THE UTILIZATION OF MASS SPECTROMETRY FOR VARIOUS APPLICATIONS IN TECHNETIUM CHEMISTRY

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The use of field desorption mass spectrometry, both in positive (1) and negative ion (2) mode, has proven to be extremely useful in establishing the nature of technetium compounds. The choice of mode depends on the particular species being investigated. With anionic complexes, for instance, negative ion mode has provided very simple spectra, giving ion currents approaching those of electron impact mass spectrometry. As an example, the monoanionic complex $[TcO(ema)]^{1-}$ (where ema = N,N'-ethylene-bis(2-mercaptoacetimidate) as its tetrabutylammonium salt gives peaks corresponding to the species A^- and CA_2^- (A = anions, C = cation) at m/z values of 319 and 803, respectively. In the case of other anions investigated, the CA_2^- peak is weak or absent. The presence of the A^- peak, however, makes the assignments unequivocal.

When using positive ion mode, a fairly strong C^+ peak is usually seen, with weaker ones corresponding to $M^+ (= CA^+)$ and C_2A^+. The unipositive cation hexakis-(t-butylisonitrile)technetium(I) as its hexafluorophosphate salt gave peaks at m/z 597 (C^+) and 742 (M^+). By contrast, the $[TcO(ema)]^{1-}$ anion gave a more complex spectrum in positive ion mode, with m/z values of 242, 321, 561, 641 and 803, corresponding to the species C^+, H_2A^+, M^+, $H_3A_2^+$ and C_2A^+ (2,3). When the complex being studied has a higher charge, the spectra become more complex and multiply charged species are often detected. As an instance, di- and tri-cationic clusters were found in the spectrum of $[n\text{-}Bu_4N]_3[TcCl_6]$, including a broad envelope (due to ^{35}Cl and ^{37}Cl) of doubly-charged ions from m/z 1072 to 1096. The elemental composition of this cluster based on high resolution mass measurements under positive ion FD conditions is $[(Bu_4N)_3(TcCl_6)Cl]_2^{2+}$. Discrimination between single and higher charge ions is provided by the peak to peak separations in peak envelopes arising from those species. Thus a doubly-charged ion shows peaks at half its mass separated by 0.5 a.m.u. intervals. It should be noted that the bulk of our studies are done under low resolution conditions, with m/z being estimated to the nearest integral value. High resolution studies, on the other hand, allow estimates to within 0.005 a.m.u. In these cases, the large mass defect and isotopic purity of ^{99}Tc allows most assignments to be made with confidence. Sensitivity studies have shown that a few microliters of a 10^{-6}-10^{-5} M solution of the material are sufficient to give usable spectra.

The technique is also not restricted to salts. The neutral technetium species $[Tc_2O_2(edt)_3]$ (where edt = 1,2-ethanedithiolate) was originally identified by FDMS, with peaks being observed at m/z of 505 and 507. In a few instances so far, either no spectrum was obtained or the ion current in FD mode was too transient. In these cases, we have used fast atom bombardment mass spectrometry (FABMS) for their identification. The FAB spectra are more complex than FD, however, because fragmentation can occur.

Thus far we have used these techniques for the following purposes: (2) the characterization of new compounds; (b) to study the nature of reactions on ligands coordinated to technetium; (c) the examination of

components present in radiopharmaceutical preparations separated by HPLC; (d) to determine the fate of technetium complexes after administration into animals.

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SOME INTERACTIONS OF TECHNETIUM LABELLED COMPOUNDS WITH ALBUMIN AND COLLAGEN

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1) Exchange of technetium between gluconate and albumin.

It is generally assumed that the technetium atom once bound to another molecule will remain bound to that molecule. The only generally considered exception to this rule being the possibility of reoxidation of the technetium back to the pertechnetate form[1,2]. However examples of transfer of technetium between molecules are known[3] and in fact some workers have evaluated the likely stability of new technetium 99m radiopharmaceuticals in the presence of strong chelating agents[4] in recognition of this possibility. We studied the exchange of technetium 99m between gluconate and albumin.

The technetium 99m stannous calcium gluconate and technetium 99m stannous albumin were prepared by our standard electrolytic technique in which the stannous ion is electrolytically generated in the presence of the pertechnetate and the chelating molecule. In a similar manner we prepared a solution of stannous calcium gluconate having the identicle composition to that of technetium stannous calcium gluconate except for the technetium. Following the adjustment of the pH the alternative chelating agent was added and then at various time intervals samples were taken and analyzed by gel chromatography on 0.9 x 60 cm column of Bio Gel P-10. The results obtained when technetium 99m stannous calcium gluconate was allowed to interact with human serum albumin are shown in the accompanying graph. The results at pH 6·5 are not shown since no exchange was observed however it is interesting to note that if sodium gluconate is substituted for the calcium gluconate in the preparation then exchange does occur in a manner represented by a single linear line showing approximately 70% exchange in 24 hours. This observation may be very important since it would tend to indicate that there may be differences in the biological clearance of technetium labelled gluconate depending on whether the calcium or sodium salts are used in the preparation.

The results obtained when technetium 99m stannous human serum albumin was allowed to interact with calcium gluconate are shown in accompanying graph. There is an initial rapid exchange, resulting in the labelling of the gluconate. However this exchange then slowly reversed with time. The exchange of the technetium onto the gluconate was greater and the reversal slower at the higher pH. If stannous calcium gluconate was substitute for the calcium gluconate the results at pH4 appeared the same while at pH 7·0 the initial exchange proceeded to a greater extent, just over 50%, and then proceeded to continue to exchange at a slow rate, approx. a further 10% in 24 hours. Note in the presence of the additional stannous ion associated with the gluconate, no reversal of the exchange occurred. These results are somewhat analogous to those for the technetium 99m exchange between albumin and pyrophosphate[5] except that in that case exchange only occurred when stannous pyrophosphate was used.

2) Interaction of technetium stannous pyrophosphate and collagen.

Technetium stannous pyrophosphate is widely employed as a bone imaging radiopharmaceutical and has been shown to localize due to association with the hydroxyapatite crystals in normal bone. However some investigators[6-9] have suggested that in some abnormal cases the localization may also envolve collagen. Here we report a study on the factors which effect the binding of technetium stannous pyrophosphate to collagen.

Technetium 99m pyrophosphate was prepared by our routine technique of electrolytic generation of stannous ion in the presence of sodium pyrophosphate and pertechnetate[10]. In order to obtain the two different ionic concentrations of

of the final product either isotonic saline or water was used in making up the volume of the initial preparation, this results in one series with an ionic strength which is isotonic and a second series in which the ionic strength is 30% isotonic saline according to the ionic strength of the preparation under study, to a volume of 2.0 ml and incubated with 10 mg collagen. Following incubation the collagen and supernatant were separated and counted. The supernatant was also subject to thin layer chromatography on silica gel with methyl ethyl ketone as the developing solvent to determine the proportion of the technetium 99m in the supernatant which was free pertechnetate. The results of these studies clearly demonstrate that the uptake of the technetium 99m onto the collagen is reduced if the ionic strength of the solution in which the collagen is suspended is increased. Thin layer chromatograms demonstrated that the technetium 99m in the supernatant at dilutions greater than that corresponding to the greater dilution edge of the maximum collagen uptake was completely in the form of free pertechnetate. Studies in various other ionic solutions demonstrated that the effect was general although minor differences were observed in the slope and shape of the curves of ^{99m}Tc uptake onto collagen versus ionic concentration.

The effect of pH on the uptake by the collagen was evaluated by varying the pH of the preparation. The results of this study indicate that the uptake of technetium 99m onto collagen from technetium 99m stannous pyrophosphate is not likely to be significant if the bone E.C.F. has a pH near neutral especially if the ionic strength of the E.C.F. is near isotonic or above. This observation is essentually consistent with the autoradiographic studies which have demonstrated that the radioactive uptake is on the hydroxyapatite. However if the pH of the ionic strength of the E.C.F. were to become lowered due to disease then significant uptake of technetium 99m onto the collagen is clearly possible and may have a significant bearing on the technetium 99m bone image obtained.

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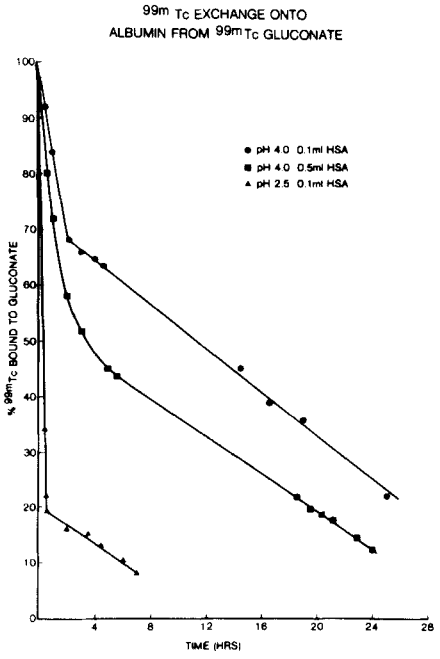


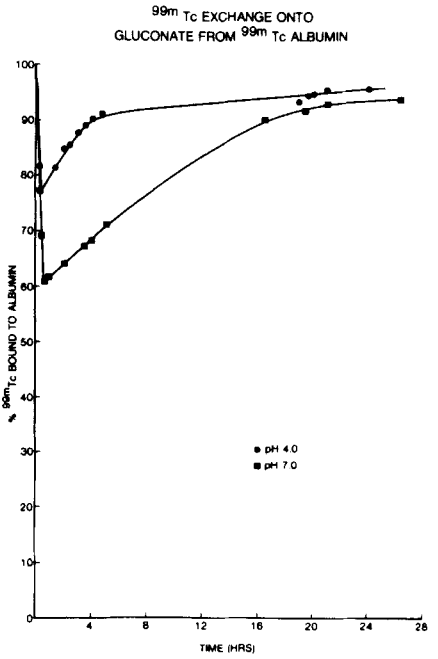


FIG 1 TECHNETIUM—99m BINDING TO COLLAGEN AS A FUNCTION DILUTION OF Tc-Sn-Pyro.

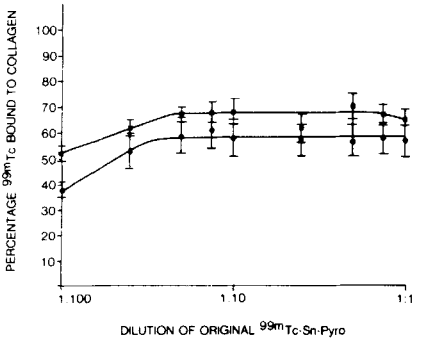
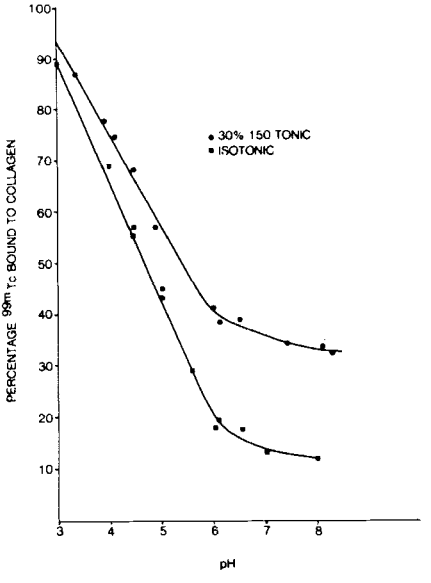


FIG 2 TECHNETIUM—99m BINDING TO COLLAGEN AS A FUNCTION OF pH



ELECTROPHILIC IODINATION OF AROMATIC RINGS

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There has been an obvious need for methods to iodinate aromatic rings other than substituted phenols and anilines by electrophilic substitutions. Although phenol and other activated aromatic rings can be readily iodinated, the resulting iodinated radiotracer usually does not have the same biochemical properties as the parent compound and nonspecific binding is increased. Many claims have been made for reactive iodinating reagents. However these are difficult to evaluate because of the widely varying reaction conditions. Therefore, we have compared four iodinating reagents: I_2 and CF_3CO_2Ag (IA), I_2 and $(CF_3CO_2)_2Hg$ (IH), NaI and N-Chlorosuccinimide (NCS), and NaI and Chloramine T (ChT) in six solvents: benzene (B), nitrobenzene (NB), ethanol (E), 95% ethanol (9E), trifluoroacetic acid (TFA), trifluoroacetic acid anhydride (TFAA), and 50% H_2SO_4 (5S) under the same reaction conditions. We have chosen benzene as a model aromatic system because it can undergo substitution by an ionic mechanism in these solvents (1), and in addition any reagent that iodinates benzene should iodinate activated aromatic rings. The purpose of this study is to determine if the reactivities reported are a function of the concentration of reagents in a particular solvent, or the rate constants per se. The moles of all components added and the temperature were held constant (2×10^{-3} mol, 10mL 70°C for 3 h). The solubility of the reagents was measured to facilitate comparison of reactivity. The determination of benzene, iodobenzene (IB), chlorobenzene (CB), and diiodobenzene (I2B) was carried out using reversed phase high pressure liquid chromatography (HPLC). In three of the solvents (E, 9E and 5S) the reactivities were so low that no IB was observed although the solubility of the reagents was high. In solvents B and NB solubility is the important factor. NaI is insoluble in B and NB as a result the yields with NCS and ChT are low, but I_2 is soluble in these solvents and as a result the yields with IA and IH are high. In TFA and TFAA, IA and IH are clearly more reactive than NCS and ChT since the solubility of all reagents in these solvents is high. In all reactions studied the yield of CB and I2B are less than 1%. IA in TFAA was also used to iodinate quinuclidinyl benzilate at equimolar ratios. The low yield (10%) of quinuclidinyl m-iodobenzilate as determined using authentic samples and HPLC is probably due to interference of other functional groups with the iodinating species or the formation of a deactivating carbonium ion in the benzilic acid structure. IH in TFAA appears to be the best iodinating agent of those tested in the various solvents. Most iodinating agents and substrates are used at high concentrations (>.2M) to achieve high yields. Therefore a solvent must be chosen that dissolves not only the iodinating agent at high concentration but also the substrate. The ability to attain high concentrations of the substrate and the iodinating species appears to be one of the most important factors in electrophilic iodination of benzene or deactivated aromatic rings.

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Table of Yields of Iodobenzene at 70°C for 3 h

Solvent	Iodinating Reagents	% Yield*	I_2 or NaI	Reagent
Benzene	I_2 & CF_3CO_2Ag	70,70	2.0	1.8
	I_2 & $(CF_3CO_2)_2Hg$	81,89	2.0	1.9
	NaI & NCS	0,0	0	1.4
	NaI & ChT	0,0	0	.40
Nitrobenzene	I_2 & CF_3CO_2Ag	24,31	1.8	1.7
	I_2 & $(CF_3CO_2)_2Hg$	24,31	1.8	1.9
	NaI & NCS	0,0	0	1.4
	NaI & ChT	0,0	0	1.0
Ethanol	I_2 & CF_3CO_2Ag	0,0	2.0	1.4
	I_2 & $(CF_3CO_2)_2Hg$	0,0	2.0	1.9
	NaI & NCS	0,0	2.0	1.9
	NaI & ChT	0,0	2.0	1.0
95% Ethanol	I_2 & CF_3CO_2Ag	0,0	1.6	2.0
	I_2 & $(CF_3CO_2)_2Hg$	0,0	1.6	1.9
	NaI & NCS	0,0	2.0	2.0
	NaI & ChT	0,0	2.0	2.0
TFA	I_2 & CF_3CO_2Ag	80,97	.42	2.0
	I_2 & $(CF_3CO_2)_2Hg$	74,44,40	.42	1.8
	NaI & NCS	14,8	1.8	2.0
	NaI & ChT	0,0	2.0	1.9
TFAA	I_2 & CF_3CO_2Ag	70,60	.40	1.4
	I_2 & $(CF_3CO_2)_2Hg$	90,91	.40	1.7
	NaI & NCS	0,0	1.8	2.0
	NaI & ChT	70,40,10	2.0	1.8

* Duplicate experiments determined by HPLC. The reactants were mixed in the solvent with the source of halogen added last.

