADRENAL UPTAKE OF I-125 FROM FREE AND ESTERIFIED COMPOUNDS.



17.19

RADIOIODINATED NORADRENALINE STORAGE ANALOGS

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Despite the physiological importance of noradrenaline as an adrenergic transmitter, no radiopharmaceutical exists that can assess catecholamine hormone accumulation and turnover in peripheral tissue. Uptake studies (1) have shown that 40-60% of small intravenous doses of noradrenaline are rapidly metabolized by catechol-O-methyltransferase (COMT) and monoamine oxidase (MAO). Although the neuron-blocking drug guanthidine (I) shares the same uptake, storage and release mechanisms as noradrenaline, it is not a substrate for COMT or MAO (2). Many aralkylguanidines (3) have pharmacological actions similar to guanethidine suggesting that radioiodinated derivatives of these compounds might be used for functional imaging studies of tissues with a high density of sympathetic innervation.

We report here: 1) a structure-distribution relationship (SDR) study of I-125- aralkylguanidines (II), 2) evidence for their accumulation in the catecholamine storage vesicles of the heart and adrenal (A.) medullae, 3) imaging of the primate heart and A. medullae with I-131- and I-123-meta-iodobenzylguanidine (M-IBG).

Nine I-125-labeled guanidines (II) were synthesized by radioiodide exchange in boiling water. Radiochemical yields were 90-98% with specific activities ranging from 0.8-1.1 mCi/mg. Specific activities of 100-200 mCi/mg could be obtained in yields of 50-60%. Purity was confirmed by both radio-TLC and radio-HPLC.

As shown in Table I, all nine guanidines showed high, selective uptake in the dog A. medullae. However, I-125-M-IBG (R_1R_2=H, n=0) showed the highest uptake and target-to-nontarget concentration ratios. Peak A. medullae uptake with M-IBG occurred at 2-3 days and was nearly double the respective peak adrenocortical uptake of the clinically useful I-131-6β-iodo-19-norcholesterol.

Pretreatment of dogs with reserpine, a drug known to selectively block vesicular uptake, lowered the A. medullae uptake five-fold. Subcellular fractionation of the A. medullae showed most of the radioactivity to be associated with the large granule fraction. Distinct images of the A. medullae of rhesus monkeys were obtained with I-123-M-IBG at 2 days.

Tissue distribution studies with the I-125-aralkylguanidines revealed that some of the compounds have heart-to-blood concentration ratios (^{H}/B) similiar to thallium-201 (Table I). The mechanism of heart localization of I-125-M-IBG was probed pharmacologically. Pretreatment of rats with d-amphetamine, a compound known to both release and inhibit the uptake of noradrenaline by nerve cells, lowers the heart concentration of I-125-M-IBG by 35-50%. Similar treatment with reserpine lowers the heart accumulation by 30-50%. These blocking studies suggest that the heart retention is mainly due to specific uptake in the adrenergic neurons where it is partially trapped in the noradrenaline storage vesicles. Dogs and rhesus monkeys gave excellent tomographic heart images from 15 min to 4 hr. using I-123-M-IBG.

Attempts to document the noradrenaline-mimicking properties of M-IBG are continuing in hopes of building a framework for its future use as a dynamic imaging agent. Further SDR studies and in vitro nuclear magnetic resonance studies of guanidine labeled chromaffin granules should help delineate the mode of retention of M-IBG in the neuronal storage vesicles.

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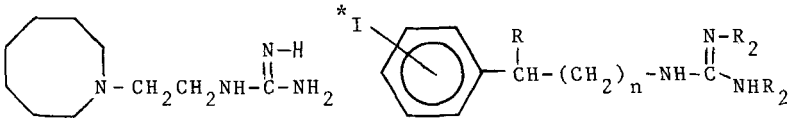

I II

TABLE I

SDR STUDY OF I-125-ARALKYLGUANIDINES* (II)

Position of Iodine	R_1	R_2	n	[Adr. Med.]	Time	[Adr. Med.]/[Liver]	H/B$^{\pi}$
ortho	H	H	0	2.7	24	80	8
meta	H	H	0	13.6	48	680	24
para	H	H	0	14.3	48	320	30
meta	H	H	0	4.4	72	240	13
para	H	H	1	4.2	24	60	26
meta	CH_3	H	0	7.9	24	190	18
para	CH_3	H	0	6.2	24	35	24
ortho	H	CH_3	0	3.6	24	55	5
3,4-DI-IODO	H	H	0	4.9	72	115	4

*Concentrations in % Kg dose/g.

$^{\pi}$ Heart-to-blood concentration ratio at 30 min.

17.20

ADRENAL UPTAKE STUDIES OF 6β-IODOMETHYL-19-NORCHOLESTEROL ANALOGUES

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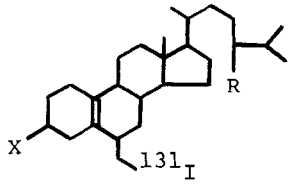
It is well known that 6β-iodomethyl-19-norcholest-5(10)-en-3β-ol (NCL-6-I) shows higher affinity for adrenal gland than 19-iodocholesterol (1-5).

To study the relationship between the structure and the adrenal uptake of NCL-6-I, we examined adrenal uptake of iodine-131 labeled 3α-hydroxy analogue (3α-OH-6-I-131) (6), 3β-methoxy analogue (3β-OMe-6-I-131) (7), 3β-fluoro analogue (3β-F-6-I-131) (7) and 6β-iodomethyl-19-norsitost-5(10)-en-3β-ol (NST-6-I-131) (8-9), respectively.

The Figure shows the concentration of radioactivity in rat adrenal after administration of respective iodine-131 labeled steroids comparing with that of NCL-6-I-131. The Table shows their tissue distributions in adrenal, liver, lung, kidney, spleen, blood, testicle and thyroid 7 days after the administration. The adrenal accumulation of 3α-OH-6-I-131 reported at the 2nd meeting of this symposium by us was shown much lower value than that of NCL-6-I-131. However, from the result of a detailed experiment performed recently, the adrenal uptake of 3α-OH-6-I-131 was approximately half in comparison with that of NCL-6-I-131 7 days after the injection. On the other hand, both 3β-OMe-6-I-131 and 3β-F-6-I-131 show much lower adrenal accumulation. Therefore, the fact suggests that 3β-hydroxy group in NCL-6-I plays an important role in the adrenal affinity.

As shown similar accumulation of NST-6-I-131 in adrenal with NCL-6-I-131, lipoid side chain analogue at the 17β-position of NCL-6-I should not have much influence on the adrenal affinity.



NCL-6-I-131	:	X=OH	, R=H
3α-OH-6-I-131	:	X=OH(α)	, R=H
3β-OMe-6-I-131	:	X=OCH_3	, R=H
3β-F-6-I-131	:	X=F	, R=H
NST-6-I-131	:	X=OH	, R=C_2H_5

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Figure Adrenal Uptake of Iodine-131 Labeled Steroids

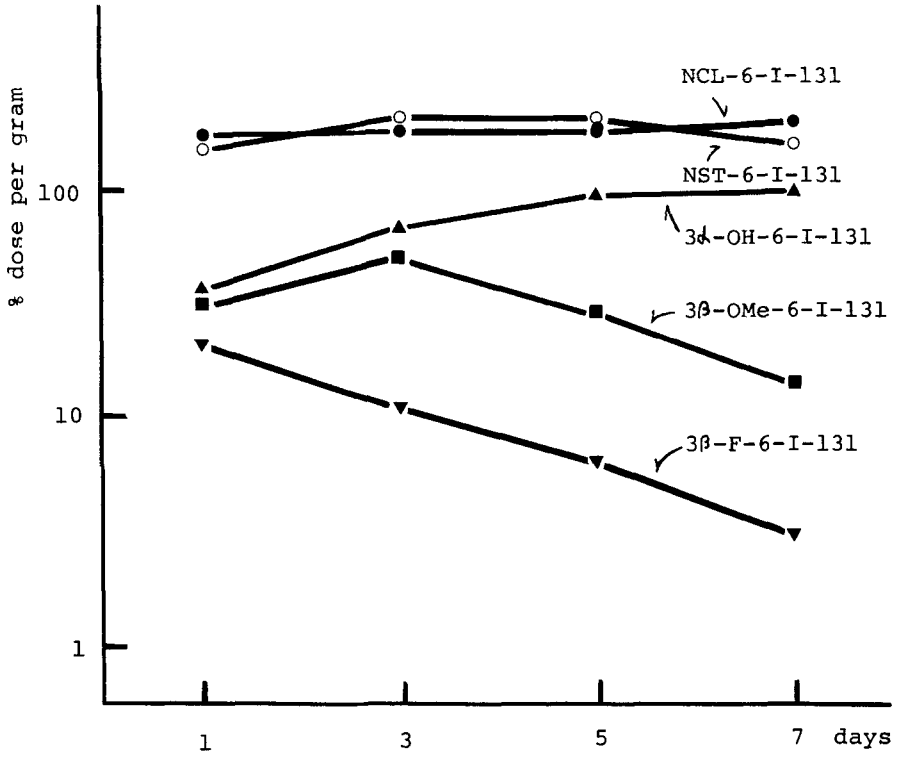

Table Tissue Accumulation of Iodine-131 Labeled Steroids[a)]

Compd.	Adrenal	Liver	Lung	Kidney	Spleen	Blood	Testicle	Thyroid
NCL-6-I-131[b)]	208	0.19	0.42	0.42	0.27	0.07	0.18	80
3α-OH-6-I-131	99	0.42	0.91	0.73	0.68	0.22	0.19	91
3β-OMe-6-I-131	18	0.33	0.26	0.27	0.33	0.05	0.12	86
3β-F-6-I-131	3	0.49	0.29	0.27	0.24	0.05	0.10	235
NST-6-I-131	168	0.35	0.71	0.43	0.52	0.18	0.23	93

a) Percent administered dose per gram of tissues after 7 days

b) Ref. (1)

17.21

A RAPID QUANTITATIVE METHOD FOR THE PRODUCTION OF ^{123}I-HIPPURAN

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^{123}I labelled o-iodo hippuric acid (Hippuran) has long been recognised as the radiopharmaceutical of choice for dynamic kidney function studies in patients using a gamma camera and associated computer system. The short half-life, 13.2 hours, absence of β-particle emission, and 159 KeV principal gamma ray, permit high quality images of the kidney to be obtained whilst the radiation dose to the patient is low. Until recently most commercially available sources have only been able to produce ^{123}I contaminated with longer life ^{124}I and ^{126}I which emit high energy gamma radiation and β-particles, resulting in an increased radiation dose to the kidney (1). However, since 1976, high purity ^{123}I produced by the decay of ^{123}Xe has been available from the UKAEA cyclotron, Harwell. This contains no ^{124}I or ^{126}I. A survey of the literature revealed that most of the methods of labelling Hippuran with ^{123}I (OIH) were complicated (2-6) and in our experience gave low and variable labelling efficiencies (7). Such time consuming and unreliable results would be unacceptable for routine use. However a later method (8) gave reproducibly high yields in a short preparation time. This method consisted of the addition of hippuran, $CuSO_4$ $5H_2O$ and ^{123}I in a vial and autoclaving for 15 minutes at 121°C.