I-123(131)-M-IODOBENZYLGUANIDINE: OPTIMIZED PREPARATION AND TISSUE DISTRIBUTION

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Recently radioiodinated aralkylguanidines were evaluated and among them meta-iodobenzylguanidine (mIBG) was proposed as adrenal medulla imaging agent (1). I-131 labeling was originally performed by reflux in water for 72 hours (2). Since we were also interested in I-123-mIBG we tried to optimize the labeling followed by determination of radiochemical purity and biodistribution.

The rate of a nucleophilic halogen exchange usually increases with substrate concentration and temperature (3) but isotopic exchange in mIBG-sulfate melt (170°, 15 min) resulted in low yields probably due to decomposition. It is known from labeling of other iodoaromatics that exchange of iodine attached to a phenyl ring may require an acidic medium (4). Addition of ammonium sulfate to mIBG-sulfate and heating above 120° (with loss of ammonia and formation of ammonium hydrogen sulfate) afforded high yields in a reasonable time (Fig. 1). Exchange yield was determined by ion exchange chromatography and TLC and strongly depended on p_H and temperature (Fig. 2, 3). Radiochemical purity was close to 100 % as analyzed by TLC (silica gel EtOH:EtAc 1:1; n-propanol:10 % ammonia 3:1) and HPLC (bonded phase-NH_2, ethanol:0,01M phosphate-buffer 20:80). mIBG can thus be labeled with I-123 (or I-131) in about 90 % yield within 1.5 hours total preparation time.

Tissue distribution was evaluated in rats and dogs (table 1). 48 hours after injection only the thyroid contained high radioactivity besides the adrenals (adrenal medulla), with all other tissued reaching only a few percent of these levels.

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Table 1: tissue distribution of I-131-mIBG in dogs 48 hours p.i. (mean ± s.d., n = 6)

whole adrenals	1.11 ± 0.19	% kg dose/g
adrenal medulla	8.32 ± 1.74	
adrenal cortex	0.29 ± 0.06	
thyroid	3.56 ± 0.54	
spleen	0.14 ± 0.03	
heart	0.035 ± 0.005	
lung	0.033 ± 0.007	
kidneys	0.025 ± 0.004	
blood	0.022 ± 0.004	
liver	0.014 ± 0.002	

Fig. 1

I-131(123)-mIBG labeling in $(NH_4)_2SO_4$: influence of reaction time (T = 140°C)

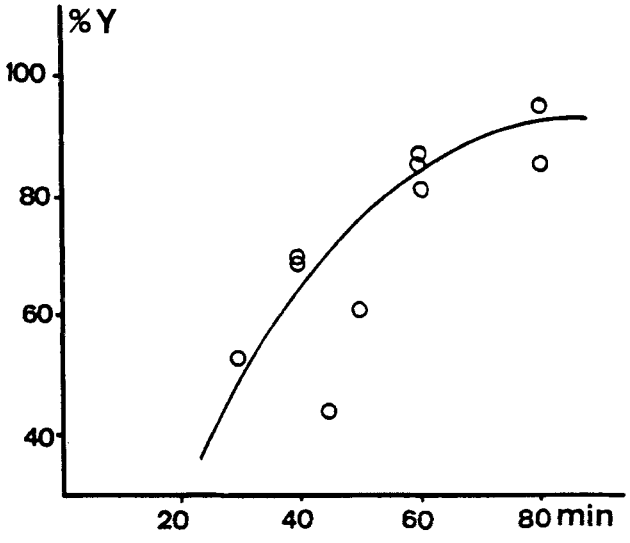


Fig. 2

influence of p_H

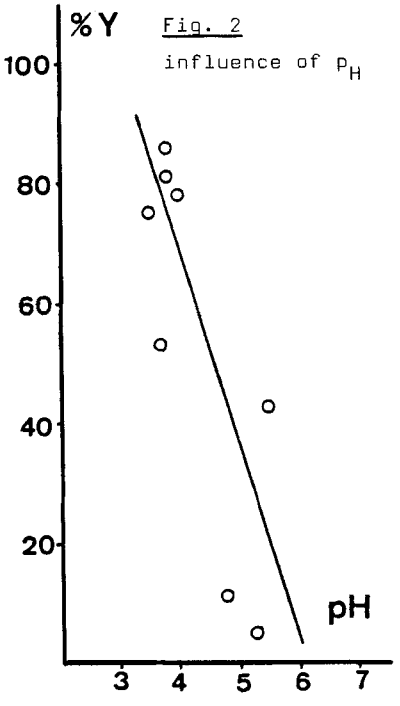
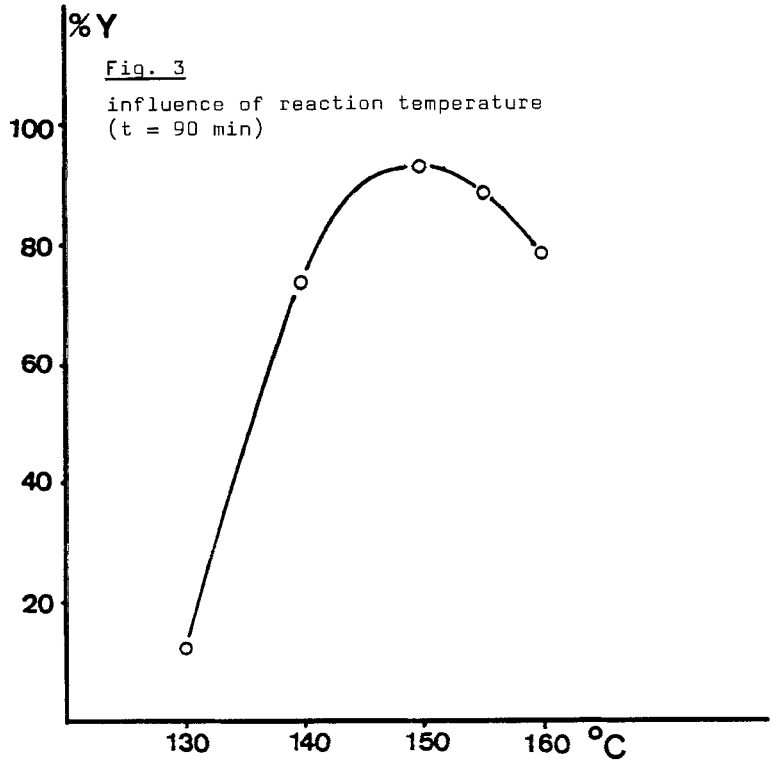


Fig. 3

influence of reaction temperature (t = 90 min)



SYNTHESIS OF Se-75 LABELED CATECHOLAMINE DERIVATIVES: ADRENAL MEDULLA TUMOR SPECIFIC RADIOPHARMACEUTICALS

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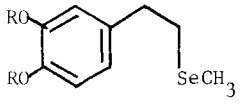
Neuroblastomas and pheochromocytomas are two tumors that originate in the adrenal medulla and can be intra- or extra-adrenal. The clinical assessment of these catecholamine-associated tumors involves the measurement of catecholamine metabolites in the urine which tend to be innacurate and do not localize the tumors. Presently, in addition to Computed Tomography (CT), two invasive procedures, selective adrenal arteriography and adrenal phlebography, are used, to preoperative localize the adrenal tumors (1).

Wieland et al (2) synthesized I-131-meta-Iodobenzylguanidine to localize adrenal medulla tumors. To date this is the only gamma-emitting radionuclide labeled compound to be shown to localize in the adrenal medulla (3).

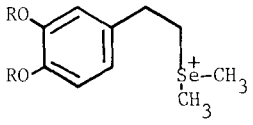
In our work, we concentrated on the development of Se-75 labeled analogues of dopamine, a catecholamine found in high concentrations in the chromaffin cells situated near the cortico-medullary junction (4).

Three classes of Se-containing catecholamines are being synthesized:

I.- Selenium replacing the amine functionality in dopamine and its derivatives (Compounds I & II). Compounds IIa & IIb were chosen because of Anderson et al's report (5) that the nitrogen atom in the dopamine molecule was not essential for dopaminergic activity, since it could be replaced by a sulfonium group.

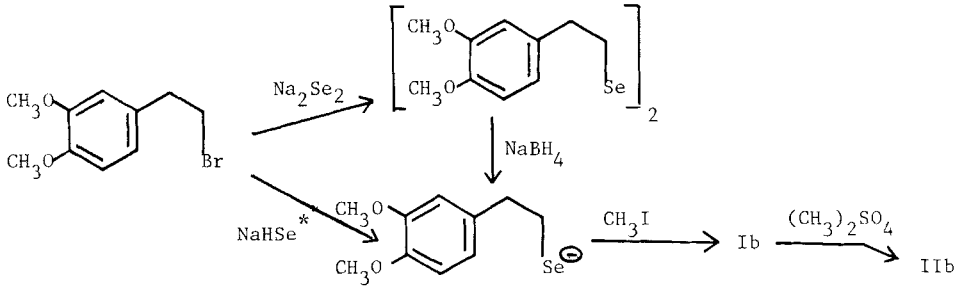


Compound I
Ia; R=H
Ib; R=CH_3



Compound II
IIa; R=H
IIb; R=CH_3

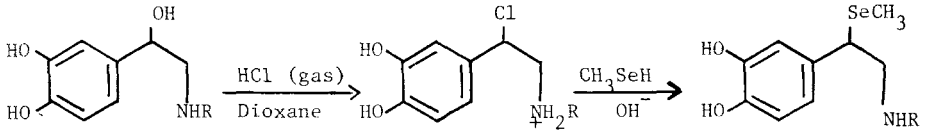
Compounds Ib & IIb were prepared and characterized utilizing the following synthetic scheme:



The radioactive scheme involves the use of Se-75 labeled nucleophile NaSeH (6) prepared from the reduction of Se-75 Selenous Acid (S.A.=136 mCi/mg Se) or Se metal (S.A.=1 mCi/mg) in pH 7.5 phosphate buffer with $NaBH_4$.

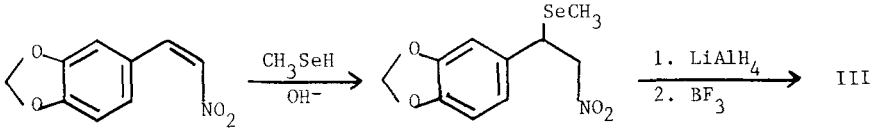
II.- The selenomethyl group replacing the -OH functionality in epinephrine and nor-epinephrine (Compounds III & IV).
Two schemes of synthesis have been utilized:

Scheme I:



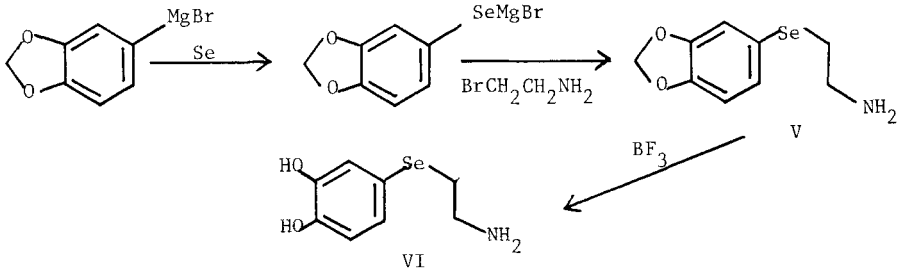
III; R=H
IV; R=CH_3

Scheme II:



The radioactive CH_3SeH is prepared from 1-2 mCi of NaSeH and equimolar amount of CH_3I at room temperature, under argon.

III.- Selenium replacing the beta carbon in the catecholamine structure (Compounds V & VI).
The following synthetic scheme has been used:



Radioactive Se metal (S.A.=1mCi/mg) was used in the radioactive synthesis of Compounds V & VI).

All compounds have been characterized by NMR, Mass Spectrometry and Elemental Analysis. The Se-75 labeled analogues were characterized by co-chromatography and co-crystallization with the non-radioactive compounds.

The radioactive compounds are being evaluated for uptake in the adrenal medulla and the brain of rats.

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SYNTHESIS OF RADIOIODINATED CIS- AND TRANS- 1,2-DIPHENYL-3,3-DICHLORO-CYCLOPROPANES: POTENTIAL RADIOPHARMACEUTICALS FOR ESTROGEN DEPENDANT TUMORS

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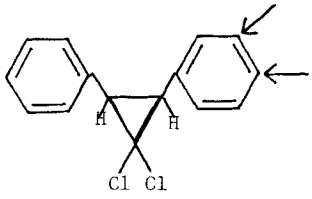
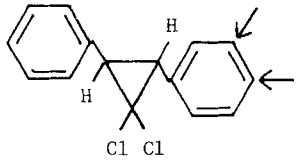
In women with breast cancer, the rate of response to anti-estrogenic treatment increases with the content of estrogen receptors in the tumor. A gamma-emitting radiopharmaceutical that would bind to the receptors in-vivo might allow a non-invasive, more accurate screening of these tumors using conventional nuclear medicine instrumentation (1).

We have adopted a systematic approach to develop and evaluate a series of compounds labeled with radioiodine that are non-steroidal in nature and have been shown to possess estrogenic and anti-estrogenic activities (2,3).

Two of the compounds we have chosen are gem-dihalo cyclopropyl analogues of cis- and trans-stilbene (Compounds I & II). Compound I has been shown to be a pure antiestrogen (3).



cis-1,2-diphenyl-3,3-dichlorocyclopropane
Compound I



Trans- isomer
Compound II

Different synthetic approaches were used to introduce radioiodine (I-125 & I-131) into only one of the phenyl rings, either para- or meta- to the substituents (arrows in diagram indicate positions of choice). The radio-iodination procedures utilized were:

I.- Exchange labeling: Attempts at radio-exchanging the p-Iodo Compound II (synthesized from p-amino trans-stilbene by diazotization, NaI treatment followed by the conversion to the dichlorocyclopropane derivative via reaction with dichlorocarbene generated from $CHCl_3$ + NaOH in the presence of a phase transfer catalyst) in different solvents e.g. ethanol, dimethyl-sulfoxide, with radioactive NaI, did not give the desired product exclusively but rather a mixture of rearrangement products with possible ring opening (the mechanism of rearrangement will be presented).

II.- Melt method labeling: A mixture of the p-Iodo Compound II and NaI (I-125 or I-131) was maintained at and above the melting point of the iodinated compound for several hours. In addition to the desired radioactive compounds, rearrangement products were also obtained.

III.- Diazotization Method: Although this method was successful in generating the non-radioactive p-iodo Compound II from the p-amino Compound II, very low yields were obtained in preparing the p-radioiodo Compound II.

IV.- Thallation-Iodination Method (4): Compound I was treated with 1 equivalent of thallium trifluoroacetate in acetonitrile at room temperature for 15 min. Radioactive NaI in water was next added and the reaction stirred for 30 min. at room temperature. The ether extract of the reaction indicated a high yield of radioactivity. Chromatography of the ether extract indicated the presence of a single radioactive compound with Rf values similar to the starting material. We are presently attempting to identify the exact location of the iodine on the phenyl ring, the p-position is presently assumed.

V.- Triazine Hydrolysis: p-Amino trans-stilbene was reacted with pyrrolidine in the presence of $NaNO_2$ and HCl at 0° C. to give the triazene derivative in good yield. This was next refluxed with HI in toluene to give p-iodo trans-stilbene. The cyclization of this compound was accomplished as described (2). The stability of the triazene was a surprise; and presently we are attempting to introduce radioactive iodine by refluxing the triazene in the presence of NaI, an organic acid and toluene.

Both methods IV and V seem to be the methods of choice in introducing a single radioiodine moiety, para- or meta- into Compounds I and II without disturbing the strained gem-dihalo substituted cyclopropane ring structure.

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CAPILLARY GAS CHROMATOGRAPHIC SEPARATION OF ISOTOPIC METHANES : A METHOD SUITABLE FOR ISOLATION OF ^{11}C METHANE

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Very high specific radioactivity is imperative when ^{11}C radiopharmaceuticals are highly toxic or when they are used for "in vivo" observation of specific receptor binding.

Strict precautions concerning pollution by atmospheric $^{12}CO_2$ at the target, gas and reagent levels have allowed a specific radioactivity for various compounds of at best 1-2 Ci/µmole at the time of use.

This order of magnitude is a limit which could not be surpassed in our laboratory during three years of routine work. It is the reason why isotopic separation of labelling agents has been undertaken.

Preliminary experiments have been performed with methanol, methyl iodide, acetone, hydrogen cyanide and methane labelled with deuterium or carbon thirteen. Chromatography (HPLC and capillary GC) has been chosen for its performance, rapidity and its ability to be automatized.

Concerning the first four compounds, it has not been possible to separate ^{13}C derivatives from ^{12}C ones, neither by capillary GC with different liquid phases (CP index ranging from 10 to 100) and highly efficient columns (N^{th} up to 600 000), nor by HPLC with normal, reverse and ion exchange phases. Nethertheless, deuterated methanol and acetone are well separated from corresponding light derivatives.

On the other hand, in the case of methane, the ^{13}C-^{12}C separation has been performed by capillary GC, at low temperature, on treated soft glass.
The original method (1) which was developped for the separation of deuterated compounds gave a poor resolution for $^{13}CH_4$ - $^{12}CH_4$ and was too slow for our purpose (5 hours). It has been modified as follows : A 100 meter soft glass capillary tube pretreated with 25 % NH_4OH at 180°C for 15 hours (2), was eluted at a pressure of 1 bar with 10 % Nitrogen in Helium at - 206 ± 1°C (obtained by adiabatic evaporation of liquid nitrogen).

With these conditions the retention time is about 1 hour and the respective resolution values are :

$^{13}CH_4$ - $^{12}CH_4$: 0,71
$^{12}CH_3D$- $^{12}CH_4$: 3,5
$^{12}CD_3H$- $^{12}CH_4$: 7,1
$^{12}CD_4$ - $^{12}CH_4$: 7,8

These results are encouraging and it now seems possible to obtain ^{11}C methane without carrier. The next step will be to try to transform the non reactive methane into methyl iodide or formaldehyde without isotopic dilution for further use in labelling radiopharmaceuticals.

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FAST METHOD FOR LABELLING OF O-IOD-HIPPURIC ACID WITH 123-I DIRECTLY FROM CYCLOTRON IRRADIATED TeO_2-TARGETS

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In this contribution we describe two procedures for labelling of o-iod-hippuric acid (o-IHA) with ^{123}I. Both methods work without an intermediate isolation of ^{123}I in form of iodide solution, unavoidable condition for all previously reported labelling methods. Our starting material is the cyclotron irradiated TeO_2-target (either $^{124}TeO_2$ irradiated with protons, or $^{122}TeO_2$ irradiated with deuterons as in our particular case). The labelled o-IHA preparation is ready for use in nuclear medicine after 30 minutes. Both methods are carried out at mild conditions, the reactions are fast and complete.

Procedure 1

In this case the same equipment is used, which is utilized to produce ^{123}I in the chemical form of iodide (1) (fig.1). The trap is filled with 1 ml of solution A. This component is the part A of an instant KIT for labelling o-IHA with radio-iodine developed at the UJV REZ Prague (CSSR) (2). This component A contains 6 mg/ml of pure o-IHA and small amounts of Cu(I)-chloride (10^{-4} mol/l) as an exchange catalyst. The pH of this solution is 4.6. The TeO_2-target is heated up to 1073 K, just to melt the targetmaterial for 1.5 - 2 minutes. The released ^{123}I is transported by an air-stream of 50 ml/min into the trap, where the ^{123}I is retained with more than 95 % yield. This solution containing $^{123}I^-$, unlabelled o-IHA and already 3 % ^{123}I-labelled o-IHA is transfered to the final ampoule and unsealed heated in a boiling water bath for 10 minutes. After adding of 9 ml KIT-component B the preparation is isotonic, neutral and ready for use. The KIT B solution contains the following components: EDTA: 1.7×10^{-5} mol/l, NaOH: 2.5×10^{-3} mol/l, NaH_2PO_4: 4.6×10^{-4} mol/l, NaCl: 1.7×10^{-1} mol/l.
With our conditions we obtain specific activities of 200 - 400 MBq/mg o-IHA (5 - 10 mCi/mg). The labelling yield is better than 99 %. (fig. 3)

Procedure 2

The equipment is shown in fig. 2. The Al_2O_3-column has to realize two functions: to retain TeO_2-traces from the target and to regulate the ^{123}I amount passing through the exchange column. This exchange column is filled with o-IHA-coated NaCl-cristals.

NaCl-cristals are mixed with an acetonic solution of o-IHA in a ratio 5 mg o-IHA/90 mg NaCl, the aceton is then removed by rotating vacuum evaporation. The starting material is the cyclotron irradiated TeO_2-target, which is heatet up to 1073 K (1). The released ^{123}I is transported by an air stream of 50 ml/min to the Al_2O_3- column kept at a temperature below 450 K. The ^{123}I is retained with yields more than 98 % at the column, which is then heated until 770 K. The ^{123}I is slowly desorbed and transported by an air stream of 10 ml/min through the exchange column loaded with 95 mg NaCl cristals coated with o-IHA. During this process, which is finished after 20 minutes, the exchange column is kept at a temperature of 405 - 415 K using an oil bath. In this heterogeneous system - gas solid - the isotope exchange takes place very fast. The cristals from the exchange column are dissolved in 10 ml water, containing a corresponding amount of alkaline for neutralisation and the preparation is ready for use after sterile filtration. An over all yield of better than 90 % was obtained, the labelled product contains less than 1 % free iodide (fig. 4).

Quality control

The method described by BIJL (3) was employed using Silufol UV 254 plates.

Results and discussion

Both procedures lead to a ^{123}I-labelled o-IHA preparation in 30 minutes with extremely high yields and high quality. Because of the unnecessity of an intermediate isolation of $^{123}I^-$-solution this methods are especially suitable for laboratories possessing small accelerators, which parameters permit the production of small amounts of ^{123}I only. The disadvantge of the methods is, for each product has to be used a separate target.

It seems to be reasonable to look at the Al_2O_3 adsorption-desorption step as a possibility for purification the ^{123}I for example from ^{121}Te, which is present when spallation reactions for ^{123}I production are employed. When the heterogeneous exchange method (procedure 2) is combined with the ^{123}I-production via spallation a large scale production of ^{123}I-labelled o-IHA is possible without any ^{123}I-isolation and purification steps. Finally it should be mentioned, that the procedure 2 offers the possibility to label other nonvolatile compounds as well as aromatic biomolecules by halogen exchange reaction with all medical useful radio-iodine isotopes.

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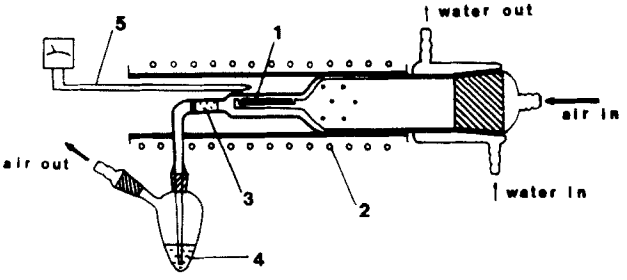
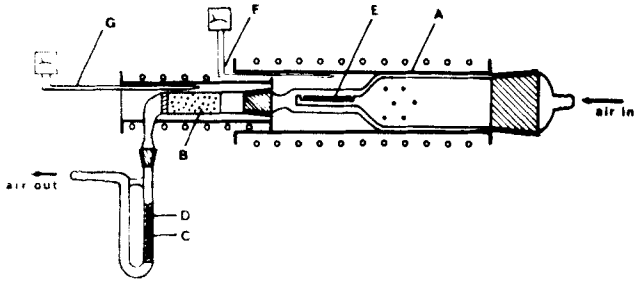


1 TeO_2-target on Pt-backing
2 oven
3 Al_2O_3-filter
4 trap with reagent solution
5 thermocouple

Fig.1 Thermochromatographic equipment for production of 123-I labelled o-IHA directly from cyclotron irradiated TeO_2-targets (procedure 1)



A oven
B Al_2O_3-column
C isotope exchange column
D o-IHA coated NaCl cristals
E TeO_2-target on Pt-backing
F,G thermocouples

Fig.2 Thermochromatographic equipment for production of 123-I labelled o-IHA directly from cyclotron irradiated TeO_2-targets (procedure 2)

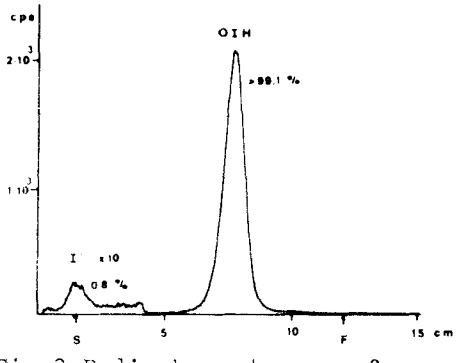


Fig.3 Radiochromatogram of 123-I labelled o-IHA, prepared by procedure 1

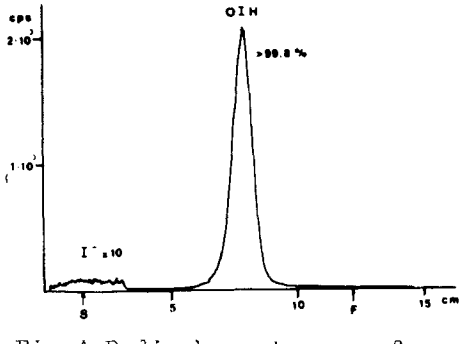


Fig.4 Radiochromatogram of 123-I labelled o-IHA, prepared by procedure 2

SYNTHESIS AND TISSUE DISTRIBUTION STUDY IN RATS OF BROMINE-82 LABELLED 6-BROMOCHOLESTEROL

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I-131 Labelled 6-iodocholesterol (CL-6-I-131), synthesized by Wang and Liu et al (1), has reported to be suitable for human adrenal imaging due to its notable stability in-vivo and in-vitro, though demonstrating lower adrenal-liver and adrenal-kidney radioactivity ratios than I-131 labelled 6-iodomethyl-19-nor-cholesterol (NCL-6-I-131)(2). This agent has been used clinically as a new diagnostic agent for adrenal gland in China. It is of interest to use a bromine labelled analogue which would give componds with more favorable characteristics. Among the potential useful neutron deficient bromine isotopes, bromine-77 is the most suitable nuclide for in-vivo studies. Also the positron emitter, bromine-75 or bromine-76 might still be useful for positron emission tomography. As a part of a continuing study of analogue synthesis of cholesterol, we planned to synthesize bromine-82 labelled 6-bromocholesterol (CL-6-Br-82) and to evaluate its ability to localize selectively in adrenals in rats.

Bromine-82 labelled 6-bromocholesterol (CL-6-Br-82) was prepared by the reaction of $^{82}Br_2$ with 6-chloromercurycholesterol in chloroform and purified by thin layer chromatography. A specific activity of 0.8 mCi/mg was obtained.

Eleven Wistar male rats were injected intravenously with 20 μCi of CL-6-Br-82. Three or five animals were sacrificed at 1, 3 and 5 days, respectively, after administration. Table 1 shows the accumulation in each organ corrected for radioactive decay. CL-6-Br-82 showed a considerabl selective localization of radioactivity in the adrenals. However, the adrenal concentration of radioactivity is less than that achieved with CL-6-I-131 or NCL-6-I-131 over the 5-day period, though it is at higher level than that of I-131 labelled 19-iodocholesterol (CL-19-I-131)(3,4). The adrenal-to-liver ratios increase from 57 at 3 days to 141 at 5 days, which are comparable to those of CL-6 -I-131, but these values are considerably lower than those observed with other agents such as CL-19-I-131 and NCL-6-I-131 (3,4). In conclusion, the substitution of radiobromine for radioiodine in the CL-6-I results in an agent which demonstrates less affinity for the adrenal gland than CL-6-I itself.

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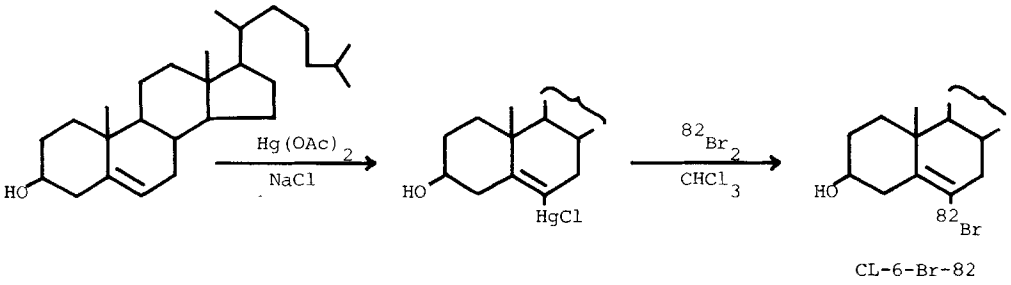

Figure 1. Reaction scheme for the synthesis of CL-6-Br-82

Table 1. Rat tissue distribution of bromine-82 labelled 6-bromocholesterol (CL-6-Br-82) at various time intervals*

	Days after administration		
Tissue	1 day	3 days	5 days
Adrenal	115.74 ± 22.02	130.78 ± 72.14	136.28 ± 41.10
Liver	21.44 ± 9.26	2.26 ± 1.04	0.96 ± 0.18
Kidney	10.20 ± 6.12	3.08 ± 0.94	2.24 ± 0.62
Lung	21.30 ± 9.66	5.34 ± 1.86	3.28 ± 0.86
Spleen	55.72 ± 21.74	4.84 ± 1.98	1.72 ± 0.32
Testicle	2.38 ± 0.38	1.44 ± 0.32	1.42 ± 0.32
Blood	6.84 ± 1.44	1.64 ± 1.16	0.46 ± 0.08
Thyroid	4.20 ± 1.06	0.92 ± 0.12	0.86 ± 0.68

* Values represent mean % administered dose per gram of tissue for 3 rats at 1 day and 3 days, 5 rats at 5 days with SD of mean.

SYNTHESIS AND IN VITRO EVALUATION OF N-PHENYL HALOGENATED DERIVATIVES OF FENTANYL AS LIGANDS FOR THE OPIATE RECEPTOR

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The biochemical identification of opiate receptors and their autoradiographic visualization has been recently described (1,2). The development of high affinity, high specific activity ^{3}H-labeled neurotransmitter receptor ligands has made it possible to determine the spatial distribution and relative regional concentration of several neuroreceptors using in vivo receptor labeling techniques in experimental animals (3-11). The quantitation and localization of opiate receptors in man using noninvasive methods, such as nuclear imaging, could provide a means of obtaining information about a variety of receptor-linked neuropsychiatric diseases as well as normal brain mechanisms regulating pain and emotions. Radiohalogenated derivatives of molecules such as fentanyl, an opiate receptor antagonist, may be useful for imaging these receptors in vivo.

Twelve derivatives of fentanyl (see figure) halogenated in the ortho, meta, or para positions of the N-phenyl ring were synthesized from appropriately substituted anilines (12) and characterized by IR, NMR, melting point, elemental analysis, and HPLC.

A series of in vitro receptor binding experiments were performed to screen the relative affinities of the derivatives for opiate receptors in male Sprague Dawley rat brain (13). The percent inhibition of ^{3}H-fentanyl binding was plotted for each compound and IC_{50} values (the concentration of a derivative which inhibits the specific ^{3}H-fentanyl binding by 50%) were obtained (see table).