A study of the labelling process was carried out. This involved determination of the optimum pH, and the required quantities of Hippuran and $CuSO_4 5H_2O$. It was also found that o-iodo-benzoic acid (OIB) was a contaminant of all commercially available sources of hippuran at a level of about 2%. It was found that this contaminant, in the absence of $CuSO_4 5H_2O$ was labelled preferentially by a factor of about 7:1 compared with the hippuran. However, addition of $CuSO_4 5H_2O$ prior to autoclaving reduced the OIB labelling to less than 1%.

A thin layer chromatography system using Benzene:Glacial Acetic Acid:Water in the volume ratios 2:2:1 with Merck 5716 TLC plates gave I(R_f= 0.01) OIH(R_f=0.4-0.5) and OIB(0.9-1.0). Thus the OIH was clearly separated from the radiochemical contaminants. It was found that some published labelling efficiencies for OIH included OIB which could not be detected by the chromatography systems used. The method was optimised for the quantities of hippuran and $CuSO_4 5H_2O$ required and a simple one step kit produced which enabled ^{123}I-hippuran to be prepared by simple autoclaving. The labelling efficiency was consistantly above 90%.

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17.22

THE SYNTHESIS AND IN VITRO STABILITY OF ORGANIC ASTATINE COMPOUNDS

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^{211}At is of potential interest for therapeutic applications such as selective immunosuppression. However, the instability of electrophilic astatinated proteins limited the applicability and has led to - in our opinion - doubtful conclusions about the stability of the carbon-astatine bond.
With the purpose of elucidating the nature of the At-protein bond, we are studying at this moment the stability of the carbon-astatine bond. We have developped a mild method for the introduction of astatine into (mainly) organic aromatic compounds. This method - astatination through chloromercury compounds - is widely applicable and a number of biomedical interesting ^{211}At-compounds, such as astatoaminoacids, astatopyrimidines, including DNA and RNA and astatosteroids have been prepared in high radiochemical yields. The measurement of the in vitro stability under different conditions indicated that the C-At bond is not much weaker than the corresponding C-I bond. These findings and preliminary measurements on At-proteins indicate that with electrophilically astatinated proteins the astatine is bound to the protein in some complex instead of by a covalent C-At bond.

17.23

ELECTROPHILIC RADIOIODODESTANNYLATION AS A NEW RADIOIODINATION METHOD

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Current methods for direct radioiodination of aromatic organic compounds with no carrier added using electrophilic iodine have several limitations. They require the presence of a powerful activating group, such as a phenol or aniline, and often yield a mixture of products which are metabolically unstable. As part of our project to develop estrogenic radioligands it became necessary to label in high yield and in high specific activity an aromatic group that did not contain an activating group.

The procedure developed involves the electrophilic iododestannylation of a trialkyltin aromatic intermediate (1). This intermediate can be prepared in high yield and in high purity from the appropriately substituted aromatic ether. The trialkyltin substitution proceeds specifically at the ortho position to give an intermediate that can be cleanly separated from the starting material (2). The use of an electrophilic ^{125}I-species such as ^{125}I/Chloramine-T, ^{125}I-I_2 or ^{125}I-ICl, generates the desired radioiodinated product rapidly and in high radiochemical yield (3). The specific activity depends only on the activity of the iodinating species. Separation of the product from the trialkyltin intermediate is clean. The location of the iodine is unambiguous, has little effect upon the biologic activity of the compound, and is relatively stable to *in vivo* metabolism.

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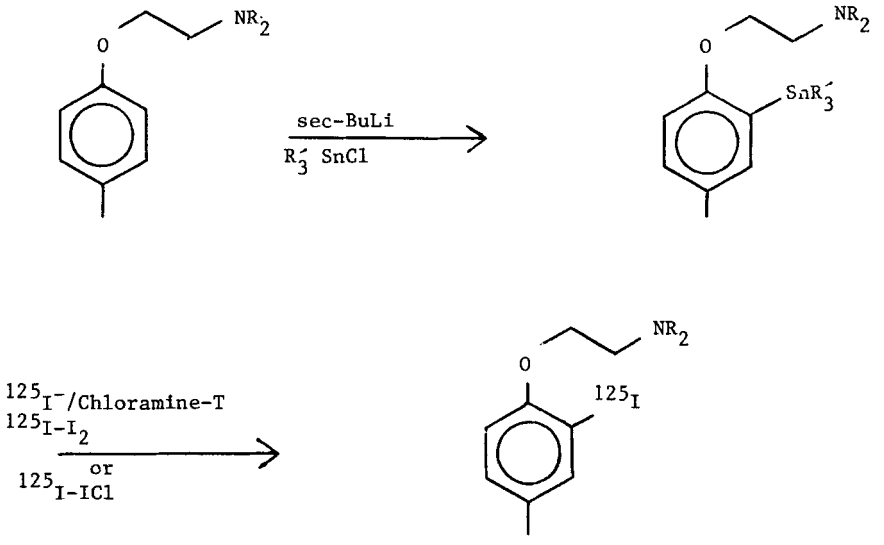

26-90% Radiochemical Yield
2.2 x 10^3 Ci/mmol maximum

18.1

SOME BASIC ASPECTS OF TECHNETIUM RADIOPHARMACEUTICAL CHEMISTRY

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Technetium is one of the most important elements in nuclear medicine. Demand for technetium radiopharmaceuticals increases astonishingly for one who knows early days of only a small scale use of ^{99}Mo-^{99m}Tc generators. A number of specialists in radiopharmacy and in nuclear medicine and biology have developed excellent techniques for labeling substances with techetium and found application to diagnosis.

Because of complexity of technetium chemistry, however, some results were not clear from the view-point of chemical identity. For example, Ikeda (1) has recognized two kinds of technetium labeled dimercaptosuccinic compounds: one of which shows an affinity to kidneys and the other to bones. These two could be prepared by similar chemical procedures, sometimes as a mixture and sometimes as one chemical species according to the change of conditions, although the structures of the two were not yet determined. This indicates that exact knowledges of technetium-labeled compounds in their chemical properties including their valence states, number of ligands and degree of polymerization are increasingly required in the recent studies on nuclear medicine using technetium. Certainly, this is one of the important fields to be clarified by the chemists who are working in radiopharmaceutical chemistry.

The other work to be done by the radiopharmaceutical chemists is, I believe, presentation of new techniques of preparing technetium compounds and of new chemical species which have possibility of application to nuclear medicine. In the latter parts of this paper I would like to show you these aspects of basic radiopharmaceutical chemistry.

Recoil implantation is known for a recent few years as a method of synthesis of some specific substances. In the technique the system usually consists of the powder mixture A and B. Recoil energy is used to break chemical bonds of the atoms which undergo nuclear transformations, and allow them to escape from the powder A and to enter into the powder B in which the recoil atoms will finally attack the molecules to give replacement products and related degradation products. One of the examples of this recoil implantation is synthesis of technetium phthalocyanine which was not described in the orthodox literature. The powder mixture of molybdenum and copper phthalocyanine (or metal-free phthalocyanine) were irradiated with deutrons using a cyclotron (2). The resulting product was separated by a wet method including sulfuric acid dissolution and by a dry method including sublimation.

$$Mo(d,xn)Tc + CuPc \longrightarrow (TcPc)^{++},$$

where Pc means a phthalocyanine skelton. The yields of the technetium complex are shown in Table 1. Recent improvement of the irradiation assembly reduced radiation damage of the target phthalocyanine in a form of pellet which was cooled with water and air without use of vaccuum conditions.

The next example of application of a recoil method is synthesis of technetium(III) acetylacetonate (3) which has not been known so far. Ruthenium (III) acetylacetonate was irradiated with γ-rays to give ^{99m}Tc and ^{95}Tc by the (γ,p) reactions. The irradiated materials were submitted to sublimotographic procedures. Figure 1 shows the results. It is obvious that Tc(III) acetylacetonate behaves as Ru(III) acetylacetonate. When the mixture of Mo and chromium(III) acetylacetonate is irradiated with deutrons in a cyclotron, a technetium radioactivity peak is detected at the position of the third eluate in the elution curve using a Sephadex C-25 column. This is assumed to be an unknown chemical species which has the ligands less than three, although its

identification in detail remains as a future problem.

A similar technique allows us to synthesize tris-benzoyltrifluoroacetylacetonato Tc(III) (hitherto unknown). A mixture of ruthenium metal and tris-benzoyltrifluoroacetylacetonato Fe(III) was irradiated with γ-rays and the irradiated mixture was subjected to sublimatographic procedures (4). A sublimatogram shows the presence of tris-benzoyltrifluoroacetylacetonato Tc(III) as a result of the following replacement reaction,

$$Ru(\gamma,p)Tc + Fe(bta) \longrightarrow Tc(bta)_3,$$

where bta denotes benzoyltrifluoroacetylacetone without one labile hydrogen atom. In addition to $Tc(bta)_3$, another unknown species appears in the sublimatogram, although it is not a major component. This may be a bis-complex containing bta-ligands.

Thus, recoil implantation (including related recoil techniques) is proved to be effective in the synthesis of some chelate compounds of technetium. Many systems other than those cited above are now intensively investigated.

One of the specific features of this method to be emphasized is the ease of preparing tervalent technetium chelates by recoil replacement reactions. Tervalent technetium chelate compounds are not so well known in chemistry of technetium. Among them there would be some interesting chemical species for radiopharmaceutical use.

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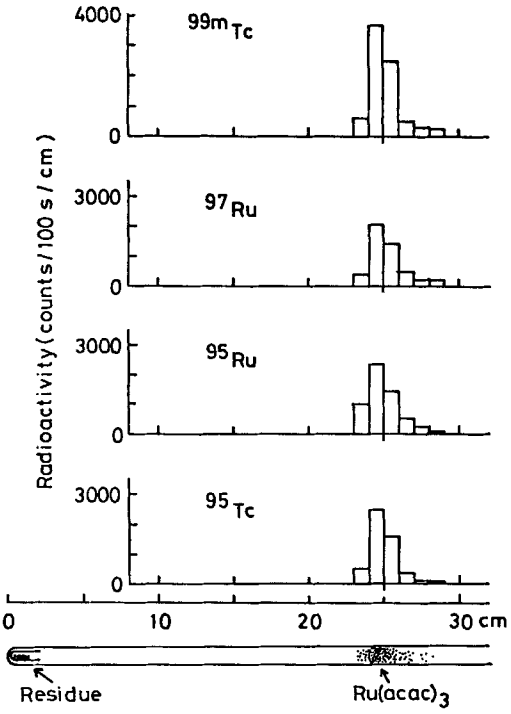

Fig. 1. Sublimatogram for $Ru(acac)_3$ irradiated with γ-rays in air.