Generally, for a particular isomer, the affinity decreases as the size of the halogen increases. For each halogen individually, the derivatives substituted in the ortho position have the highest affinity for the receptor while substitution in the meta or para position substantially decreases the affinity.

The derivative of fentanyl fluorinated in the ortho position of the N-phenyl ring has an IC_{50} of 0.35 nM (K_I = 0.26 nM). This represents an affinity three times higher for the opiate receptor than fentanyl, the parent compound. We are currently investigating procedures for aromatic ^{18}F-fluorination in the ortho position to yield a high specific activity radiotracer for in vivo imaging of opiate receptors.

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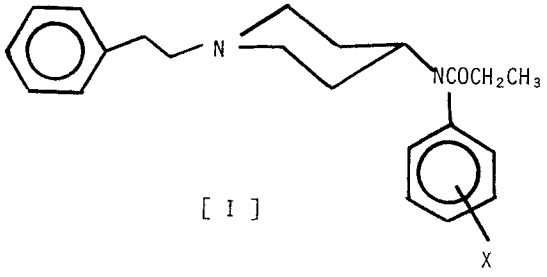
HALOGENATED DERIVATIVES OF FENTANYL



[I]

X = o;m;p - F;Cl;Br;I

Table of Halogenated Derivatives of Fentanyl

X	Chemical Yield	IC_{50} (nM)†
Fentanyl		0.95
2-F	24%	0.35
3-F	81%	2.88
4-F	71%	2.33
2-Cl	26%	1.4
3-Cl	55%	3.77
4-Cl	84%	7.5
2-Br	24%*	---**
3-Br	68%	3.57
4-Br	76%	16.67
2-I	39%	4.5
3-I	70%	4.33
4-I	86%	20.25

* Requires further purification.
** Not determined.
† Results represent mean of two to four experiments.

ω-DIFLUOROAMINO CARBOXYLIC ACIDS: READILY ACCESSIBLE FLUORINE-LABELED FATTY ACID ANALOGS

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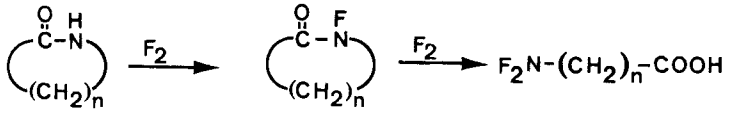
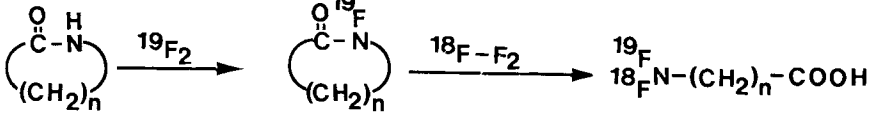
Radiolabeled fatty acids are used extensively to study myocardial function in both the normal and diseased heart. The availability of positron-based imaging systems has stimulated the development of ^{11}C-carboxyl-labeled hexadecanoic acid (1). For studies requiring a longer half-life, or for which the loss of radio-label through beta oxidation might be important, we sought an efficient, general method of introducing an ^{18}F label at a site distant from the carboxyl group (2).

To this end we have synthesized $F_2N-(CH_2)_{11}-COOH$ and $F_2N-(CH_2)_{14}-COOH$ as analogs of tridecanoic and hexadecanoic acids. These compounds were obtained in approximately 50% yield by direct fluorination of the corresponding lactams in acetonitrile/water (3).



While the difluoroamino alkyl group has been shown to be attacked by bases (4), the fatty acid analogs could be dissolved in aqueous bicarbonate solution, pH 8.1, without apparent decomposition after 1 hour, indicating that the label should be stable at physiological conditions. These compounds were quite stable toward acid, indicating the possibility of subsequent synthesis of complex ^{18}F-labeled lipids, e.g., triglycerides and phospholipids. Liquid chromatography (silica and reverse phase) indicated polarity and partitioning behavior intermediate between hexadecanoic acid and 16-iodohexadecanoic acid. Since the latter has been shown to be taken up by the myocardium (5), it is likely that the difluoroamino analog will also be taken up by the heart. On the basis of chromatography the ω-F_2N- analog of hexadecanoic acid is markedly less polar than the ω-FCH_2- analog. Thus, in general, it appears that the F_2N- group can be considered a readily available bio-analog of the CH_3- group.

The intermediate fluoro lactams were also isolated. They were found to react readily with F_2 to yield the difluoroamino fatty acids and were easily separated chromatographically from the latter. This promises an efficient route to the ^{18}F-labeled difluoroamino fatty acids from the "cold" fluoro lactams:



Details of the synthesis, isolation, analytical chromatography, spectra and physical properties of the difluoroamino acids and intermediate fluorolactams are given.

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RADIOIODINATED 6-IODOCHOLESTEROL. NEW STRUCTURAL EVIDENCE

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The preparation of 3β-hydroxy-6-iodo-Δ^5-cholestene 1 was first reported by Merz (1) and later by Levin et al (2). In both cases the remarkable stability of the carbon-iodine bond was recognized but no detailed structural evidence for the assignment of the iodine to the 6-position was published. Renewed interest in 1 is attributable to the reported observation (3) of localization of iodine-131-labelled 1 in adrenal glands. Additional kinetic evidence for the unusual nature of the carbon-iodine bond in 1 was recently provided by Bo-Li et al (4).

We have investigated in detail the synthesis of 1 and have developed a rationale for the unusual mechanism by which this reaction proceeds to the chloro mercuri intermediate 2 (Scheme). This intermediate is unusual in that it represents a substitution of a vinylic hydrogen by mercury; a result usually only observed in aromatic systems. A detailed spectroscopic analysis including 400 MHz PMR and 100.5 MHz ^{13}CMR analysis confirms the originally postulated structure for 1 and 2. (TABLES 1 and 2)

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TABLE 1

^{13}CMR Values for 6-SUBSTITUTED CHOLESTEROLS[1]

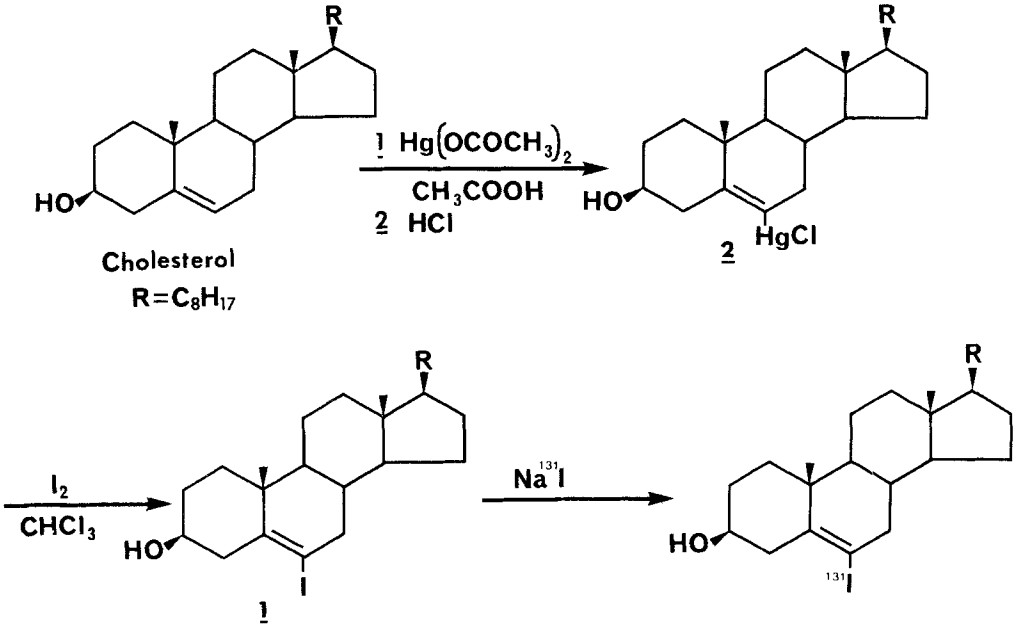
Carbon	1	2	Carbon	1	2
1	37.4	37.3	14	56.2	56.6
2	31.6	31.4	15	24.2	24.2
3	70.7	71.2	16	28.2	28.3
4	49.0	48.9	17	56.2	56.2
5	143.9	147.3	18	11.9	11.9
6	100.7	146.4	19	19.8	20.1
7	47.2	41.6	20	35.8	35.8
8	35.6	33.9	21	18.8	18.8
9	49.9	49.7	22	36.3	36.2
10	41.3	40.8	23	23.9	23.9
11	21.4	21.1	24	39.6	39.6
12	39.8	39.6	25	28.0	28.0
13	42.5	42.5	26	22.6	22.6
			27	22.8	22.8

TABLE 2

Significant perturbations for 6-substituted cholesterols compared to cholesterol[1]

		6-ICh	6-HgClCh
α	C-6	-20.7	+25.4
β	C-5	+3.2	+5.4
	C-7	+15.2	+9.7
γ	C-4	+6.8	+6.7
	C-8	+4.6	+2.0
	C-10	+3.8	+4.3
σ	C-3	-0.9	-0.7
	C-9	-0.2	-0.5
	C-14	-0.6	-0.5
	C-19	+0.4	+0.7

1) All values quoted are in PPM.


R
HO
1 Hg(OCOCH3)2
CH3COOH
2 HCl
HgCl
Cholesterol
R=C8H17
I2
CHCl3
Na131I
131I

NUCLEOPHILIC IODINATION OF 3-QUINUCLIDINYL BENZILATE (QNB)

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Nucleophilic halogenation of an aromatic ring can best be carried out using the diazonium salts or their stable intermediate such as the triazene (1-5). In order to optimize radioiodine incorporation using quinuclidinyl p-triazenobenzilate (QTB) (Fig.) each of four sources of iodide [NaI or KI, tetrabutylammonium iodide (TBAI), trimethylsilyl iodide (TMSI), and crown ether/KI (CI) were tested in five solvents: trifluoroacetic acid anhydride (TFAA), trifluoroethanol (TFE), carbon tetrachloride, bromobenzene (BB), and acetonitrile (ACN). The reactions were run for 1 hr at 75°C using 10 mM QTB, 10 mM or 40 mM iodide in 5λ 0.1 N NaOH and 10 mM methanesulfonic acid (MSA) at pH 6 to 6.5. The desired product, quinuclidinyl p-iodobenzilate (pIQNB) was analyzed by thin layer chromatography (silica gel in n-BuOH;acetic acid:water (4:1:1) and high pressure liquid chromatography [reversed phase C_{18}, ACN:MeOH;H_2O (36:30:40), 5 mM octanesulfonic acid pH 3.5]). Yields were measured by using tracer I-125 or by UV absorption. With all sources of iodide except CI in CCl_4, a precipitate formed and no product was detected. With CI, less than 1% pIQNB was found. In ACN and BB, TMSI gave yields of up to 20% pIQNB. The other sources of iodide gave yields of <5% in both solvents. In TFAA, yield was not detected because of the insolubility of QTB in TFAA. However with TFE, yields of up to 25% were obtained with KI, TMSI and CI whereas TBAI gave less than 5% pIQNB. Likewise no-carrier-added I-125 and I-123 were reacted with 10 mM QTB, 5 mM MSA and sufficient MSA to neutralize the base with TFE as the solvent. Comparable yields (ca. 15%) were obtained with both radionuclides showing that the yield was independent of the concentration of iodide from 40 mM down to the no-carrier-added level. Higher yields for the triazene reaction have been reported using triazenes containing fewer functional groups (2,3). The lower yields of radioiodinated product in the case of QTB may be due to the competing reactions of the intermediate with other functional groups. Bromination of QNB in TFE also occurred at equimolar ratios but quinuclidinyl p-bromobenzilate was not obtained when no-carrier-added Br-77 was used. One of the most important factors is the solubility of the triazene derivative. QTB appears to be most soluble in TFE and furthermore TFE prevents free radical chain reactions. It also has one additional important property, low nucleophilicity (6). For these reasons TFE appears to be the best solvent.

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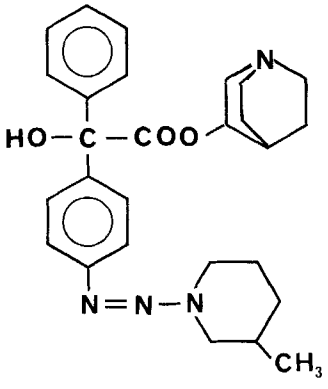
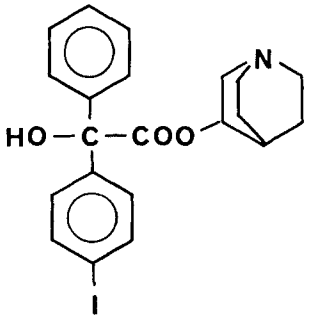
Table: Yield of quinuclidinyl p-iodobenzilate at 75°C and 1 hr.





	% Yield of p-IQNB*				
Source of Iodide	KI	KI	TBAI	TMSI	CI
Concentration (mM)	10	40	40	40	10
Weight (mg)	.179	.716	1.6	.86	.179
TFAA	QBT insol.				
TFE	15-20	15-20	<5	20-25	5-15
CCl_4	insol	insol	insol	insol	<1
BB	<5	<5	<5	20	<5
ACN	<5	<5	<5	15-20	<5

* 10 mM triazene with 5λ of the listed iodide in 0.1 N NaOH in 100 µL of solvent. Five mM MSA and sufficient MSA to neutralize the base was also added. Duplicate determinations.

THE SYNTHESIS OF [1-^{11}C] -BUTANOL

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Characterisation of tumor pathophysiology at cellular and molecular levels in vivo, has become significant in medical diagnosis. The determination of substrate transport and metabolism has to take into account the nutritional blood flow in region of interest. The quantitative information is important for the investigation of the local substrate utilisation. Labelled alcohols have been investigatedfor their use in measurements of cerebral blood flow[1,2] and in isolated myocardium[3]. ^{11}C-labelled alcohols may also provide reliable results in the estimation of extraction fractions[2] and were used in distribution studies[4].

It has been suggested that the characteristics of ^{11}C-labelled alcohols comply with requirements of a tracer for studies of local substrate utilisation. On the basis of recent experimantal data, it was postulated that the ability of ^{11}C-labelled alcohols to diffuse would increase with increasing length of the carbon chain.

As a part of our program to develop metabolic tracers labelled with short-lived radionuclides, we report the synthesis of carbon-11 labelled butanol, for application as a tracer in the assesment of regional blood supply for substrate utilisation in tumors[5].

The $^{11}CO_2$ was produced via the ^{14}N (p,α) ^{11}C reaction on the Heidelberg Compact cyclotron. The production parameters and the trapping system, trapping efficiency and

specific activity for produced $^{11}CO_2$ are described.The semi-automated preparation has been evaluated for no carrier labelling of n-butanol with the positron emitting carbon-11 ($T_{1/2}$= 20.3 min) radionuclide. The generally known organic synthesis has been adopted(6). The synthesis is based on the carboxylation of n-propylmagnesium chloride with ^{11}C-labalied carbon dioxide, and the subsequent reduction of the resulting free [1-^{11}C]-butyric acid with $LiAlH_4$ dissolved in anhydrous ether.

The method gives reproducible, high yields of [1-^{11}C]-butanol suitable for in vivo applications. The described procedure is, of course, applicable to the synthesis of ^{11}C labelled alcohols in general. The total [1-^{11}C]-butanol synthesis including separation and purification required 25 minute from the end of bombardment. Gas chromatography and HPLC were used to estimate the purity of the product. To maintain no carrier added conditions, atmospheric carbon dioxide was rigorously excluded from all reagents and every part of the apparatus.

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SYNTHESIS OF ^{75}Br-LABELLED ESTRADIOLS

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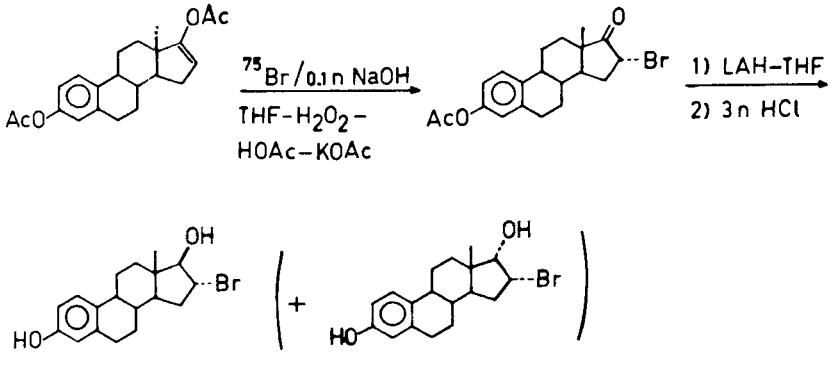
Radiopharmaceuticals, derived from estrone, have become available for the scintigraphic investigation of estrogen-receptor containing tumor tissues. Among these radiopharmaceuticals are 16α-{^{77}Br}bromoestradiol-17β, prepared by Katzenellenbogen et al (1), and 16α-{^{125}I}iodoestradiol-17β, prepared by Hochberg and Rosner (2). Each of these estradiols has a receptor binding affinity which is higher than the binding affinity of estradiol itself.

Besides our interest in ^{11}C-steroids, we are also investigating 16α-{^{75}Br}bromoestradiol-17β, and its 11β-methoxy analogue. These compounds should be useful in positron-emission tomography (^{75}Br, $t_{\frac{1}{2}}$ = 1.6 h, β^+ 76%). For our experiments the ^{75}Br was produced by two nuclear reactions: i) ^{76}Se(p,2n)^{75}Br, and ii) ^{75}As(^{4}He,4n)^{75}Br. The first reaction yields ^{75}Br with only 1.4 % ^{76}Br as a contaminant at EOB, when enriched selenium is used as target material. For animal experiments natural selenium can also be used, because the only consequence will be a higher degree of contamination with ^{76}Br, which also is a positron emitting radionuclide ($t_{\frac{1}{2}}$ = 16.2 h, 57% β^+). When the α-particle induced reaction on As_2O_5 is used, the contamination at EOB with ^{76}Br is 6%.

Heating of the irradiated material with H_2O-H_2SO_4-$K_2Cr_2O_7$ removes the bromine isotopes, which are collected in 0.1 N NaOH. This solution is used for the bromination of the enoldiacetate of estrone (cf. scheme), similar to the reaction devised by Katzenellenbogen (1). Reduction of the intermediate α-bromoestroneacetate with LAH at -80°C gives a mixture of α-bromoestradiols -17α and 17β. Twofold HPLC purification (Lichrosorb Si-60, then Lichrosorb Diol) gives 16α{^{75}Br}bromoestradiol-17β, free from impurities (except for the ^{76}Br-analogue) in 20-35% chemical yield. A similar sequence has been applied to prepare 11β-methoxy-16α-{^{75}Br}bromoestradiol-17β, a compound that has an even higher receptor binding affinity than 16α-bromoestradiol-17β.



The main problem associated with this approach to the ^{75}Br-estradiol lies in the fact that it takes ca. 5 h (from EOB) to obtain the pure radiopharmaceutical. Since available production methods for ^{75}Br lead to coproduction of at least 1.4% ^{76}Br at EOB (3), this means that the final product contains at least 10% of the ^{76}Br-analogue. For that reason, less time-consuming routes to the ^{75}Br-estradiol have to be found. Such routes are currently under investigation in our laboratory.

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RADIOARSENIC LABELED MERCAPTOETHANOL ANALOG: SYNTHESIS AND BIODISTRIBUTION

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It has been possible to obtain a number of dimethylarsinous acid esters of sulfhydryl containing biomolecules like sugars, amino acids, and steroids (1,2). Dimethylchloroarsine (DCA) has been used in the syntheses of many of these compounds as the intermediate which was generated from cacodylic acid. Based on the method of Witten (3), a procedure for small scale production of DCA has been developed where arsenic trioxide can be used as the starting material. This was accomplished by combining two reaction steps into a single operation using a specially designed glass apparatus. A series of compounds, especially lipophilic in nature, were then synthesized as prerequisite for the derivation of radiolabeled compounds. Dimethylarsinothiol-derivatives were made from mercapto alcohols and also from linear alkyl mercaptans. These molecules are likely to cross the blood brain barrier, and may be useful for the measurement of regional blood flow when labeled with an appropriate radionuclide. Previously, carbon-11 labeled alcohols have been evaluated which were found to be extracted by brain (over 90%) by a single passage through the capillary system (4). However, F-18 labeled fluoroethanol was not extracted by the brain so efficiently (5). Considering these factors, further work was undertaken to synthesize a radioarsenic labeled alcohol analog: dimethylarsinomercaptoethanol (DAM). Arsenic-76 was used in the present study which was obtained by (n,γ) reaction on arsenic trioxide in a nearby nuclear reactor.

The irradiated target material (arsenic trioxide containing As-76) was transferred to a 25 ml side armed round-bottom flask, and mixed with anhydrous potassium acetate. A glass trap containing ferric chloride in concentrated HCl was fitted over the reaction flask. A slow stream of carbon dioxide gas was allowed to flow through the side arm, and the flask was heated to 360° C for 4 hours. The reaction led to the production of dimethylarsineoxide which was carried up by the gas and trapped in the ferric chloride solution where it reacted to produce dimethylchloroarsine (DCA). The As-76 labeled DCA was then extracted with chloroform, mixed with an equimolar amount of mercaptoethanol in the presence of an equimolar amount of triethylamine in chloroform. The mixture was heated for 1 hour at reflux temperature where dimethylarsinomercaptoethanol (As-76 labeled DAM) was generated. The labeled compound was purified by chromatography, and extracted with isotonic saline for biological tracer studies.

Biodistribution studies were carried out in mice with intravenous tracer doses. The animals were sacrificed in groups of 5 at different time intervals. Blood sample and various organs were obtained from each mouse, and assayed for radioactivity to determine percent dose per organ. Blood clearance was rapid (about 90% cleared by 15 min) but total body retention remained high for over an hour. Liver, lungs, kidneys, and GI tract had high accumulation of the radioactivity. Results indicated an apparent relation to the tissue blood flow as may be expected for an alcohol. It is possible to incorporate a cyclotron produced radioarsenic suitable for radionuclide imaging, such as As-71 (6), where radioarsenic may be converted to trioxide during target processing. This may lead to the development of a new group of radiopharmaceuticals from SH-containing biomolecules.

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SYNTHESIS OF VERY HIGH SPECIFIC ACTIVITY RADIOIODINATED AND RADIOBROMINATED MATERIALS VIA ORGANOBORANE CHEMISTRY

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Organoboranes have proven to be useful intermediates for incorporating radionuclides (1,2). We recently reported that iodine (3,4) and bromine (5) can be incorporated into organic molecules via the reaction of the appropriate halide with organoboranes in the presence of mild oxidizing agents:

$$R_3B \xrightarrow[[O]]{Na^*X} R-{}^*X$$

We have utilized these reactions to synthesize alkyl, aryl, and vinyl halides. [Due to the mildness of the reaction conditions, a variety of functional groups are tolerated.]

$^{123}ICH_2(CH_2)_{17}CO_2H$ CH_3-C$_6$H$_4$-^{82}Br $^{125}ICH{=}CHCH_2CH_2Cl$

We wish to report that the reactions can be used to prepare very high specific activity organic bromides and organic iodides. In a typical reaction, 2mCi of ^{123}I—labeled sodium iodide [Medi+Physics] is added to 1 millimole of organoborane in 0.5 mL of tetrahydrofuran; methanolic chloramine-T is added and the product is immediately isolated in >50% yield by chromatography.

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6-FLUOROETHYL-19-NORCHOLESTEROL AS A TOMOGRAPHIC ADRENAL IMAGING AGENT. A SYNTHETIC ROUTE FOR THE LABELLING WITH FLUORINE-18

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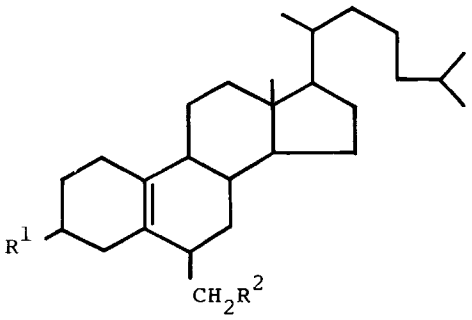
The limitations of diagnostic imaging with radioiodinated 6ß-iodomethyl-19-norcholest-5(10)-en-3ß-ol, a scanning agent of adrenal glands (1-4), are the time required to complete the study and the high radiation dose to patients. With the object of further improving adrenal specificity, some of structually modified 19-norcholesterol analogues labelled with radioiodine, radiobromine, or radioselenium have been also reported (5-8).

Fluorine-18 has attractive properties for use in nuclear medicine. Thus, 19-norcholesterol analogues suitably labelled with this nuclide are expected to provide the tomographic images of adrenal glands in short intervals and reduced radiation dose. Although it appears that 6ß-fluoromethyl-19-norcholest-5(10)-en-3ß-ol (NCL-6-F) is an obvious candidate for synthesis with fluorine-18, attempts to prepare NCL-6-F were unsuccessful. Continued interest in preparing fluoro norcholesterol analogues turned to the synthesis of fluorine-18 labelled 6ß-fluoroethyl-19-norcholest-5(10)-en-3ß-ol and the evaluation of its potential utility as adrenal imaging agent. The present paper deals with the development of a synthetic route for the labelling with fluorine-18 of 6ß-fluoroethyl-19-norcholest-5(10)-en-3ß-ol (III).

6ß-p-Toluenesulphonyloxymethyl-19-norcholest-5(10)-en-3ß-ol acetate (I) derived from cholesterol acetate was converted into the 6-hydroxyethyl(II) in a five-step process. Our initial attempt in the fluorination of this compound (II) utilized diethyl(2-chloro-1,1,2-trifluoroethyl)amine (FAR) as a fluorinating agent. When II was allowed to react with the fluoroamine (FAR) in dry methylene chloride at room temperature, the desired 6-fluoroethyl acetate was isolated in 38% yield and subsequent conversion to III was achieved by alkaline hydrolysis in good yield. The chemical forms of fluorine-18 readily available for labelling are alkali-metal fluorides and silver fluoride. Therefore, the simplest method applicable to the fluorine-18 labelled steroid would be the nucleophilic displacement of a good leaving group.

Treatment of II with triphenylphosphine dibromide and triphenoxymethylphosphonium iodide and subsequent hydrolysis with base gave the 6-bromoethyl (IV) and 6-iodoethyl (V), respectively. When V obtained thus was heated for 90 min at 50°C with a suspension of excess anhydrous silver fluoride in dry acetonitrile, the required III was obtained in 74% yield. On the other hand, no success was encountered in diplacing V with KF or CsF in acetonitrile, N,N-dimethylformamide, ethylene glycol, or hexamethylphosphoramide. Fluorination using silver fluoride gave a high yield of III based on V, but the use of silver fluoride to prepare useful amount of fluorine-18 labelled 6-fluoroethyl compound (III) was considered inappropriate from the view of practical radiochemical yield (up to 50% radiochemical yield).

Consequently, the more preferred approach to III appeared to be *via* the sulphonic ester of the 6-hydroxyethyl (II). However, all attempts to effect sulphonylation of II with p-toluenesulphonyl chloride, trifluoromethanesulphonyl chloride or trifluoromethanesulphonic anhydride in pyridine or triethylamine-methylene chloride were unsuccessful. In seeking an alternative method, we were thus prompted to examine the response of the 6-iodoethyl (V) towards silver p-toluenesulphonate. When this reaction was carried out in acetonitrile for 2 hr at room temperature, the desired tosylate ester (VI) was obtained in 27% yield. Although the tosylate ester (VI) proved to be markedly resistant to exchange using KF or CsF in acetonitrile or with a crown ether, the conversion to the 6-fluoroethyl (III) was achieved in 76% yield by heating at 125-130°C with KF for 2 hr in diethylene glycol, which is a readily applicable method for labelling with fluorine-18.



(I): R^1=$OCOCH_3$ R^2=OTs
(II): R^1=$OCOCH_3$ R^2=CH_2OH
(III): R^1=OH R^2=CH_2F
(IV): R^1=OH R^2=CH_2Br
(V): R^1=OH R^2=CH_2I
(VI): R^1=OH R^2=CH_2OTs

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LABELING WITH REACTOR PRODUCED FLUORINE-18

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Introduction of fluorine-18 in organic synthesis by nucleophilic displacement is not a simple procedure. So it seemed attractive to prepare a fluorine-18 labeled precursor in which the carbon-fluorine bond is already present. A rather promising reagent seemed to be $^{18}FCH_2I$ (1). Successive electrophilic (2) or nucleophilic (3) introduction should lead to the fluorine labeled compounds.

Different routes will be described for the preparation of this reagent, but none of them were successful.

The second part of the research deals with the labeling of aliphatic amino acids. As is shown in the scheme 4-hydroxy-L-proline was treated successively with *p*-toluenesulfonylchloride (4), diazomethane (4) and trifluoromethanesulfonic anhydride (5).
Nucleophilic introduction of fluorine-18 was accomplished by reaction with tetraethylammonium fluoride-18 (6,7), leading to the proline-derivative in a radiochemical yield of 18%.

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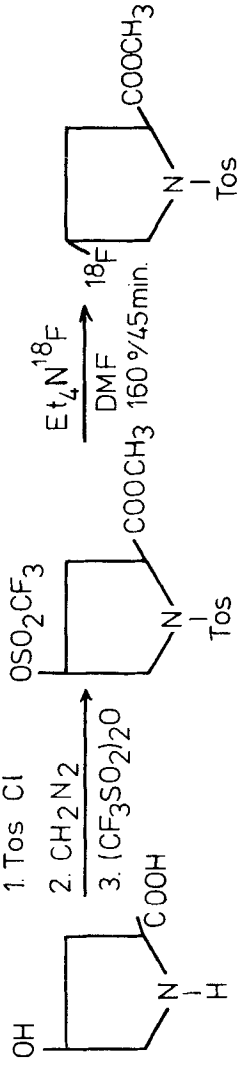
Scheme


OH
COOH
N
H
1. Tos Cl
2. CH_2N_2
3. $(CF_3SO_2)_2O$
OSO_2CF_3
$COOCH_3$
N
Tos
$Et_4N^{18}F$
DMF
160°/45min.
^{18}F
$COOCH_3$
N
Tos

NEW TRENDS IN SELENIUM-LABELLED COMPOUNDS SYNTHESIS

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The development of research on $^{73-75}Se$- selenocompounds as potential radiopharmaceuticals originated in our Center in 1975. Our most recent work will be reported here including the biological activities. Four different main directions have been successfully investigated. In general, the introduction of selenium in organic species may be carried out essentially by two different chemical pathways : On one hand through the selenide anion (Se^{--}) and on the other hand through the diselenide anion (Se_2^{--}).

1. Anion Se^{--}

a. Labelled selenoamines.

We have shown that the selenide anion is able to close a ring with bis (β-chloroéthyl) amines leading to selenomorpholines derivatives. In a similar way ω-haloalkylamines produce bis ω-aminoalkyl selenides.

b. Selenium analogs of fatty acids.

When the same reaction is done with ω-haloalkanoïc acids we are able to obtain monoselenaalkanoïcdiacids. (C_{23}-C_{25}).

2. Anion Se_2^{--}

a. Selenium analogs of fatty acids.