18.2

PREPARATION AND BIOLOGIC EVALUATION OF LIPOPHILIC DERIVATIVES OF Tc-99m(Sn)PYRIDOXYLIDENEPHENYLALANINE: AN APPROACH TO STRUCTURE/BIO-DISTRIBUTION RELATIONSHIP OF TECHNETIUM COMPLEXES.

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We have previously reported a stannous reducing method for the preparation of Tc-99m-labeled pyridoxylideneaminates, and selected Tc-99m (Sn)pyridoxylideneisoleucine [Tc-99m(Sn)PI] as one of the promising hepatobiliary imaging agents (1-5). We have continued to study the relationship between the complex's structure and its bio-distribution to find agents with rapid blood clearance, quick hepatobiliary transport and low urinary excretion.

Recently, Yokoyama et al. reported that the valency state of Tc under our stannous reducing method would be tetravalent and the complexes would be mono-nuclear (6). Our separate work (7) on some mixed-ligand complexes in this system demonstrated that two molecules of pyridoxylideneaminates would coordinate to a Tc atom. Above findings allow us to draw a possible structure of Tc-99m(Sn)pyridoxylidenephenylalanine [Tc-99m(Sn)P.Phe] as shown in FIG.1. This time, we evaluated some halo- and alkyl-derivatives of phenylalanine (FIG.2) as the amino acid constituent, so as to reveal the structure/bio-distribution relationship in this system.

The preparation and the storage (frozen at -30 °C) of each kit reagent were identical to that reported for Sn-PI (2, 5). Technetium-99m complexes were prepared by mixing each kit reagent with an equivalent volume of $^{99m}TcO_4^-$ solution and incubating for 1.5 hr at 25 °C. A chromatographic study (silica gel TLC; MeOH:H_2O:MEK=9:1:10) (2) showed that the labeling efficiency for each preparation was practically 100 %. The lipophilicity of the complexes was evaluated by the measurement of their n-octanol/buffer partition coefficient(D$_o$ value) and their in vivo distribution was studied in Sprague-Dawley rats (TABLE 1).

The time course distribution study (FIG.4, FIG.5) revealed that the ortho-substituents (o-F, o-Me) were quite effective in accelerating the hepatobiliary transport, and the opposite results were obtained with the para-substituents (p-Cl, p-Me, p-iPr).

A linear relationship (FIG.3) was observed between the amount of the cumulative urinary excretion (FIG.6, FIG.7) and the D$_o$ value of the Tc-complexes. The o-F, o-Me and m,m'-diMe derivatives fit themselves on a lower line compared with the others; the fast hepatobiliary transport of these three derivatives would diminish the amount of radioactivity which go through the kidneys in the early phase, and hence reduce the urinary excretion.

Above results allowed us to consider the structure/bio-distribution relationship of these technetium complexes. The para-substituents effectively increase the global lipophilicity of the complex molecule and hence reduce the urinary excretion. The ortho-substituents, on the other hand, would interfere with the rotation of the benzene ring and hence increase the rigidity of the complex's structure. The increase in the rigidity of the structure seems to be effective in accelerating the hepatobiliary transport of Tc-99m(Sn)pyridoxylidenephenylalanine derivatives.

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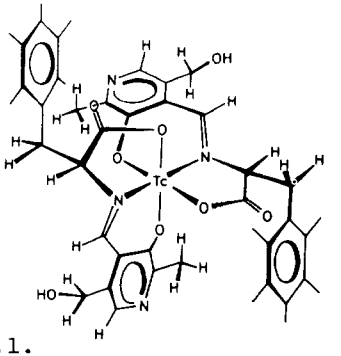


FIG.1.
Tc-(Sn)-Pyridoxylidenephenylalanines:
-Possible Structure-

TABLE 1. Lipophilicity* and in vivo distribution§ of Tc-99m(Sn)pyridoxylideneaminates.

	amino acid constituent									
			phenylalanine derivative							
	Ile	Phe	o-F	m-F	p-F	p-Cl	o-Me	m,m'-diMe	p-Me	p-iPr
D_o value*	0.58	2.1	2.4	3.0	4.1	7.7	5.0	6.3	7.1	10.9
Liver	0.78	5.63	0.58	5.67	5.01	12.01	0.62	1.52	6.88	7.48
Intestine	84.88	79.19	87.30	79.38	82.51	77.98	90.39	90.97	85.16	87.57
Kidneys	0.70	0.50	0.42	0.94	0.84	0.61	0.43	0.32	0.50	0.41
1 ml Blood†	0.033	0.072	0.042	0.083	0.076	0.058	0.039	0.056	0.039	0.086
Urine	12.09	10.09	8.36	9.56	9.23	5.03	5.98	4.49	5.39	2.13

*n-Octanol/buffer partition coefficient.
§Each value represents mean result (%ID/organ) for 5 rats at 1 hr after the i.v. administration.
†Normalized to a body weight of 200 g.

FIG.2.

Halo and alkyl derivatives of phenylalanine for the preparation of ^{99m}Tc-(Sn)-prydoxylidene-aminate for hepatobiliary imaging

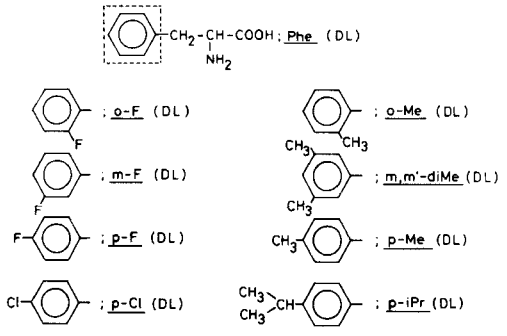


FIG.3.

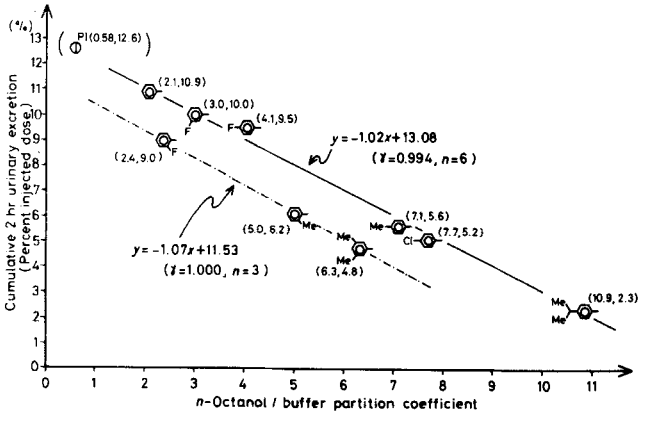


FIG.4.

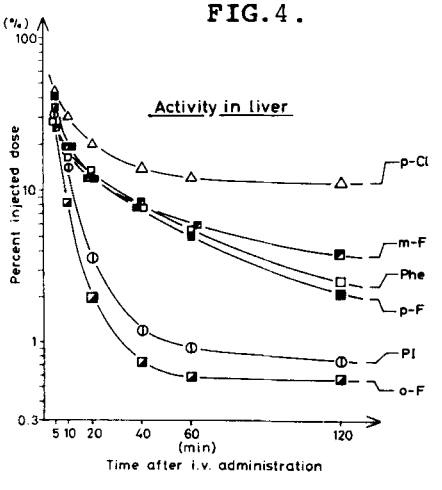


FIG.5.

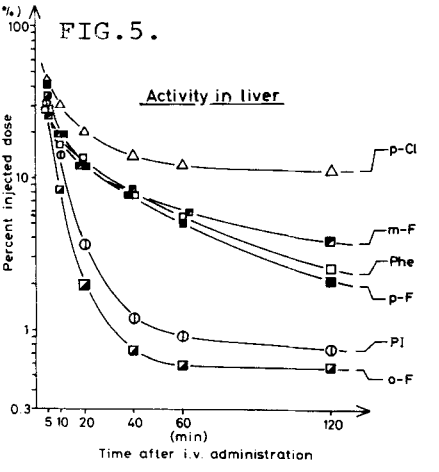
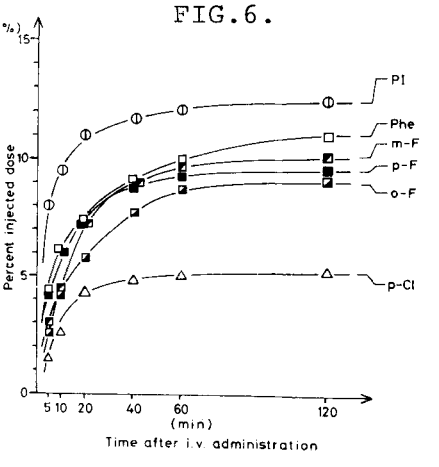
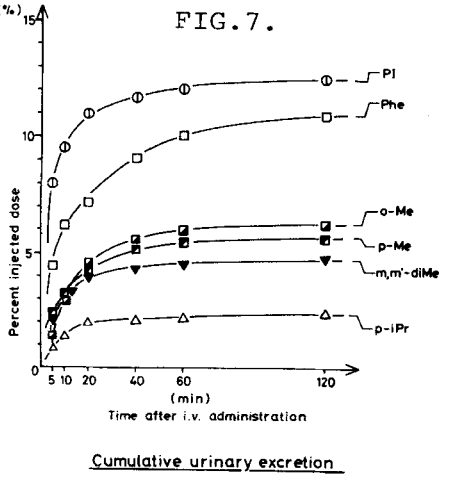


FIG.6.



Cumulative urinary excretion

FIG.7.



Cumulative urinary excretion

18.3

Tc-GLUCOHEPTONATE, CHEMICAL STRUCTURE AND TISSUE DISTRIBUTION

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^{99m}Tc-glucoheptonate is used in nuclear medicine as a kidney and brain imaging agent. To establish the chemical structure of the complex carrier ^{99}Tc was used. In a comparative study between ^{99m}Tc-glucoheptonate and ^{99}Tc-glucoheptonate, it was shown by means of TLC, electrophoresis and tissue distribution studies, that identical complexes were formed in physiological saline.