Reaction of haloalcanes with this anion leads to the formation of dialkyldiselenides which can be easily cleaved to the corresponding alkylselenols.
These intermediates are then coupled with a ω-haloalkanoïc acid to give monoselenaalkanoïc acids. (C_{13}-C_{24}).
An other possibility is the direct reaction of the Se_2^{--} anion with ω-haloalkanoïc acids producing diselenaalkanoïcdiacids. (C_{24}-C_{26}).

b. 1-methylseleno 1-deoxy-D-glucose.

In a similar way dimethyldiselenide is obtained by reaction of methyliodide with Li_2Se_2. After reduction in situ and coupling with 1-bromo 1-deoxytetraacetyl-D-glucose, we isolated the tetracetyl derivative of the title compound; this compound being obtained through desacetylation.

c. PZ 51.

The anion Se^{--} has also been proved to be a nucleophilic reagent for arene-diazonium salts. This reaction has been used to obtain in a "one pot synthesis" the labelled PZ 51 (N phenylbenzisoselenazolinone), a new potential drug, which is now under screening.
Biological activities of all these previous compounds are compared on animals.

STRUCTURE-DISTRIBUTION STUDIES WITH FLUORINE-18 LABELED PREGNENOLONES: EFFECT OF STRUCTURE ON ADRENAL UPTAKE AND ELIMINATION

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Distribution data obtained from rats following the administration of 21-F-18-fluoropregnenolone-3-acetate (I) demonstrated an initial high adrenal/blood fluorine-18 concentration ratio which declined rapidly (1,2). Subsequently, there was a substantial uptake of fluorine-18 activity by bone, presumably resulting from the uptake of fluoride ion following metabolism of the label from the steroid molecule. A dual isotope study using a 7-H-3 label and a 21-F-18 label further demonstrated the metabolic loss of the fluorine-18 label in the adrenal glands (1). In a search for radiopharmaceuticals which would be useful for adrenal imaging, we sought to prepare fluorine-18 labeled analogs of pregnenolone in which the fluorine-18 label would not be susceptible to metabolic defluorination. Hopefully such compounds would reach and maintain higher target/nontarget activity ratios than compound I.

The synthesis of pregnane derivatives labeled with fluorine-18 in the 21-position using crown ether assisted nucleophilic substitution which gave radiochemical yields of 20-25% for compound I (3), was used to prepare 16-dehydro-21-F-18-fluoropregnenolone-3-acetate (II), 16-methyl-16-dehydro-21-F-18-fluoropregnenolone-3--acetate (III), 16α,17α-cyclopropyl-21-F-18-fluoropregnenolone-3-acetate (IV), and 17α-methyl-21-F-18-fluoropregnenolone-3-acetate (V), with similar yields.

The effects of these structural modifications on the in vivo distribution of these fluorine-18 labeled steroids were studied in 200±10 gm male Wistar rats. Animals were sacrificed at 5, 10, 15, 30, 60, and 120 minutes after the intravenous administration of the labeled steroids. Representative results of the distribution studies are shown for blood, adrenals, kidneys, and liver in Figure 1. Only compound III resulted in a decrease in adrenal uptake. Compounds II, IV, and V all reached and maintained higher adrenal concentrations of fluorine-18 activity than did compound I, at least through the first 60 minutes. In addition to adrenal uptake and excretion, one must also be concerned with the activity in surrounding nontarget tissues. Compound II reached a high kidney concentration at 5 minutes, indicating that it would not be suited for adrenal imaging.

In vivo defluorination as measured by bone uptake of fluorine-18 activity was observed in the following order: II<III<I<V<<IV. Using the adrenal/kidney activity ratio as an index of radiopharmaceutical potential, compound V was shown to be the best compound in this series and worthy of further studies.

From these studies it is evident that structural modifications at the 16 and 17 positions of pregnenolone can alter the uptake and excretion of the steroid by the adrenal glands. These results also indicate that such modifications can alter the rate of in vivo defluorination. Further studies are planned to investigate the effects of structural modifications at other sites on the steroid molecule on adrenal uptake, elimination, and metabolism. Structure-distribution studies such as this may identify radiopharmaceuticals which are useful for the study of specific metabolic pathways.

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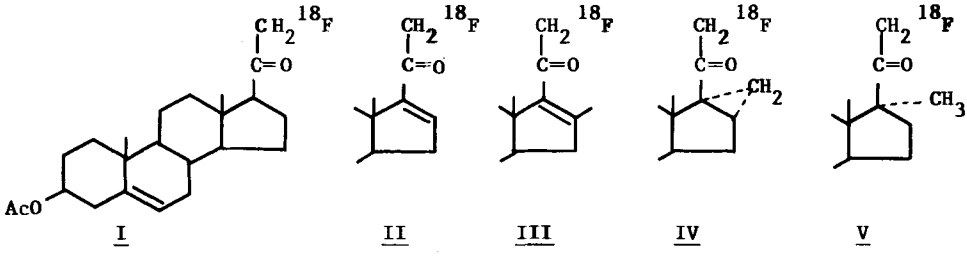
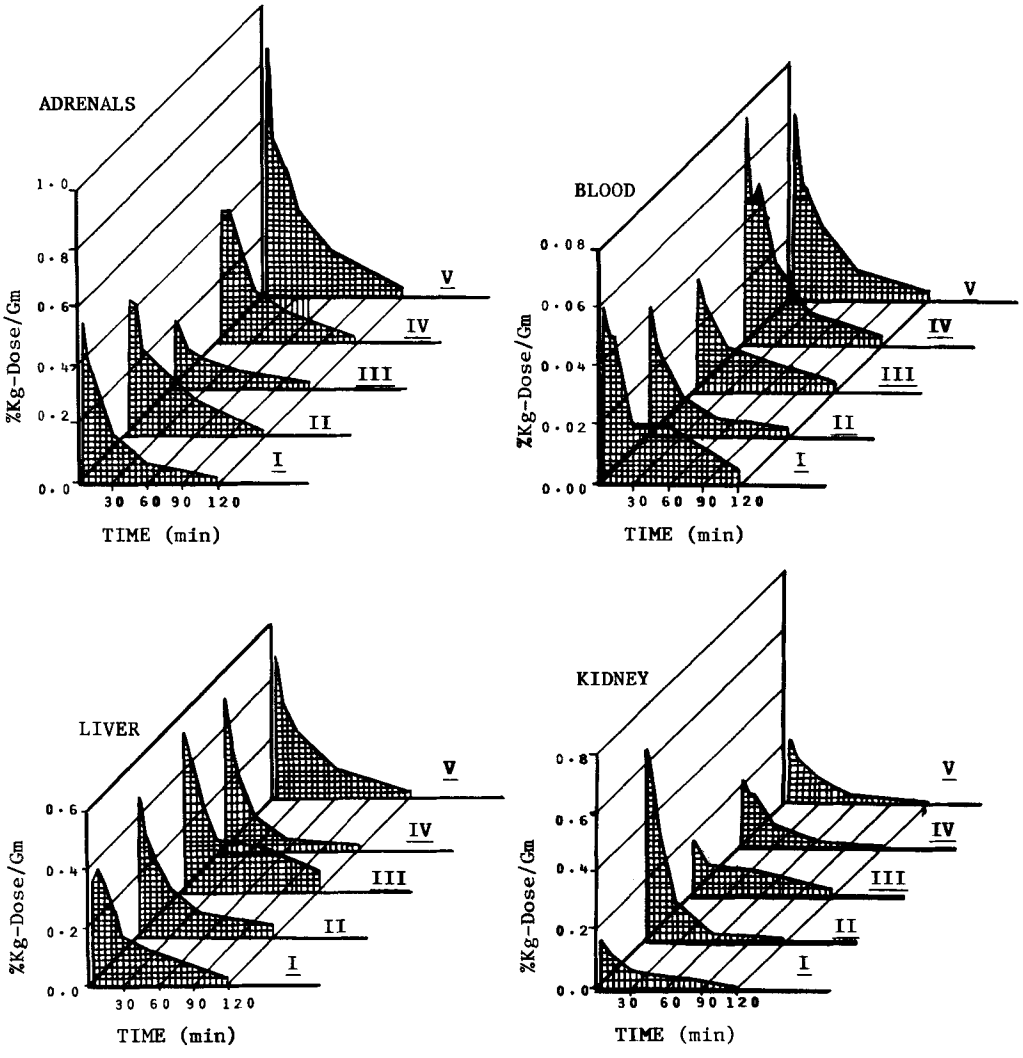


Figure 1. Tissue Concentrations of F-18 at Various Times After Administration of Fluorine-18 Labeled Steroids.



APPLICATION OF NIH-EPA CIS CHEMLAB PROGRAMS FOR DESIGN OF RADIOPHARMACEUTICALS

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The NIH-EPA Chemical Information System (CIS) (1) consists of a collection of disk-stored data bases and a battery of interactive computer programs. The Chemical Modeling Laboratory (CHEMLAB) program is the third generation of the CAMSEQ (2) series of molecular processing programs and represents a package of molecular structure calculation routines tied together by a centralized controller program.

We have applied CHEMLAB programs for analysis of a group of muscarinic antagonists. The following options have been exercised:
1. Linear Free Energy with suboptions: a) octanol/water partition coefficient (log P); b) free energy of solvation in water; c) molecular volume; d) connectivity indexes; and e) polarizability.
2. Conformational Analysis
3. Surface Areas (solvated and unsolvated).
4. Dipole Moment.
5. Shape Analysis.

Conformational analysis was performed to determine the lowest free energy conformations of the investigated 13 antagonists of general formula I. The obtained conformers were in turn compared to X-ray crystallographic studies of 3-quinuclidinyl benzilate (QNB). The conformation of one of the investigated antagonists: 3-quinuclidinyl xanthene-9-carboxylate (QNX) with a rigid skeleton, served as another reference. In addition to the calculated log P values we determined the capacity factors (k') obtained by reversed phase HPLC (3).

The data obtained from CHEMLAB, association constants obtained in in vitro studies using rat ventricular muscle and rabbit caudate muscarinic receptor fractions, the results of the in vivo displacement studies, and experimentally determined k' were analysed together in search of pattern recognition that is, correlations among the above parameters (Tables 1 and 2).

The preliminary results permit the following conclusions:
a. 3-Quinuclidinol provides the most compact, rigid aminoalcohol moiety with the greatest accessibility of the lone electron pair on the nitrogen atom and therefore any search for a better aminoalcohol moiety will be difficult.
b. The replacement of one of the phenyl rings of QNB with a cyclopentyl group leads to substantial improvement of affinity in the heart and displaceability in the brain. When the benzylic acid part is replaced by xanthene-9-carboxylic acid, a significant difference in affinity to the receptor in the two tissues is observed. In these cases the shape, volume and lipophilicity of the molecules play the determining role.

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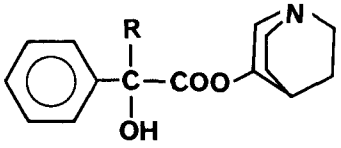
Table 1. Physicochemical and Biochemical Properties

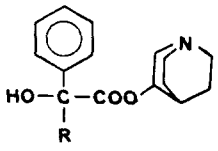


R	K_A $10^9 M^{-1}$ [a]		k' [d]	Calc	Surface area Å^2		Mol. vol.	FH_2O [e]
	RVM [b]	RC [c]		Log P	unsolv.	solv.	Å^3	Kcal/mol.
QNB	5.28	---	1.6	2.87	136.13	315.56	169.10	-0.38
C_6H_{11}	3.51	1.99	3.9	3.58	167.62	337.54	246.30	1.56
C_5H_9	8.21	3.58	2.5/2.9	3.05	146.69	318.02	211.10	1.36
C_4H_9	2.91	3.44	2.1/2.4	2.99	144.20	324.24	192.30	2.20
$HC{\equiv}CCH_2$	0.03	1.26	1.3	2.17	98.29	269.71	115.00	0.63
QNX	0.225	3.56	1.5	2.74	225.56	439.46	314.70	-8.43
$3\text{-}FC_6H_4$	5.03	3.91	2.3	3.10	140.96	322.60	172.2	-4.54
$4\text{-}FC_6H_4$	3.03	3.97	2.3	3.03	140.96	322.60	172.4	-4.54
$2\text{-}BrC_6H_4$	0.598	0.66	3.3	3.71	155.39	344.11	192.0	----
$3\text{-}BrC_6H_4$	0.433	2.2	3.5	3.83	162.49	355.93	192.0	----
$4\text{-}BrC_6H_4$	0.608	1.81	4.3	4.06	162.49	355.93	192.0	----
$3\text{-}IC_6H_4$	0.482	1.52	4.0	4.05	168.19	365.22	202.5	----
$4\text{-}IC_6H_4$	1.22	2.37	5.0	4.30	168.19	365.22	202.5	----

[a] Association constant [b] Rat ventricular muscle [c] Rabbit caudate

[d] C_{18} column, 5 mM octane sulfonic acid, pH 4, MeOH:THF:H_2O (26:16:58)

[e] Free energy of solvation



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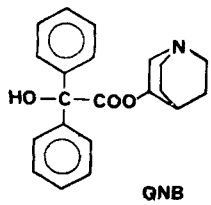
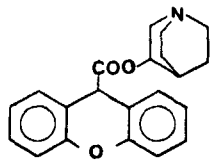
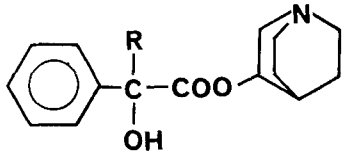


QNB



QNX

Table 2. Physicochemical Properties



R	Connectivity Indexes						Polarizability	Dipole Moment Debye
	X_0	X_1	X_2	X_{3-p}	X_{3-c}	X_3		
QNB	4.39	2.41	1.32	0.94	0.17	1.11	4295.9	23
C_6H_{11}	5.11	3.39	2.05	1.89	0.29	2.18	3592.8	--
C_5H_9	4.40	2.89	2.10	1.64	0.29	1.93	3079.6	--
C_4H_9	4.12	2.41	1.35	0.71	0.00	0.71	2707.1	--
$HC\equiv CCH_2$	2.99	1.52	0.70	0.29	0.00	0.29	2367.7	41
QNX	8.60	5.22	3.25	2.99	0.43	3.42	8751.5	--
$3\text{-}FC_6H_4$	4.09	2.21	1.14	0.79	0.13	0.92	4408.3	--
$4\text{-}FC_6H_4$	4.09	2.21	1.12	0.81	0.13	0.94	4408.3	--
$3\text{-}IC_6H_4$	5.31	2.82	1.84	1.17	0.33	1.51	----	--
$4\text{-}IC_6H_4$	5.31	2.82	1.82	1.22	0.33	1.55	----	--

IODO BEADS:EVALUATION OF AN IMMOBILIZED PROTEIN IODINATION REAGENT

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Iodobeads* are polystyrene beads covalently modified with a Chloramine B analog (N-Chlorobenzene Sulfonamide Na salt). Each spherical bead is about 1/8"diameter, non-porous and has an oxidative capacity of 0.5 μmole./bead. Any protein or peptide (containing a tyrosine group) can be iodinated in neutral buffer media using this reagent. We evaluated this reagent for iodinating several proteins of different molecular weights including 1) Glucagon, 2) Lysozyme, 3) Lactoferrin, 4) HSA, 5) Rabbit IgG and 6) Fibrinogen.

In a typical experiment, 100 μg of protein in 0.5 ml, pH 7.5, 0.2 M phosphate buffer solution is taken in a test tube and required quantity of I-125 or I-131 was added (0.1 μCi -3 mCi) along with 1-5 Iodobeads and mixed once, let stand for 1-10 minutes. The protein solution is then withdrawn from the test tube and assayed for labeling yield. For each compound the labeling yields were determined for the following variables 1) Time of incubation (1-10'), 2) protein concentration (10-1000 μg), 3) pH (7-8) and 4) number of beads (1-5). Reaction volume was kept constant at 0.5 ml for all the experiments.

The labeling yields in each experiment were determined by HPLC using a Gel filtration column. (Spectraphysics model 3500B and BioRad TSK-250 column) These were further verified by thin layer chromatography (paper/methanol etc.) and ion exchange separations. The results are shown in four tables.

Three to five beads for a volume of 0.5 ml was found to be adequate for any labeling procedure. There were no significant differences in labeling yield at pH 7-8. A protein concentration of even 10 μ gm seemed to be adequate. Incubation time of 1 minute was enough to reach almost maximum labeling yield.

Rabbit immunoglobulin (same batch) was separately labeled with either I-125 using Iodobeads and with I-131 using the lactoperoxidase technique and the blood clearance of both compounds were simultaneously studied in rabbits over a period of 7 days. No significant differences were found in their blood clearance rates. These results indicate that Iodobead method of labeling of proteins is an excellent one and more convenient than any other method available to date.

*Pierce Chemical Co., Rockford, Illinois, USA.

PROTEIN IODINATION USING IODOBEADS. PERCENT LABELING YEILD.

100 µgm Protein in 0.5 ml
0.2 M $NaPO_4$ Buffer pH 7.5
3 Iodobeads

Time	1'		5'		10'	
	$\bar{x}$	± S	$\bar{x}$	± S	$\bar{x}$	± S
GLUC	40.7	0.4	---		39.1	4.7
LYZ	86.1	0.7	84.8	1.5	84.4	1.2
LF	53.9	4.6	61.7	2.0	61.9	1.5
HSA	77.4	2.2	75.7	2.1	79.7	4.0
IgG	46.5	7.4	43.4	4.1	51.4	2.4
FIB	33.1	0.4	41.6	7.0	30.1	2.0

100 µgm Protein in 0.5 ml
0.2 M $NaPO_4$ Buffer pH 7.5
10 min Incubation

# Beads	1		3		5	
	$\bar{x}$	± S	$\bar{x}$	± S	$\bar{x}$	± S
GLUC	----		35.8	2.0	54.6	3.3
LYZ	63.5	9.1	76.6	2.1	81.5	4.2
LF	35.1	2.0	56.9	1.6	70.7	4.6
HSA	40.2	1.7	78.8	1.4	83.0	1.2
IgG	28.5	1.2	57.7	2.8	76.6	0.3
FIB	14.5	3.3	24.4	1.3	29.6	2.1

Total Volume of 0.5 ml
0.2 M $NaPO_4$ Buffer pH 7.5
3 Iodobeads; 10 min. Incubation

Protein	10 µgm		100 µgm		1000 µgm	
	$\bar{x}$	± S	$\bar{x}$	± S	$\bar{x}$	± S
GLUC	47.9	3.0	39.7	2.5	----	
LYZ	62.7	1.5	78.6	0.9	80.3	0.7
LF	64.8	3.9	78.8	1.7	85.3	0.2
HSA	69.3	1.6	71.5	3.1	54.3	1.2
IgG	49.4	3.6	56.0	0.9	58.9	6.6
FIB	30.7	0.7	27.2	2.1	49.8	0.3

100 µgm Protein in 0.5 ml
0.2 M $NaPO_4$ Buffer
3 Iodobeads; 10 min. Incubation

pH	7.0		7.5		8.0	
	$\bar{x}$	± S	$\bar{x}$	± S	$\bar{x}$	± S
GLUC	44.3	1.8	41.9	3.5	38.4	0.2
LYZ	78.7	1.5	77.2	0.3	79.2	1.0
LF	67.2	3.5	60.5	4.1	62.8	2.3
HSA	74.5	0.9	75.8	2.4	76.4	2.7
IgG	56.5	5.0	54.5	4.2	54.6	3.6
FIB	34.8	1.6	35.4	0.5	37.4	1.1

THE SYNTHESIS OF VARIOUS N-[^{11}C-METHYL]-PHARMACEUTICALS USING ^{11}C-METHYL IODIDE

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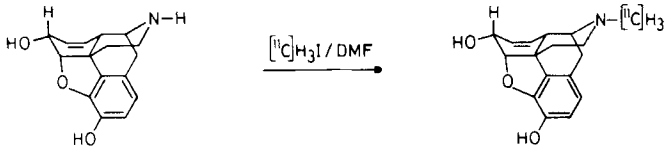
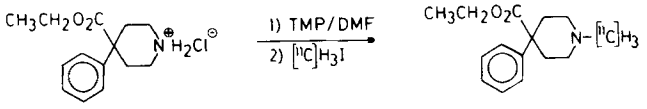
The synthesis of various N-[^{11}C-methyl]-containing pharmaceuticals have been performed using ^{11}C-methyl iodide.(1) In this paper the synthesis of two analgesics, morphine and pethidine, labelled according to Scheme 1 and 2 is reported.



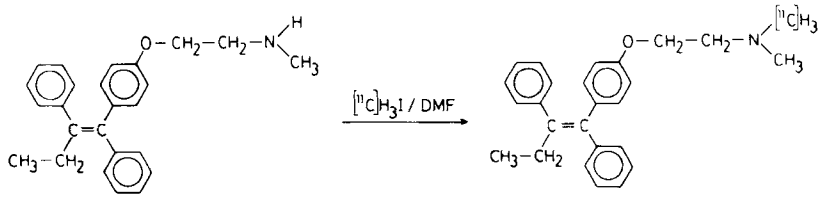
Scheme 1



Scheme 2

The synthesis of ^{11}C-morphine has previously been performed by reductive alkylation, using ^{11}C-formaldehyde (2) and by N-alkylation using ^{11}C-methyl iodide in ethanol (3). The latter reaction was also used here in the synthesis of N-[^{11}C-methyl]-morphine (Scheme 1) and N-[^{11}C-methyl]-pethidine (Scheme 2). The use of dimethylformamide (DMF) instead of ethanol as solvent gave radiochemical yields in the order of 70 and 80 % respectively. The use of tetramethylpiperidine (TMP) made it also possible to liberate the appropriate norcompound from its hydrochloride *in situ*. The total time required for the syntheses, counted from the trapping of ^{11}C-methyl iodide was 20 min for each compound.

The synthesis of N-[^{11}C-methyl]-tamoxifen, a non-steroidal oestrogen analogue with affinity for oestrogen receptors and with anti-oestrogenic activity *in vivo* (4), was also carried out by using ^{11}C-methyl iodide, produced as previously described (5), in DMF as shown in Scheme 3. The synthesis of the nortamoxifen (6) will also briefly be reported.



Scheme 3

The radiochemical yields were in the order of 50-70 % and the product was obtained within 10-20 min of trapping ^{11}C-methyl iodide.

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(4) Capony, F. and Rochefort, H., Mol. Cell. Endocrinol. 11, 181 (1978).

(5) Långström, B. Doctoral thesis, Acta Universitatis Upsaliensis No. 555, 1980.

(6) Någren, K., Sohn, D., Sjöberg, S. and Långström, B. manuscript in preparation.

SYNTHYSES OF ^{73}Se- OR ^{75}Se-LABELED COMPOUNDS BY THE ISOTOPIC EXCHANGE WITH ELEMENTAL SELENIUM-73 OR -75

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Selenium-73 was produced by the nuclear reaction ^{75}As(p,3n)^{73}Se, or Ge(^{3}He,xn)^{73}Se, as reported previously (1). For the purpose of medical use of selenium-73, a positron emitter with a half-life of 7.1 hr, the syntheses of ^{73}Se-labeled compounds has been studied. The isotopic exchange is one of the advantageous methods for labeling with a short half-life nuclide. Selenium-73 is separated from the targets as elemental selenium with a minute amount (5 μg) of the carrier without difficulty. It is well known that sulfur atoms in some thio-compounds such as 2-thiouracil and 6-mercaptopurine can be labeled by isotopic exchange with elemental sulfur-35.

As for selenium which is a sulfur analogue, we already proved that selenium atoms in some seleno-compounds such as 2-seleno-uracil and 6-selenopurine are also labeled by isotopic exchange with elemental selenium-73 or -75 (2). In this presentation, experimental results are shown for the isotopic exchange labeling of 2-selenouracil, 6-methyl-2-selenouracil, 2-selenothymine and 6-selenopurine.

The exchange reaction was carried out in the mixture of carbon disulfide and pyridine as the solvent in a degassed sealed tube. Suitable conditions in the reaction with selenium-73 were determined by the use of selenium-75 whose half-life is 120 days. The results are shown in Table 1 and Fig. 1. Because of the short half-life of selenium-73, the maximum activity yield may be given at a reaction time of about 1-2 hrs. About 1.3 mCi of ^{73}Se-2-selenouracil was obtained by the above method in 1 hr from 5 mCi of elemental selenium-73. ^{73}Se-2-selenouracil was administered intravenously into a rat, and its distribution was measured. ^{73}Se-2-selenouracil was found to be taken up mainly in the liver and kidney.

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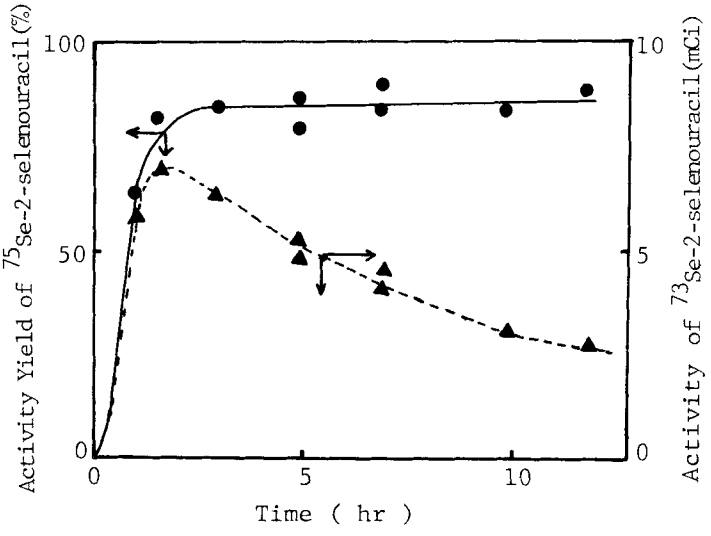

Fig. 1 Isotopic exchange yield of 2-selenouracil vs Reaction time, 2-selenouracil (1 mg, 5.7×10^{-3} mmol) and elemental selenium-75 (4.9 μg, 6.2×10^{-5} mmol) in CS_2-Pyridine(1:1) at 130°C.

Activity yield for ^{75}Se-2-selenouracil(%)

Activity for ^{73}Se-2-selenouracil(mCi)

Table 1 Isotopic Exchange Yield for Various Seleno-compounds

	Time(hr)	Temp(°C)	Yield(%)
^{75}Se-2-selenouracil	7	110	70
^{75}Se-6-methyl-2-selenouracil	7	110	66
^{75}Se-2-selenothymine	7	110	52
^{75}Se-2-selenopurine	11	110	65

Seleno-compound; 1 mg Selenium-75; 5×10^{-3} mg

Solvent; 2 ml of CS_2-Pyridine(1:1)

ENZYME-INHIBITOR MEDIATED RED CELL LABELLING

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A rational approach to selective cell labelling involves the use of carrier ligands which bind to specific receptors. The ligand may be an antibody, an enzyme-inhibitor, a hormone, neurotransmitter or some other chemical effector, or a metabolite. Since few enzymes are restricted to only one cell type and tight-binding inhibitors are known to only some of these, the enzyme-inhibitor approach to selective nuclide targeting has found limited application. However, the red blood cell is an appropriate system which may serve as the paradigm for all other studies since red cells contain 90% of the body's complement of the enzyme carbonic anhydrase to which aromatic sulphonamide inhibitors bind very tightly.

The disappointing results first reported for this system (1) can be accounted for by an unfortunate choice of inhibitor. An aromatic sulphonamide is required which is liphophilic and will therefore afford rapid cell labelling, which binds tightly to carbonic anhydrase at physiological pH, which has pKa>7.4 to inhibit plasma protein binding, and which is amenable to facile radiolabelling. p-Iodobenzene-sulphonamide (PIBS) fulfills these requirements.

Radioiodinated PIBS was prepared by three methods; solid phase radioiodide exchange in the presence of ammonium sulphate consistently gave the highest radiochemical yields. The radiolabelled sulphonamide was purified on Dowex ion exchange and alumina columns and checked for purity by tlc on silica in $CHCl_3$/MeOH 2:1.

p-Iodobenzenesulphonamide has pKa 10.1, and partitions between chloroform and phosphate buffered isotonic saline with $\log_{10}P = 1.2$. In vitro inhibition studies indicated that pIBS

bound to the high activity carbonic anhydrase isozyme tightly, K_D = 3 x 10^{-7} mol.dm^{-3} at pH 7.4.

[^{125}I]-pIBS labelled red cells in whole blood rapidly and efficiently (95 + 5% at [pIBS] = 1 x 10^{-6} mol.dm^{-3}); the labelling was instantaneous and temperature independent suggesting passive diffusion across the red cell membrane. Sephadex G75 gel filtration of labelled red cell lysate was consistent with the binding of the radiolabel to carbonic anhydrase. Comparison of pIBS uptake with that of [^{125}I]-p-iodobenzene-N-methylsulphonamide, a weaker carbonic anhydrase inhibitor, also confirmed the true enzyme-inhibitor nature of red cell labelling by pIBS.

After intravenous administration of [^{131}I]-pIBS to a rat all the radiolabel was found in the blood pool, associated with the red cells; however, there was slow elution of the label from the cells ($t_{1/2}$>30h).

Radioiodinated p-iodobenzenesulphonamide may have applications in short term blood pool imaging; tighter binding sulphonamides may have even greater potential.

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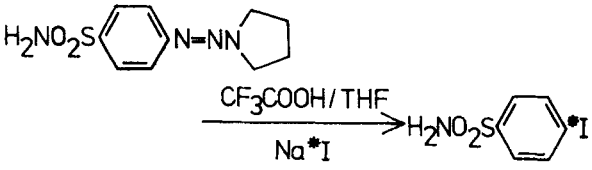
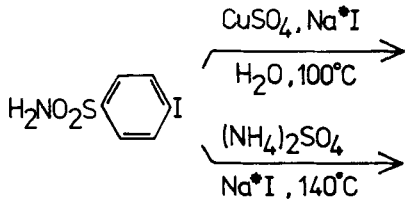
Table. Synthesis of [^{125}I]-p-Iodobenzenesulphonamide from 1mg starting material and 100 mBq $Na^{125}I$.

Method	Radiochemical Yield %	
	30	(2)
	30	
	90	(3)

SYNTHESIS OF RADIOIODINATED METYRAPONE - A POTENTIAL ADRENAL IMAGING AGENT

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Metyrapone, a well known inhibitor of steroid 11ß-hydroxylation in adrenal cortex mitochondria and liver microsomes (1) was assumed to concentrate in the adrenal cortex better and faster than I-131-iodocholesterol. Attempts to label metyrapone with radioiodine resulted in the synthesis of 4'-bromo-metyrapone, a presursor that is labelled with I-131(123) by halogen exchange before use. (2)

Since metyrapone (I) consists of 2 pyridine rings on a propanone chain the best position for a gamma label appeared to be on one of the pyridine rings. Our synthetic route includes activation of ring B by formation of metyrapone-N-oxide II (3). The activated ring system easily undergoes nitration in the 4'-position giving III (4).

To evaluate routes for the direct substitution of III with halogen, 3-methyl-4-nitropyridine-N-oxide was adopted as a model compound (5). It was converted to 3-methyl-4-bromopyridine-N-oxide resp. 3-methyl-4-bromopyridine by known reactions (6,7). Both compounds were treated with hydroiodic acid yielding 3-methyl-4-iodopyridine (8). This provided evidence that halogen exchange of 4-bromo-substituted pyridines is independent of the N-oxide group (9). Attempts to displace the nitro-group by bromine with simultaneous reduction of the N-oxide of III using PBr_3 failed to produce halogenation but gave 4'-nitro-metyrapone VII (10) only. We chose the direct halogenation of III with acetylbromide and subsequent reduction of the N-oxide of IV with Raney nickel/H_2 to produce 4'-bromo-metyrapone V. Each compound was identified by NMR-spectra (^1H, ^{13}C) and mass spectroscopy.