Owunwanne et al (1,2) by an ion-distribution method determined that the net charge of ^{99m}Tc-glucoheptonate was -1. The conditions of complex formation between reduced ^{99}Tc (reducing agent: Sn(II) and glucoheptonate were investigated by UV-Vis spectroscopy. Within a few seconds after reduction a red-violet ^{99}Tc-glucoheptonate complex is formed, which is stable in saline for more than a half year at 25°C. Absorbance is observed in the UV-Vis spectrum at 502 nm ($\varepsilon_{\max}$=65) and 270-280 nm ($\varepsilon_{\max}\sim$2800), assigned to a d-d electron transition and a charge transfer transition respectively. A 25x molar-excess glucoheptonate is recommended to obtain the ^{99}Tc-glucoheptonate complex. In order to identify the oxidation state of ^{99}Tc in ^{99}Tc-glucoheptonate, $^{99}TcO_4^-$ was titrated spectrofotometrically with $SnCl_2$ in saline in an inert nitrogen atmosphere. The oxidation state of ^{99}Tc proved to be +5. This agrees with the oxidation state reported for ^{99}Tc-gluconate(3). Tc(V) complexes frequently contain a Tc=O unit (4-7). The existance of Tc=O in ^{99}Tc-glucoheptonate has been demonstrated by IR-spectoscopy (Tc=O stretches at 930 cm^{-1} and 970 cm^{-1}). The IR spectrum also demonstrated co-ordination of ^{99}Tc by the carboxyl-oxygen of glucoheptonate.

The number of glucoheptonate ligands, which co-ordinates with one Tc(V)-ion was investigated by a mol-ratio method. The curve of the mol-ratios glucoheptonate/TcO_4^- vs. absorbance at 502 nm demonstrates a bend at glucoheptonate/TcO_4^- = 2.0. Thus two glucoheptonate molecules co-ordinate with one Tc(V) ion. The complex can be described as an oxobis(glucoheptonato)technetate(V) anion.

Experiments with the reducing agent $NaBH_4$ demonstrated the formation of an identical complex, suggesting the absence of Sn(II or IV) in the ^{99}Tc-glucoheptonate product. The absence of the incorporation of Sn(II or IV) is in agreement with the suggested structure of the Tc-gluconate complex (8). The in vivo distribution in rats of ^{99}Tc-glucoheptonate complexes prepared with different agents (Sn(II) or BH_4^-) indicated that the presence or absence of Sn (II or IV) did not influence the biological behaviour.

pH measurements combined with acid and base titrations demonstrated the reaction:

$$TcO_4^- + 2\ (C_7H_{13}O_8)^- + Sn^{2+} + H_2O \rightarrow [TcO(C_7H_{12}O_8)_2]^- + 2H^+ + [SnO_2(OH)_2]^{2-}$$

The Sn(IV) product can be a mixture of Sn(IV) hydrolysis products. ^{13}C- and ^{1}H-NMR studies of glucoheptonate in D_2O demonstrated a linear glucoheptonate molecule. In the complex a 5- or 6- membered ring can be formed, with the oxygen of the carboxylgroup and the oxygen of the hydroxylgroup of C_2 or C_3 as the donating atoms.

By means of TLC, electrophoresis and density gradient centrifugation it was shown that in human blood, ^{99m}Tc-glucoheptonate is distributed in the plasma (60%), erythrocytes (6%) and leucocytes (34%).

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18.4

^{99m}Tc-Cu-EHDP, ANOTHER FORMULATION FOR SKELETAL IMAGING

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Technetium-99m-labelled stannous ethane-1-hydroxy-1,1-diphosphonate(^{99m}Tc-Sn-EHDP) has been first reported as a potential radiopharmaceutical for skeletal imaging by Yano et al.(1). After that a current of EHDP (2-4) have been used for the skeletal evaluation and other bone lesion. Cuprous chloride has first been used in the preparation of ^{99m}Tc-Cu-HEDSPA by Chervu et al.(5). However, the present study entails the application for the first time the method of induced cuprous ions in the mixture (6) as a labelling tool for EHDP with technetium-99m. The rapid formation of ^{99m}Tc-Cu-EHDP in the mixture, suggests the probable reduction of pertechnetate to Tc(IV), in comparison with that in ^{99m}Tc-Cu-EDTA (6).

Gelchromatography Column Scanning (GCS) technique was used for the determination of reduced hydrolyzed-Tc-99m, free pertechnetate, and ^{99m}Tc-Cu-EHDP complex (7,8). Experimentally, the optimal concentrations of $CuCl_2$, EHDP and ascorbic acid in the final preparation were determined and found to be not less than 0.5, 2, and 38 mg/ml respectively. The labelling yield is not less than 95%.

The study of the influence of pH value on the amount of ^{99m}Tc-Cu-EHDP fraction shows that pH 1.6-1.7 gave the best labelling results. The GCS-profiles obtained from samples taken at various pH values are shown in Fig.1. The formation rate and stability of the compound were studied by analyzing the samples at different periods of time with GCS-method, Table 1.

In vivo study of the degree of binding the ^{99m}Tc-Cu-EHDP and ^{99m}Tc-Sn-EHDP with the plasma protein have been also performed. No significant difference was found in the plasma protein binding of the two compounds in rats as assessed by GCS-technique.

Comparative biokinetic studies of ^{99m}Tc-Cu-EHDP and ^{99m}Tc-Sn-EHDP in rabbits and mice showed a high bone uptake and fast elimination of ^{99m}Tc-Cu-EHDP from the skeleton. The clearance rate of ^{99m}Tc-Cu-EHDP and ^{99m}Tc-Sn-EHDP from the blood seems relatively the same.

On the basis of the high bone uptake of ^{99m}Tc-Cu-EHDP in rabbits and mice, the possible explanation is releated to the copper which has itself a greater affinity for bone than tin and not to the reduced technetium-99m.

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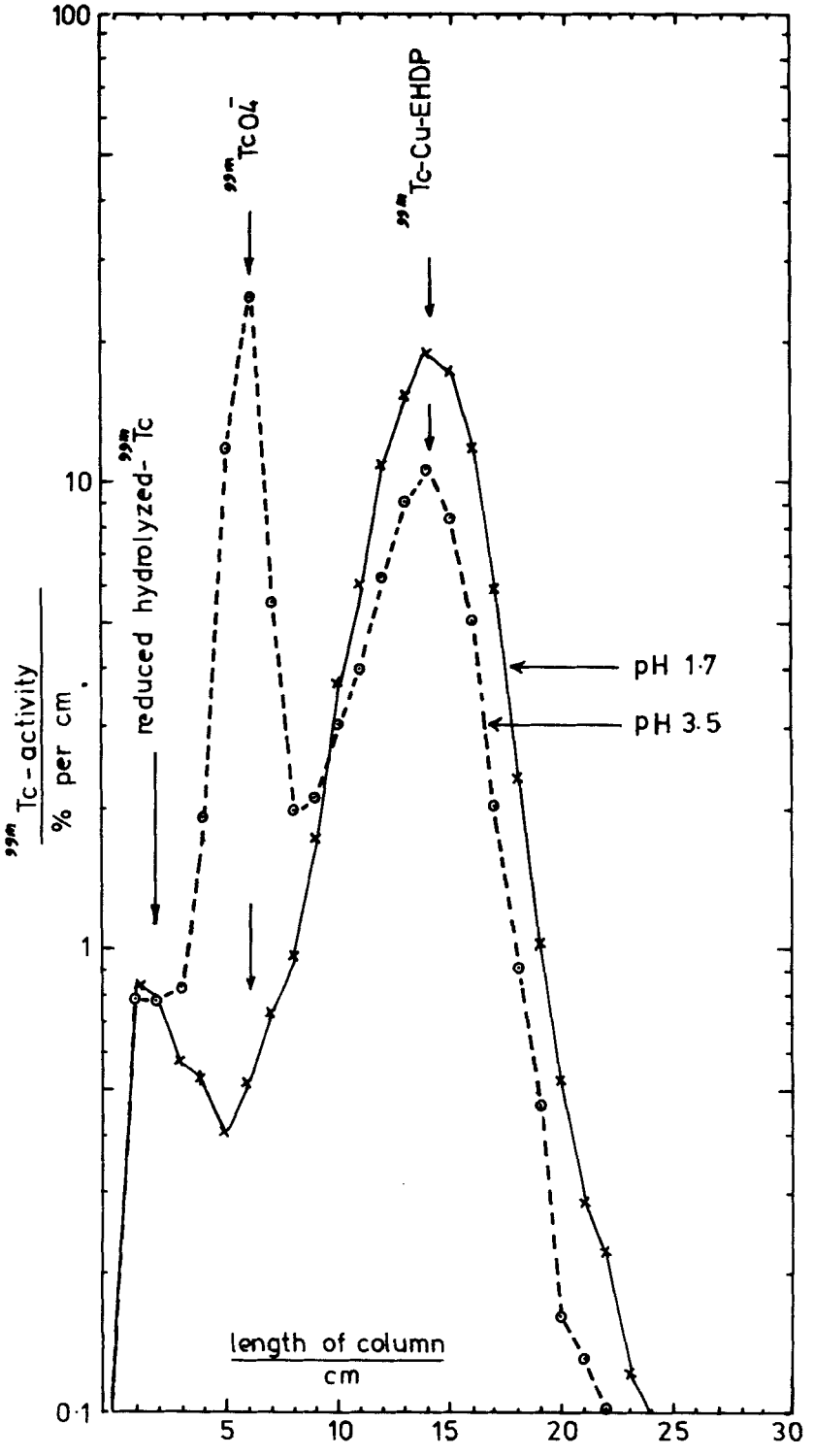
Table 1. The formation rate and stability of ^{99m}Tc-Cu-EHDP complex as function of time using GCS-method.

Time after mixing (min)	Percent of ^{99m}Tc-activity corresponding to reduced hydrolyzed-^{99m}Tc	TcO_4^-	^{99m}Tc-Cu-EHDP
3	1.4	4.2	94.3
5	1.3	3.7	94.8
10	1.3	3.2	95.5
15	1.3	3.4	95.1
30	0.9	2.8	96.2
60	1.3	2.8	95.9
120	1.4	2.7	95.8
240	1.5	2.7	95.8

FIG 1. Gel chromatography column scanning profiles obtained from ^{99m}Tc-Cu-EHDP preparations of various pH values.



18.5

TECHNETIUM 99m EXCHANGE BETWEEN HUMAN SERUM ALBUMIN AND PYROPHOSPHATE

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This paper is a report of an investigation into the transfer of technetium between human serum albumin and pyrophosphate. The recent literature contains two reports (1, 2) of the interaction of technetium phosphorous compounds and serum proteins in which the binding between the labelled technetium phosphorous compound and the protein is reported. However other reports in the literature indicate that technetium may suffer a chelate exchange between different ligands (3). In this investigation we demonstrate that technetium is actually exchanged between pyrophosphate and human serum albumin.

(A) Technetium 99m pyrophosphate and albumin.

Technetium stannous pyrophosphate solutions were prepared using the electrolytic generation of stannous ion in a solution of pertechnetate and sodium pyrophosphate. After conducting thin layer chromatography on a sample of the preparation to ensure that essentially complete labelling of the pyrophosphate had been achieved, human serum albumin was added to the preparation and the solution allowed to stand. Periodically over the next 24 hours a small sample (0.1-0.3 ml) was taken and applied to the top of a 60 cm Bio gel P-10 gel chromatography column. The column was then eluted with saline and the eluant collected in 40 drop (1.3 ml) samples. Under such conditions the human serum albumin is found to elute in a peak centered in the sixth fraction (void volume), the pyrophosphate in a peak centered in the sixteenth fraction and the free pertechnetate in a peak centered in the twenty-sixth fraction. Throughout the study if the radioactivity in excess of 3% of the total radioactivity was found in the free pertechnetate peak that set of data was disgarded on the basis of oxidation of the technetium due presumably to air infiltration of the sample vial. Such studies were carried out at a variety of pH's, the pH of the technetium 99m pyrophosphate solution being adjusted prior to the addition of the human serum albumin. The results obtained, shown in figure 1, indicate that at pH4.2 or greater there is a rapid initial exchange of technetium between the pyrophosphate and the albumin, followed by a second phase in which a slower exchange occurs. At pH2.3 no exchange was observed. It further appears that the duration of rapid initial exchange is pH dependent although the rate of exchange is not. The rate of the slower second phase also seems relatively independent of pH. Further studies in which the quantity of albumin added to the preparation was varied (at pH4.2) indicate that the rate but not the duration of the initial rapid exchange is dependent on the initial concentration of albumin; while a temperature study indicated that an increase in temperature resulted in an extention of the duration of the initial rapid exchange without any change in the rate of exchange, but increased the rate of exchange during the second slower phase.

(B) P-32 pyrophosphate and human serum albumin.

An identical experiment was carried out in which a tracer quantity of P-32 pyrophosphate was added with the sodium pyrophosphate during preparation of the technetium pyrophosphate. Following counting of the fractions from the gel chromatography for technetium 99m the fractions were stored for 5 days to allow the technetium 99m to decay, liquid scintillation solvent added to each and then counted for P-32. None of the P-32 was observed in the human serum albumin peak at any time interval. The P-32 was found totally in the pyrophosphate peak.

(C) Technetium 99m human serum albumin and sodium pyrophosphate.

Studies were carried out in which the technetium 99m human serum albumin was prepared using electrolytic generation of stannous ion in a solution containing albumin and pertechnetate and sodium pyrophosphate added to the labelled albumin

preparation. These studies showed no exchange of the technetium between the albumin and the pyrophosphate at any pH studied with a variety of concentrations of the reactants.

(D) Technetium 99m human serum albumin and stannous pyrophosphate.

Studies were carried out in which the technetium 99m human serum albumin was prepared as above and stannous pyrophosphate was prepared by electrolytic generation of stannous ion in the sodium pyrophosphate solution. After adjustment of the pH of both solutions they were mixed and allowed to stand with samples being taken at various time intervals. The resultant exchange, shown in figure 2, indicates that the exchange of the technetium takes place in a single rapid exchange the extent of which is pH dependent. It was further observed that a change in the relative concentrations of albumin and pyrophosphate (albumin up and pyrophosphate down) resulted in a decrease in the extent of the exchange, as would be expected, however since the rate of exchange is so rapid, being virtually complete within 1/2 hour, it is impossible to tell whether it is the rate or the duration of the exchange which is altered.

In summary it may be said that it has been demonstrated that technetium 99m may be exchanged between pyrophosphate and human serum albumin however if the stannous ion is not present with the pyrophosphate no exchange will occur from the albumin onto the pyrophosphate. Since exchange in both directions has been demonstrated it is clear that the phenomenon being observed is not that of binding of the technetium pyrophosphate to the albumin molecule. This is also indicated by the lack of P-32 pyrophosphate found in the albumin peak when tracer quantities of P-32 pyrophosphate was incorporated into the technetium pyrophosphate preparation.

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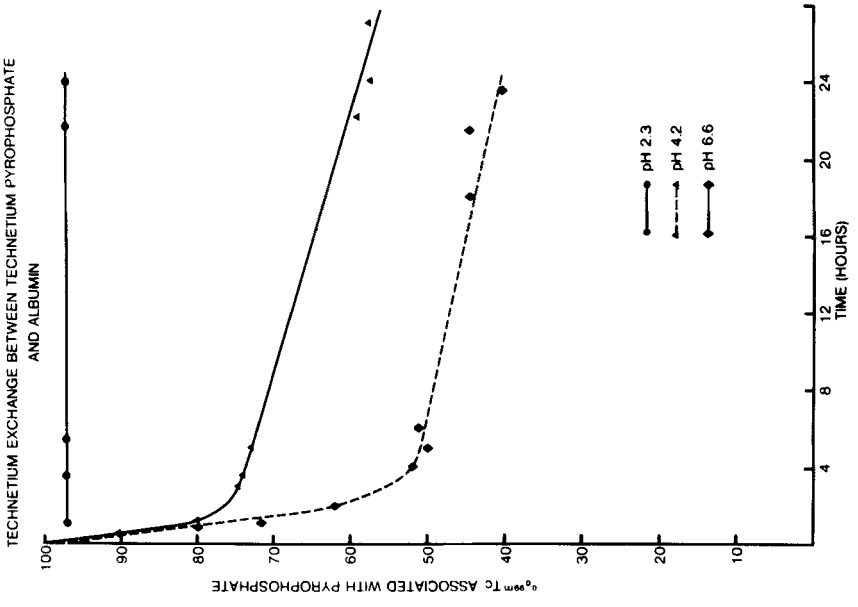
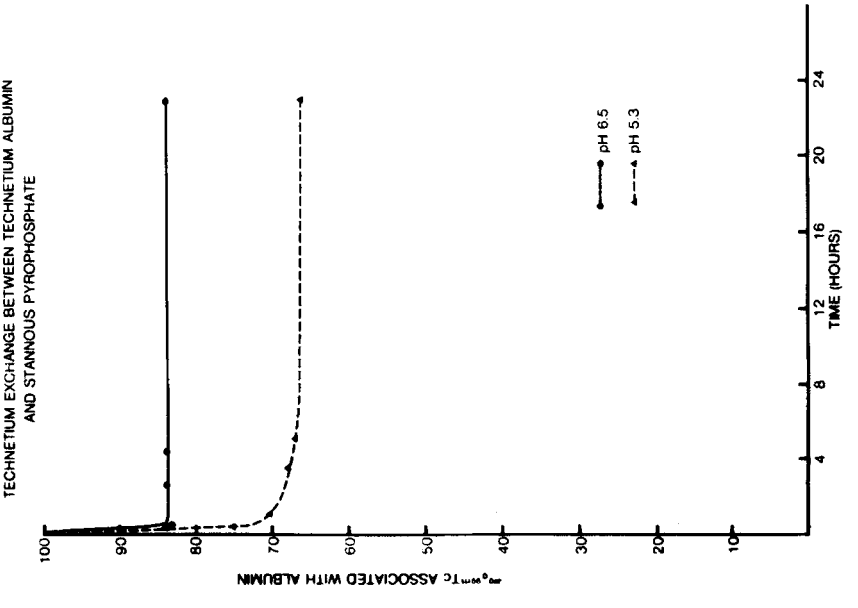

TECHNETIUM EXCHANGE BETWEEN TECHNETIUM PYROPHOSPHATE AND ALBUMIN
% 99m Tc ASSOCIATED WITH PYROPHOSPHATE
TIME (HOURS)
pH 2.3
pH 4.2
pH 6.6


TECHNETIUM EXCHANGE BETWEEN TECHNETIUM ALBUMIN AND STANNOUS PYROPHOSPHATE
% 99m Tc ASSOCIATED WITH ALBUMIN
TIME (HOURS)
pH 6.5
pH 5.3

18.6

IMPROVEMENTS OF THE LABELLING OF EHDP WITH ^{99m}Tc BY REDUCTION WITH Sn^{II}. Identification of the compounds formed and measurements of their adsorption efficiency.

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Optimization of the labelling.

The labelling of 1-hydroxy-ethylidene-1,1-disodium phosphonate with ^{99m}Tc by reduction with Sn^{II}, added as $SnCl_2.2H_2O$ is performed (1,2).
The compounds formed are separated by gel permeation chromatography on a 23 x 1.5 cm column of Biogel P4. A sample of one ml is separated into two fractions: one for the Tc-complex (0-30 ml) and one for the free pertechnetate (30-60 ml).
Both an EHDP solution and a physiological saline solution (0.9% NaCl) are used as the eluent. An EHDP solution has the advantage of preventing the decomposition by hydrolysis of the compounds formed (3).
The labelling percentage is determined radiometerically, using ^{99m}Tc.
The significance of the parameters: [EHDP], [Sn(II)], pH and their combinations is found by analysis of variance. The optimum of the labelling is obtained by the construction of the regression equation.
It is found that the labelling percentage is always high; a value of 97% is observed at a pH of 5.8 and a [Sn(II)]/[EHDP] ratio of at least 0.2 at very low EHDP concentration (4).

Compounds of Tc, Sn and EHDP

By using the same method of separation (Biogel P4 column 1.6 x 84 cm), ^{32}P labelled EHDP, $^{99}Tc/^{99m}Tc$ and $^{113}Sn \rightarrow {}^{113m}In$, it is possible to detect various components formed and to determine their relative composition (5). To this end the peaks in the chromatogram are submitted to interactive gaussian curve fitting with a fixed half width.
Occasionally peaks may be disturbed by isotopic exchange with the eluate and a possible decomposition of the complexes by hydrolysis.
Four compounds may be detected. Their probable composition is:

Tc(1): $Tc(Sn(IV))EHDP_3$ Tc(3): $Tc(Sn(IV))EHDP$
Tc(2): $Tc(Sn(IV))EHDP_2$ TC(4): Tc EHDP

The relative occurrences of the different compounds may be varied by changing the experimental conditions. The most obvious choice is the pH. Another variable is the sequence in which the reagents are introduced to prepare the $^{99m}Tc(Sn)EHDP$ complexes (6). It is observed that the distribution of ^{99m}Tc over the various complexes does not change within one hour after the pH is changed to physiological conditions. Thus different experimental conditions may lead to different products for medical application, without influencing the labelling efficiency.
The results can be summarised as follows:

Table: The labelling efficiency and the relative occurence of the different ^{99m}Tc complexes.

Elution parameter $(V_e-V_{BD})(V_{Sn}-V_{BD})^{-1}$							
			Tc(0)	Tc(1)	Tc(2)	Tc(3)	Tc(4)
			0.06±0.02	0.59±0.03	0.84±0.03	1.02±0.04	1.24±0.05
	Relative occurence of the different ^{99m}Tc(Sn)EHDP						
pH	sequence	Lab %	Tc(0)	Tc(1)	Tc(2)	Tc(3)	Tc(4)
2	1	100.0		43.2±1.1	49.8±1.1		7.0±0.4
2	1	99.8		40.9±0.7	51.9±0.7		7.2±0.3
5	1	98.8		38.8±3.7	43.9±3.6		17.3±1.3
5	1	98.4		42.6±2.6	44.0±2.5		13.8±0.9
7	1	98.7		41.8±2.3	53.8±2.4		4.4±0.7
7	1	99.1		38.9±0.9	56.1±3.0		5.5±0.5
9	1	97.9		19.5±0.6	74.9±1.3		5.6±0.4
9	1	99.9		19.5±0.5	75.5±1.3		5.1±1.0
2	2	99.5	36.7±2.7	18.4±2.5	22.9±3.8	16.9±3.9	5.0±1.2
2	2	98.3	45.9±2.4	15.7±1.9	20.1±2.6	14.3±0.9	4.0±0.9
11.5	2	99.1	2.8±0.2	16.7±0.9	45.0±1.4	24.4±1.2	11.2±1.0
11.5	2	98.8	2.0±0.2	14.2±0.3	41.6±0.5	33.4±0.5	8.8±0.3

sequence (1): 5.10^{-3}M EHDP/10^{-3}M Sn^{2+}/pH = x/$^{99m}TcO_4^-$/pH = 7.4/77.10^{-3}M NaCl

(2): 10^{-3}M Sn^{2+}/pH = x/$^{99m}TcO_4^-$/5.10^{-3}M EHDP/pH = 7.4/77.10^{-3}M NaCl

eluent 5.10^{-3}M EHDP/10^{-3}M Sn^{2+}/77.10^{-3}M NaCl/pH = 7.4

Ve = elution volume, V_{BD} = elution volume blue dextran, V_{Sn} = elution volume SnEHDP.

The adsorption of ^{99m}Tc complexes to hydroxyl apatite.

The percentage of adsorption to hydroxyl apatite will be measured at a constant temperature of 37°C and after a constant reaction time for the solutions of labelled products obtained under these various different experimental conditions.
The aim is to find the maximal adsorption efficiency and thus the most useful preparation technique.

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18.7

A NEW TYPE OF OXOTECHNETIUM(V) COMPLEX

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As a result of an investigation undertaken to evaluate the acetamidomethyl group ($-CH_2NHCOCH_3$) (1) as a water-solubilizing protecting group for thiolate ligands, a new type of oxotechnetium(V) complex has been synthesized and characterized. This neutral complex has been formulated as cis-$[Tc_2O_2(SCH_2CH_2S)_3]$, with one ethanedithiolate forming a bridge between the two technetium atoms, and the two oxygens cis to one another.

The reaction of one equivalent of $[n\text{-}Bu_4N][TcOCl_4]$ with two equivalents of bis-(acetamidomethyl)ethanedithiolate in methanol at room temperature rapidly gives orange crystals of the complex in 82% yield. Recrystallization from CH_2Cl_2/hexane allows the isolation of high quality crystals. The x-ray crystal structure determination is currently under way. Using an 18-fold excess of the protected thiolate, the same complex was produced in 75% yield, with no evidence for the presence of the known monomeric species $[TcO(SCH_2CH_2S)]^-$ (2) among the products. The presence of the protected ligand thus appears to be important in the reaction leading to the 3:2 complex. For example, addition of two drops of unprotected ethanedithiolate to the optical cell in the latter case produced an instantaneous change to the spectrum characteristic of the monomer (λ max, 403 nm). That this is not the sole factor governing the reaction, however, is shown by the fact that the addition of exactly 1.5 equivalents of unprotected dithiolate to one of $[TcOCl_4]^-$ gives orange crystals of the dimer instantly and in high yield. Furthermore, an analogous experiment with unprotected 2,3-toluenedithiolate ligand produced only the previously characterized monomer $[TcO(tdt)_2]^-$ (3). The dimeric neutral complex has also been synthesized by an indirect ligand-exchange synthesis in aqueous solution.

The material has been characterized by elemental analysis, infra-red and optical spectroscopy, and nuclear magnetic resonance spectroscopy. The exact mass has been determined by field desorption mass spectrometry (FDMS). The relative peak intensities at $^m/_e$ = 505 and 507 (due to the presence of ^{32}S and ^{34}S) confirm the presence of six sulfur atoms in the complex.

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Analysis:

		C	H	S
elemental:	calc	14.23	2.39	37.99
	found	14.76	2.63	36.85

(average of three determinations)

infra-red (KBr disc): ν (Tc = O) 953, 946 cm^{-1}

optical (in CH_2Cl_2):

λ (nm)	ε
424	6300
286	9600
242	shoulder, > 10,000

conductivity: a nonconductor in CH_3CN

^1H nuclear magnetic resonance: 250 MHz, in $CDCl_3$.

AA'BB' pattern, rel. int. 1, ∂ 3.22-3.34
3.41-3.53

ABCD pattern, rel. int. 2, ∂ 3.53-4.12

field desorption mass spectrometry:

m/e	rel. int.	rel. %
505	8	100
507	2.8	35 (^{34}S)

18.8

THE PREPARATION AND CHARACTERIZATION OF SOME NITROSYL COMPLEXES OF ^{99}Tc

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The reaction of $^{99}TcO_2 \cdot xH_2O$ with nitric oxide in 4M HBr leads to the isolation of red crystals of $[n\text{-}Bu_4N][Tc(NO)Br_4]$. This material is formulated as a low-spin d^5 paramagnetic complex that shows a 10-line solution EPR spectrum at room temperature, and which demonstrates only technetium hyperfine interaction. This complex is a useful starting material for the synthesis of other low valent technetium complexes.

The reaction of $[Tc(NO)Br_4]^-$ with excess NCS^- allows the isolation of ink-blue crystals of $[n\text{-}Bu_4N]_2[Tc(NO)(NCS)_5]$. This complex can be reduced electrochemically ($E_{\frac{1}{2}} = 0.14$v. with respect to S.C.E.), or chemically with hydrazine, to yield rust-colored crystals of $[n\text{-}Bu_4N]_3[Tc(NO)(NCS)_5]$.

All three of these complexes have been characterized by elemental analysis, infra-red and optical spectroscopy, and conductance measurements. The magnetic properties and infra-red spectral studies show that the nitric oxide ligand is coordinated as an NO^+ group in these materials, which are thus to be regarded as complexes of
Tc(+2) $\{[n\text{-}Bu_4N][Tc(NO)Br_4]$ and $[n\text{-}Bu_4N]_2[Tc(NO)(NCS)_5]\}$ and
Tc(+1) $\{[n\text{-}Bu_4N]_3[Tc(NO)(NCS)_5]\}$.

Analysis:

$[n\text{-}Bu_4N][Tc(NO)Br_4]$

		C	H	N	Br
elemental analysis:	calc	27.81	5.25	4.05	46.25
	found	27.36	5.56	4.15	46.06

I.R. (KBr disc): ν_{NO} 1795 cm^{-1}

conductivity: Λ_M = 168 $cm^2 \cdot ohm^{-1} \cdot mole^{-1}$ (1 mmol in CH_3CN)

$[n\text{-}Bu_4N]_2[Tc(NO)(NCS)_5]$

		C	H	N	Br
elemental analysis:	calc	49.15	8.02	12.39	17.73
	found	49.27	8.16	12.47	17.49

infra-red (KBr disc): ν_{NO} 1785 cm^{-1}

conductivity: Λ_M = 256 $cm^2 \cdot ohm^{-1} \cdot mole^{-1}$ (1 mmol in CH_3CN)

$[n\text{-}Bu_4N]_3[Tc(NO)(NCS)_5]$

		C	H	N	Br
elemental analysis:	calc	55.51	9.49	10.99	13.98
	found	55.20	9.30	11.28	13.55

infra-red (KBr disc): ν_{NO} 1690 cm^{-1}

conductivity: Λ_M = 362 $cm^2 \cdot ohm^{-1} \cdot mole^{-1}$ (1 mmole in CH_3CN)

18.9

MAGNETIC MOMENTS OF SOME TECHNETIUM COMPLEXES IN D_2O

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The position of a particular band in the NMR spectrum of a liquid sample is determined by the bulk susceptibility of the solution .In the presence of a paramagnetic solute there will be a shift in the band position, which,according to Evans, permits the calculation of the magnetic mass susceptibility of the solute.(1) Evans made differential measurements, introducing a capillary containing a paramagnetic salt and a reference compound in D_2O into a NMR tube containing only the reference compound (t-butanol) and D_2O. The arrangement eliminates diamagnetic corrections.

We have used the following form of his equation, eliminating two minor terms, and substituting moles for grams:

$$\chi_M = \frac{3\Delta f}{2\pi f m} \qquad (1)$$

In this equation, χ_m is the molar susceptibility of the paramagnetic species, Δf is the frequency separation of the t-butanol proton bands in Hz (cycles/sec), f is the frequency of the spectrometer in Hz, and m is the number of moles of paramagnetic substance in 1 ml. of solution. This in turn permits the calculation of the effective magnetic moment, μeff., from the Curie law, for a particular absolute temperature T:(2)

$$\mu_{eff.} = 2.84\sqrt{\chi_M T} \quad \text{(in Bohr Magnetons)} \qquad (2)$$

Measurements were made on a Varian T60A, operating at 60 mega-Hz; the sample temperature was 38°C. A double NMR tube was used, with identical solutions (except for the paramagnetic species) inside and out. Each solution had 2% t-butanol as the reference. The frequency separation was measured on the instrument recorder paper. The reproducibility of the separation was 0.1 Hz, but the instrument was not sensitive enough to show the bands if the separation was 0.4 Hz or less (determined with known solutions of $KCr(SO_4)_2 \cdot 12\ H_2O$).

Table I shows the performance of the instrument with four known compounds in solution. The term $\mu_{lit.}$ refers to literature values.

Table I
Test of Evans' Method

Compound	Molarity	Δf,Hz	$\chi_m \times 10^6$	μeff	$\mu_{lit.}$
$K\ Cr(SO_4)_2 \cdot 12H_2O$	0.0125	10.0	6370	4.0	3.84(3)
$Ni(NH_4)_2(SO_4)_2 \cdot 5H_2O$	0.162	83.0	4079	3.2	3.26(4)
$CuSO_4 \cdot 5H_2O$	0.0788	14.6	1475	1.9	1.95(5)
$(NH_4)_2TcCl_6$ in DCl	0.0112	9.7	6895	4.2	4.05(6)

The first three literature values refer to the moments of solids at 300°K. The fourth refers to a measurement of $(NH_4)_2TcCl_6$ in HCl solution. The experimental values and the literature values are in good agreement.

A number of technetium-99 compounds of radiopharmaceutical interest were prepared in D_2O. The citrate [7] and mannitol [8] complexes were made by reduction of pertechnetate with excess $SnCl_2$. The others were prepared by controlled potential electrolysis; the phosphate and pyrophosphate solutions were then air-oxidized.[9] The oxidation state of technetium is +4 in the first five compounds. It has the d3 configuration, which should mean a minimum of complicating factors in interpreting the results. The mannitol complex is a Tc(V) compound.[8] The results are shown in Table II. The term μcalc. represents the expected value, which is the spin-only value corrected for spin-orbital coupling.

Table II
Magnetic Properties of Tc(IV) and Tc(V) Complexes in D_2O

[Tc], mM	Ligand	Δf, Hz	μeff.	μcalc.
2.60	0.4M citrate, pH7	2.0	3.9	3.6 [10]
2.69	1M phosphate, pH7	0.0-0.4	0-1.66	3.6
2.69	0.2M pyrophosphate, pH7	0.7	2.3	3.6
2.65	0.49M EHDP, pH5.1	0.5	2.0	3.6
2.65	0.46M MDP, pH7	1.0	2.8	3.6
2.55	0.17M mannitol, pH 12.4	0.0-0.4	0-1.66	1.5 [11]
11.3	0.71M mannitol, pH 13	0.0-0.1	0-0.4	1.5

It was concluded that the citrate is a normal Tc(IV) complex, that the phosphate probably has antiferromagnetic properties, and hence is a dimer or polymer, and that this may also be true of the pyrophosphate, EHDP and MDP Tc(IV) complexes. The mannitol complex similarly may be a dimer; it may, however, be a low-spin Tc(V) complex.

Other Tc complexes will also be discussed.

We thank the Proctor and Gamble Company for the EHDP and MDP compounds

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18.10

Tc-99-CYCLAM

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Earlier studies (1,2) have shown that Tc-99m and macrocyclic amines react to form stable complexes in high yield. We are investigating the potential use of these complexes and their derivatives as radiopharmaceuticals. A part of that investigation is a study of the reactions of one of those amines, cyclam (1,4,8,11-tetraazacyclotetradecane), with Tc-99 in order to understand better the nature of the carrier-free complexes of Tc-99m.

Both $SnCl_2$ and $Na_2S_2O_4$ were satisfactory reducing agents when the complex was prepared at pH $\sim$ 11. Complexes prepared with either reagent exhibited identical elution volumes from Biogel P-2 columns, R_f values in paper and thin-layer chromatography, and electrophoretic migration patterns. In one series of experiments Tc-99 spiked with Tc-99m was complexed using Sn-113 labeled $SnCl_2$ as the reducing agent. Fractions of the eluent from a Biogel P-2 column were collected and both Tc-99m and Sn-113 were counted in each fraction. Two over-lapping peaks were observed but there was a clear difference between the distributions of Tc-99m and Sn-113. Electrophoresis of the complex was performed at 300 V for one hour in an acetate buffer at pH 5. Tc-99 migrated towards the anode while the Sn-113 remained at the origin. These results show that Sn is not a part of the complex.

The complex is formed only in the presence of large excesses of cyclam. Complexing of 90% or better at pH $\sim$ 11 required cyclam concentrations of $\sim 10^{-2}$ M, 2×10^{-3} M, 1.5×10^{-4} M, when Tc-99 concentrations were 10^{-3} M, 10^{-4} M, and 10^{-5} M, respectively. The excess cyclam could be extracted into $CHCl_3$ from 10^{-2} M NaOH with negligible extraction of Tc-99-cyclam.

Radiometric titrations of 1.35×10^{-4} M Tc-99 pertechnetate in 1.0 M cyclam at pH 11 using standardized $SnCl_2$ solutions in a N_2 atmosphere showed that a two-electron transfer is necessary to form the complex leading to a Tc oxidation state of +5. Electrophoretic migration patterns of complexes prepared with a substoichiometric amount of $SnCl_2$ were identical to those of complexes prepared with an excess of $SnCl_2$ showing that further reduction did not occur after complexing was complete.

Efforts were made to determine the charge of the complex by an electrophoretic method similar to that developed by Marzilli, Worley, and Burns (3). The complexes $[Co(III)\ cyclam\ Cl_2]^{+1}$, $[Co(III)\ cyclam\ (N_3)_2]^{+1}$, $[Co(III)\ cyclam\ (SCN)_2]^{+1}$, $[Ni(II)cyclam]^{+2}$, $[Co(III)cyclam\ (NH_3)_2]^{+3}$, $[Co(III)(en)_3]^{+3}$, and $[Co(III)(NH_3)_6]^{+3}$ were prepared following literature methods. C-14 labeled cyclam was synthesized using C-14 dibromoethane as a starting reagent. Cyclam is doubly protonated at pH's near 7 and can be represented as cyclam-H_2^{+2}. All these complexes as well as Tc-99-cyclam complex were subjected to electrophoresis under identical conditions. Migration of Ni^{+++} complexes was determined by color of the moving band, Co^{+++} by both color and Co-57 tracer, of cyclam by C-14, and of Tc-99 by Tc-99m tracer. The Tc-99-cyclam complex migrated the same distance as $[Co(III)cyclam\ Cl_2]^{+1}$, $[Co(III)cyclam\ (N_3)_2]^{+1}$, and $[Co(III)cyclam\ (SCN)_2]^{+1}$, about one-half as far as cyclam-H_2^{+2} and $[Ni(II)cyclam]^{+2}$, and about one-third as far as $[Co(III)(NH_3)_6]^{+3}$ and $[Co(III)(en)_3]^{+3}$. These results indicate the charge of the Tc-99 complex is +1. However, $[Co(III)cyclam\ (NH_3)_2]^{+3}$ migrated only as far as $[Ni(II)cyclam]^{+2}$. This result is not yet fully understood and this method of charge determination may not be unequivocal. If, however, the complex charge is +1 as the evidence suggests, a complex formula consistent with that result and with the +5 oxidation state of Tc is $[TcO_2\ cyclam]^{+1}$.

In an attempt to prepare a compound of the complex for IR analysis, 15 mg of Tc-99 as NH_4TcO_4 dissolved in a solution containing a 20-fold excess of cyclam was reduced using a 4-fold excess of $Na_2S_2O_4$. The solution turned light brown upon

addition of the $Na_2S_2O_4$. Excess cyclam was extracted into $CHCl_3$ after making the solution 0.1 M in NaOH. A two-fold excess of $NaClO_4$ was added and orange crystals appeared following slow evaporation. The crystals were collected and washed with water. Paper chromatography on a portion of the redissolved solid showed the Tc-99 to be greater than 95% complexed. Electrophoretic patterns were identical with those of freshly prepared cyclam complexes of both Tc-99 and Tc-99m. An IR spectrum performed in a Nujol mull revealed no band near the one at 950 cm^{-1} observed by DePamphilis, Jones, Davis, et al. (4) in $TcO[SCH_2C(O)S]_2^{-1}$ and attributed by them to the Tc=O stretch. There was, however, a strong band at 790 cm^{-1}. Johnson, Lock, and Wilkinson (5) observed similar bands at ~ 810-820 cm^{-1} for compounds containing $[Re(py)_4O_2]^{+1}$ and $[Re(en)_2O_2]^{+1}$ and attribute them to the O=Re=O bond. The 790 cm^{-1} band observed here may therefore be due to a O=Tc=O bond. Experiments are underway to synthesize larger quantities of the complex for elemental analysis and structure determination.

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18.11

REVERSED PHASE HPLC OF Tc-99m IMINODIACETATE HEPATOBILIARY AGENTS AND A QUESTION OF MULTIPLE PEAKS.

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The chromatography of Tc-99m iminodiacetate hepatobiliary radiopharmaceuticals on paper and thin layer systems has been described (1,2). Although intermediate R_f values have been obtained, the bands of radioactivity are often broad, and, in contrast to a single peak on HPLC reported for Tc-99m-N-(2,6-dimethylacetanilide)iminodiacetate (Tc-dimethyl-IDA), often show overlapping multiple bands present. The objective of this study, therefore, was to evaluate the HPLC behavior of several of these complexes under a variety of solvent conditions. Technetium-99m complexes of N-(2,6-diethylacetanilide)iminodiacetate (Tc-diethyl-IDA), N-(2,6-diisopropylacetanilide)-iminodiacetate (Tc-diisopropyl-IDA), and N-(p-butylacetanilide)iminodiacetate (Tc-p-butyl-IDA) usually resulted in a major and a minor peak and parameters which might explain the multiple peaks were also studied.

The radiopharmaceuticals, Tc-diethyl-IDA, Tc-diisopropyl-IDA, and Tc-p-butyl-IDA were separable from each other on a reversed phase octadecylsilyl column using 0.02M phosphate, pH 6.8, and acetonitrile over a 10 to 60% acetonitrile gradient (Table 1). Subsequently, it was found with Tc-diethyl-IDA that separation of the two normally appearing peaks was maintained or increased as the gradient was reduced to as much as 20 to 40% acetonitrile with a reduction in flow rate to 2.35 ml/min (Table 2). No loss of separation or peak sharpness was noted as the buffer pH was altered from 5.5 to 6.8. Use of methanol or ethanol as the primary solvent reduced retention times to less than 2 min and resulted in loss of peak resolution. Water as the primary solvent and methanol or ethanol as the secondary resulted in comparable retention times to phosphate buffer and acetonitrile, but with broadened peaks.

The question of multiple peaks from these complexes is important since it has been observed for Tc-diethyl-IDA, Tc-diisopropyl-IDA and Tc-p-butyl-IDA. Moreover, a pair of peaks has been present in two commercial sources and our in house Tc-diethyl-IDA in variable ratios. Although presently only in a preliminary stage, experimental results suggest that the peaks may be due to different oxidation levels. Addition of small amounts of sodium borohydride (ca. 0.2mg) reduced the intensity of the later appearing peak (Figure 1). Correspondingly, the earlier peak intensity was increased. It has been reported that Tc-dimethyl-IDA has an oxidation level of 3 after stannous ion reduction (3). It is possible that under some conditions such as lower kit pH, sterilization by radiation, and storage time, which is the case for the manufacturer with larger amounts of the latter peak, that some complex may be formed at a higher oxidation level which was converted to the lower by hydride reduction. Other work which also supports differing oxidation levels has resulted from the Tc-99m complex of N,N'-bis(benzoylmercaptoacetamido)-ethylenediamine. The use of different reducing agents also resulted in two peaks which are common with each reducing agent but in variable ratios. Studies to establish this point are continuing.

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TABLE I. RETENTION TIMES OF Tc-99m-IMINODIACETATE COMPLEXES ON REVERSED PHASE HPLC*

Tc-99m-Complex	Retention times (min)
N-(2,6-diethylacetanilide)iminodiacetate	8.6(major), 10.4 (minor)
N-(2,6-diisopropylacetanilide)iminodiacetate	10.0(major), 10.9 (minor)
N-(p-butylacetanilide)iminodiacetate	10.3(major), 9.5(minor)

*Conditions were 0.02 M phosphate and acetonitrile with a gradient of 0 to 50% acetonitrile in 10 min at 3.5 ml/min flow rate on an ODS column.

TABLE 2. RETENTION TIMES OF Tc-DIETHYL-IDA UNDER VARYING GRADIENT AND FLOW RATE CONDITONS*

Condition				
Gradient	10-50% B	15-50% B	18-42% B	20-40% B
Flow Rate	3.0 ml/min	2.75 ml/min	2.5 ml/min	2.35 ml/min
Retention Time				
Peak 1	5.9 min	5.5 min	5.7 min	5.2 min
Peak 2	6.8 min	6.4 min	6.8 min	6.7 min

*Solvent A was 0.01 M phoshate, pH 5.8, solvent B acetonitrile. Gradient was over 10 min period. Column was 15 cm ODS.

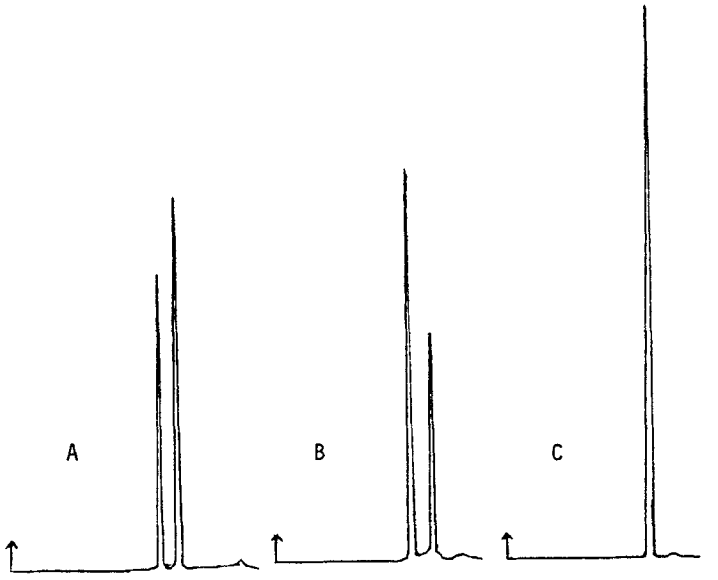

Figure 1. HPLC of Tc-diethyl-IDA on an ODS column eluted with 0.01 M phosphate and acetonitrile (10 - 50% gradient, 3 ml/min). A is control Tc-diethyl-IDA, B is after addition of 0.2 mg $NaBH_4$, and C is after further addition of 0.4 mg $NaBH_4$. Peak heights are adjusted for dilution effects. Note loss of second peak after addition of reducing agent.

18.12

PRELIMINARY STRUCTURE-DISTRIBUTION RELATIONSHIPS (SDR's) OF 99m-Tc HEPATOBILIARY AGENTS. (1) PROTEIN BINDING OF HIDA's.

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Hepatobiliary agents, based on substituted acetanilido iminodiacetic acid derivatives (the HIDA's) have, since their discovery, rapidly gained acceptance for routine clinical use.

Many different derivatives have been made to try and optimise the properties of the HB agents and it has become apparent that more than one compound will be needed to investigate the complete spectrum of hepatobiliary disease (1).

Structure-distribution relationships (SDR's) are required to develop a logical program of synthesis of these new agents. Some information along these lines has been reported (2,3), however, it really only gives a general idea of what a compound should be in order that it be excreted via the HB system. It does not give much information as to what a compound should look like to yield high contrast images in the various clinical situations.

The different arenas in which HB agents will be used can be very generally divided into use in jaundiced patients and use in non-jaundiced patients. In the latter group, it can be argued that, provided hepatic uptake and excretion is adequate, renal excretion is the major factor influencing the acquisition of good images. In the jaundiced group, competition with bilirubin becomes a dominant factor. The deleterious effect of bilirubin on the hepatic uptake of some of the HIDA's in rabbits has been demonstrated (4) and similar results have been obtained using isolated rat hepatocytes (1).

In order to simplify the determination of SDR's for the HIDA's under different physiological conditions, we have chosen to divide the problem into a number of parts. These are:

1. SDR of protein binding - including the effect of bilirubin and common drugs.
2. SDR of isolated hepatocyte uptake.
3. SDR of liver transport kinetics.

From the individual SDR's we hope to determine an overall SDR which will allow us to predict and develop an optimum HIDA for the whole spectrum of HB disease. It should also be possible to develop a model for the HIDA binding site on albumin.

This paper reports preliminary work performed to determine SDR's for protein binding. As the major transport protein in the blood is albumin, we chose to investigate albumin - 99m-Tc HIDA interactions. An affinity chromatography method was used because it is rapid, but more important, it is a dynamic system which is more akin to the *in vivo* situation (than the slower kinetics of dialysis, etc.). For this reason also 99m-Tc rather than 99-Tc complexes were used in these studies and thus no attempt has been made to determine the number of binding sites or binding constants. The system consists of an agarose support material to which has been attached directly, (using CNBr Sepharose 4B), or indirectly, (using CH Sepharose 4B), human serum albumin. The affinity of the HB agents for the albumin can be determined by observing their retention characteristics after application to and elution from columns of the affinity matrix with 0.9% Nacl. The methodology and advantages of the system have been reported elsewhere. (5,6).

Approximately, thirty HIDA derivatives have been synthesised bearing

substituents, ranging from the halogens to short chain alkyls (and combinations of the same) in the 2 to 6 positions.

The results so far show that there is a difference in the interaction with albumin depending upon the position of substitution and the substituent.

With no substitution (R_1 - R_5 = H) and when R_1 - R_5 = F there is negligible interaction with the albumin and the radiopharmaceutical is eluted close to the void volume of the column. No stearic effect would be expected by F for H substitution and it appears that F-hydrogen bonding interactions are not important.

Derivatives with 2 or 6, substitution also show little interaction with the albumin. This remains true even when doubly substituted derivatives are used containing short chain alkyls or higher halogens.

Derivatives substituted in the 4 position or the 3 and 5 position, show a strong interaction with the albumin. This interaction increases with increasing chain length and between the different halogens.

The 4-Butyl derivative is very strongly bound to the albumin. Attempts at displacing bound activity with phenol red, (which is known to bind to albumin) were unsuccessful. However, the activity could be eluted by the addition to the column of either cold 4-Butyl HIDA or various other substituted derivatives. The Tc-HIDA complexes are known to be resistant to ligand exchange so this result confirms that the interaction of the complexes with the albumin is through the ligand and also demonstrates that the uncomplexed ligand can compete with the complexed ligand for the binding site despite the differences in charge and molecular weight. Thus it appears that, as with the previously reported biliary excretion of HIDA with substitution of Cr(III) for Tc(III)(3), the metal atom plays an inert role during interaction of the HIDA's with albumin. The nature of the HIDA binding site is being investigated and the results of studies with bilirubin and drugs that influence the binding of bilirubin to albumin will be reported. The influence of fatty acids bound to the albumin and the relationship between octanol/water partition coefficients (determined by HPLC) and binding, will be described. The relevance of the results to the design of HB agents will be discussed.

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18.13

SPECIFIC SEQUESTERING AGENTS FOR TRIVALENT METALS

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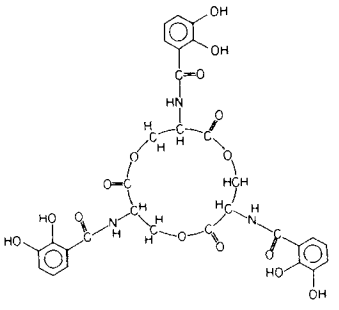
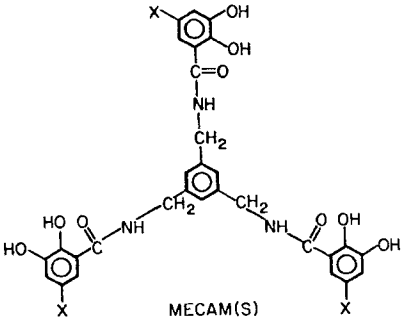
Enterobactin is one of a class of compounds called siderophores which are produced by microorganisms to obtain the amounts of iron essential for growth (1,2). Enterobactin and the other siderophores form extremely stable complexes of high-spin Fe(III). Similar stability is observed for other trivalent metal ions such as Ga(III) and Cr(III) (3).

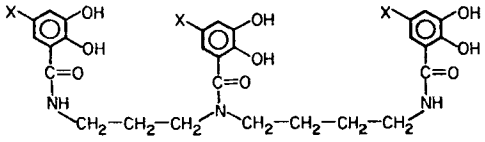
The synthesis of a series of compounds which are analogous to enterobactin will be described (4-7). The formation constants of metal complexes of these compounds have been determined and this information is used in their modification and improvement.



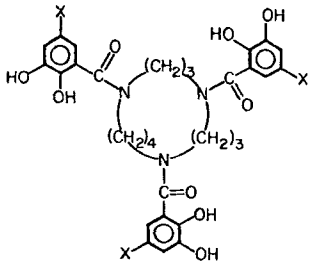
ENTEROBACTIN



MECAM(S)

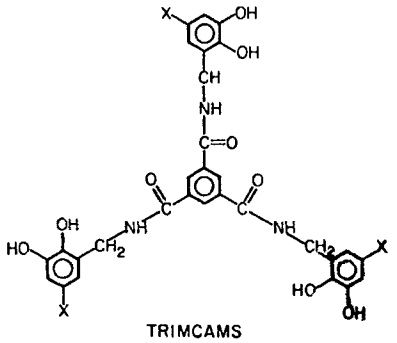


3,4-LICAM(S)



CYCAM(S)

X = H, (SO_3^-)



TRIMCAMS

The tris-catechol derivatives of 2,3-dihydroxybenzoic acid (DHB), x = H in the above figures) are in some cases nearly as effective in complexing Fe(III) and Ga(III) as is enterobactin. The sulfonated derivatives ($x = SO_3^-$) are even more effective and offer the advantages of increased water solubility, greater acidity (hence better competition of the metal ion versus protons) and improved stability toward oxidation.

Alkylation of the nitrogen atoms in the above compounds substantially increases hydrophobic character of these materials.

The biodistribution properties of the Ga(III) complexes of these sequestering agents will be described in a separate paper at this meeting (8).

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