I-131(123) is introduced by halogen exchange in the melt at 165°C for 2 hr. Labelling is primarily affected by the radioiodine solution and the heating temperature (11). Carrier-free iodide with minimum salt content in dry form is essential for a satisfactory yield. (65-75% of VI) At 120°C labelling was below 20%. Labelling increased with temperature and showed maximum values between 165-170°C. Free iodide was separated either by extraction with chloroform or Cellex-D column chromatography. Radiochemical purity was confirmed by radio-TLC in two solvent systems.

Studies of the biodistribution of I-131-metyrapone (rat,dog) indicated a high concentration (0.91%kg dose/g) in the adrenal gland corresponding to data reported for H-3-metyrapone.(12) However, absolute uptake in the normal adrenal is low. In a patient with bilateral,nodular hyperplasia we have produced the first adrenal scintigram using 1.25mCi of I-123-metyrapone. It is expected that high specific activity labelling with I-123 will provide adequate radioactivity for external visualization of the gland.

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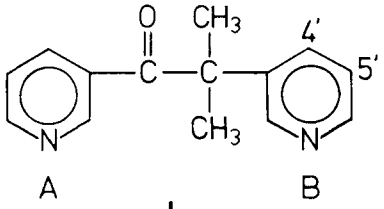

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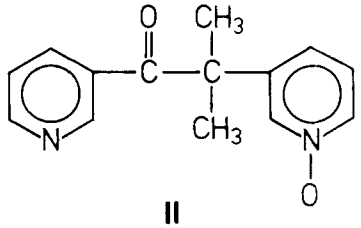

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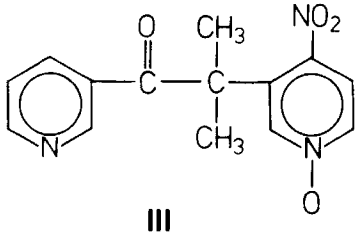

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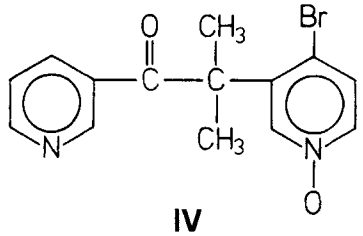

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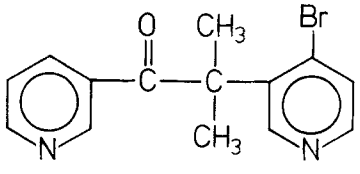

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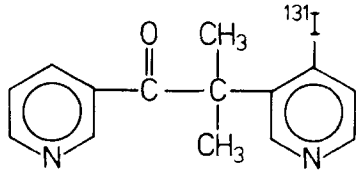

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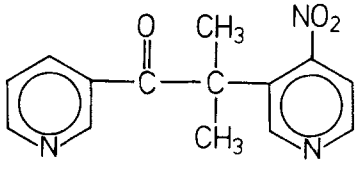

VII

A RAPID HIGH SPECIFIC ACTIIVTY METHOD FOR LABELLING LONG-CHAIN FATTY ACIDS WITH RADIOIODINE (I-131 OR I-123)

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Long-chain fatty acids, labelled with radioiodine in the terminal (ω-) position, have been shown to be good agents for measuring myocardial functions (1,2). Numerous methods have been investigated to obtain high yields of high specific activity ω-halofatty acids (3-9).

We report here another reliable method for routine preparation of high specific activity 16-^{123}I-hexadecanoic acid (16-^{123}I-HdA) and 17-^{123}I-heptadecanoic acid (17-^{123}I-HdA) from commercially available Na$^{123/131}$I.

The adopted procedure depends on the exchange reaction between lyophilized radioactive sodium iodide (1-10 mCi) and inactive 16-Br-HdA or 17-I-HdA in anhydrous redistilled methyl n-propyl ketone at 100^0 in the presence of small specific amount of elemental cold iodine. A radiochemical yield of 90-95% can be obtained within 5-15 min. Direct labelling at 30 to 100^{0}C gives a yield ranging from 23 to 93%. The different parameters affecting the rate of the isotopic exchange reaction (namely: exchange medium, concentration of elementary cold iodine, temperature and time of exchange) were investigated.

Fig. 1 demonstrates that in methyl n-propyl ketone high chromatographic yields are obtained in short times, whereas longer times are needed for methyl ethyl ketone or benzene.

Fig. 2 demonstrates that yields improve significantly upon addition of carrier, whereas Fig. 3 shows that good yields can only be obtained at about reflux temperature (100^{0}C).

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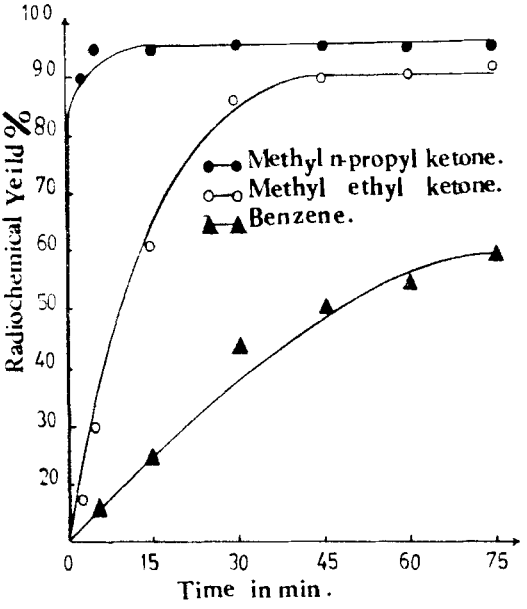

Fig. 1 Effect of exchange and time on the yield of labelling of 17-I-HdA

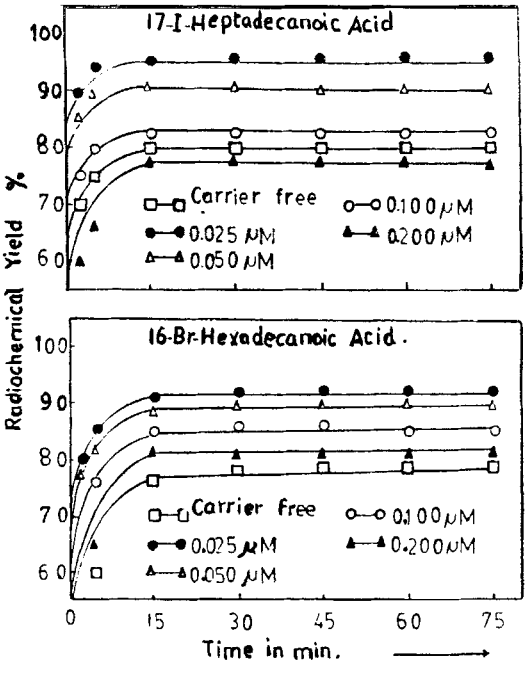

Fig. 2 Effect of carrier iodine on the yield of the isotopic exchange reaction

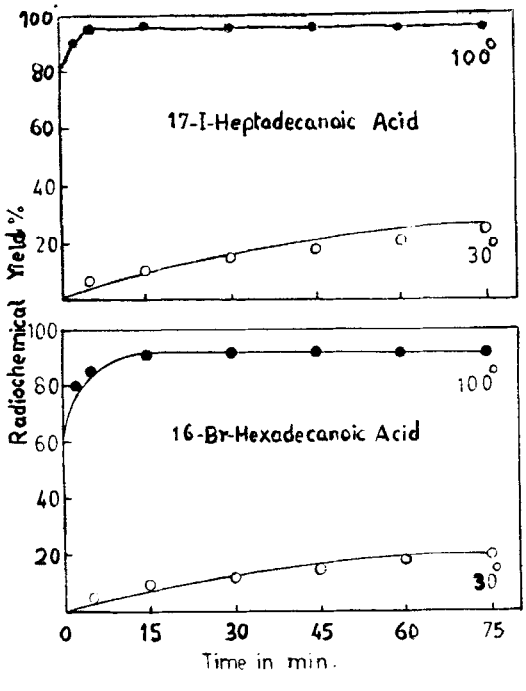

Fig. 3 Effect of temperature on the rate of the isotopic exchange reaction

FORMATION KINETICS AND PRODUCTION OF FREEZE-DRIED TECHNETIUM(TIN-HIDA)-KIT USING SODIUM STANNITE

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Technetium-99m-labelled stannous N-(2,6-dimethylphenylcarbomoylmethyl)-iminodiaacetic acid (Tc-99m-HIDA) has been prepared by many authors for hepatobiliary imaging (1-4). The application of stannite ions in the preparation of Tc-99m-EHDP (5), encourages us to apply the stannite procedure to develope a simple method for the production of freeze-dried kit containing 20 mg of HIDA and 0.4 mg of $SnCl_2 \cdot 2H_2O$. A stable Sn-HIDA chelate is prepared advantageously by dissolving the compound in the stannite solution. Gelchromatography column scanning (GCS) technique was used for the determination of Tc-99m-HIDA complex, free pertechnetate and the reduced hydrolyzed-Tc-99m (6,7).

The formation conditions of Tc-99m-labelled-HIDA was found to be dependent mainly on the pH of the reaction mixture as assessed by the GCS-method. When the reaction was carried out at pH 10.4, the GCS-profile of the samples taken after (15 min) of technetium adding, showed only one peak corresponding to the area of free pertechnetate. At pH 8.9, most of the technetium was observed on the front of the gel bed. However, the formation of Tc-99m-HIDA was observed at pH 7.4 and increased significantly when the pH was shifted down as shown in the figure. The optimal pH for a high yield Tc-99m-HIDA was found to be in the range 5.5-6.0.

However, a mixture of stannite-HIDA of pH 10.4, lowered to the optimal pH, showed turbidity and when the Tc-99m-eluate has been added to the filtered mixture, only one peak of pertechnetate was observed on the GCS-profile, because the hydrolysis of tin is faster than the formation of Tc-99m-HIDA. Simultaneously, the GCS-profile obtained from the analyzed samples of a Tc-99m-stannite mixture at the same pH showed a reduced hydrolyzed-Tc-99m and free pertechnetate. Therefore, we can conclude that at high pH the presence of HIDA is lowering the redox potential of the system forming a stable tin-HIDA chelate, but does not incorporate technetium. This contention could be supported by the redox potential variation in the ferrous-phenanthroline system reported by Eckelman et al. (8).

The Tc-99m-labelled-HIDA was found stable for at least (6 hr) using the GCS-method. The organ distribution of the preparation was studied on Swiss albino mice. At the times 5, 10, 15, 30 and 60 (min) after injection via tail vein, the animals were sacrificed and the results of the distribution in the organs of interest are listed in the table. Tc-99m-HIDA injection is rapidly cleared from the blood within (5 min).

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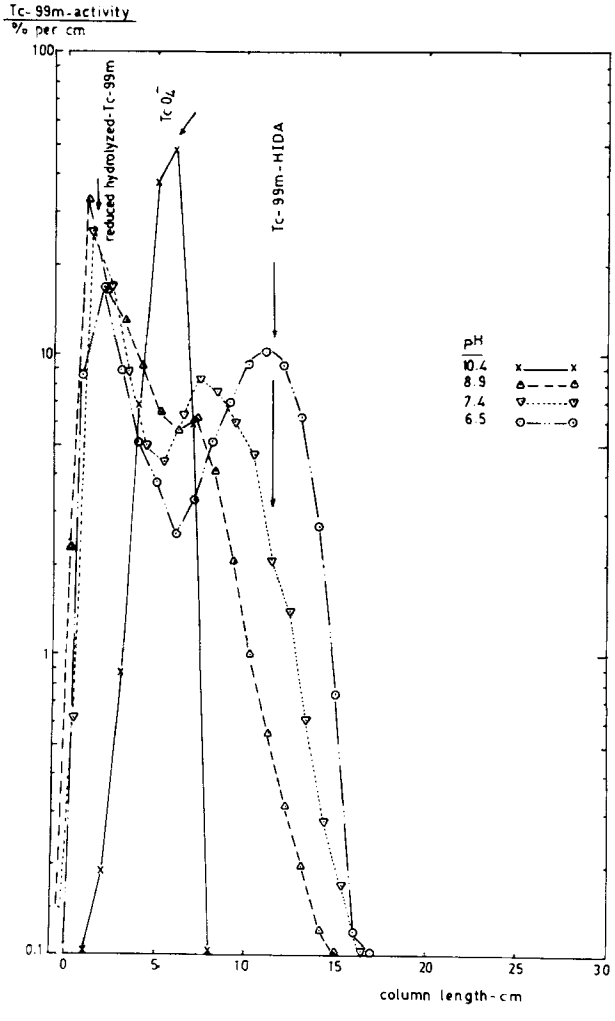
Biodistribution data of Tc-99m-HIDA in mice

Name of organ	% of Tc-99m activity at (min)				
	5	10	15	30	60
Liver	32.74	20.97	15.40	3.58	1.64
Intestine	45.52	49.88	59.34	78.96	65.89
Kidneys	2.74	1.98	1.81	1.12	0.87



Formation kinetics of Tc-99m-HIDA as a function of pH

AN EVALUATION OF ANALYTICAL TECHNIQUES USED IN THE DEVELOPMENT OF RADIONUCLIDE GENERATORS

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The need for short-lived isotopes in diagnostic nuclear medicine has promoted the development of many radionuclide generators. In addition to the well characterized Mo-99/Tc-99m system (1), reports continue to appear on Rb-81/Kr-81, Ge-68/Ga-68, and Sr-82/Rb-82 generators. Each is based on a chromatographic column which firmly retains the parent isotope and provides the daughter isotope in the eluate.

The development of new radionuclide generator systems requires the selection of a suitable adsorbent-eluent combination and the design of a chromatographic column to provide an eluate of desired radioconcentration and radiochemical purity.

A number of analytical techniques are often used to select and evaluate a chromatographic system. Three techniques have been used and evaluated: Batch equilibration, eluate analysis, and column scanning.

Batch equilibration of adsorbent and eluent provides information on the adsorption of parent and desorption of daughter isotope. Results are typically expressed in terms of a distribution coefficient (Kd). Because of the slow kinetics of many inorganic systems, the Kd is often measured as a function of time. When problems of adsorption capacity must be considered, adsorption isotherms will provide useful information. These techniques are useful for checking a large number of adsorbent-eluent combinations. The system with the greatest potential for further development can be selected quickly. The data obtained is generally reliable and can be used to select the dimensions and estimate the characteristics of a radionuclide generator.

The analysis of eluates from small generators can be used to experimentally determine breakthrough, yield, and radioconcentration. This technique provides more precise data than batch equilibration, which is primarily a comparative technique. Results from eluate analysis can be used to modify the initial generator design.

Column scanning determines the distribution of isotopes on a column. The technique is simple and rapid. It can be used to complement data obtained from eluate analysis. Scans can often provide unique information on generator performance which will not be determined by analysis of eluates. It may be used to measure isotope radioconcentration and to evaluate chemical and physical changes which may occur during elution.

Each of these techniques is well suited to a particular aspect of generator development. In order to totally characterize a generator system, they should all be used to complement one another.

Since the use of column scanning has received little attention to date, this report concentrates on its application in some recent work. Three examples are cited below:

1. The potency limitations of a particular column design had to be determined. Column scanning of simulated generators was used to rapidly evaluate several potencies. The scans (Figure 1) established satisfactory performance at 2220 mCi Mo-99.

2. Reduced Tc-99m yield in high activity generators is related to the short range radiation dose from the decay of Mo-99 (2). A formulation was desired which would reduce the radioconcentration of Mo-99 by dispersion. The column scan (Figure 2) was essential in estimating the degree of dispersion.

3. Specification limits for the manufacture and use of a new generator system had to be set. Column scanning techniques were adapted to evaluate generator preparation and elution. The data obtained were used to establish those limits.

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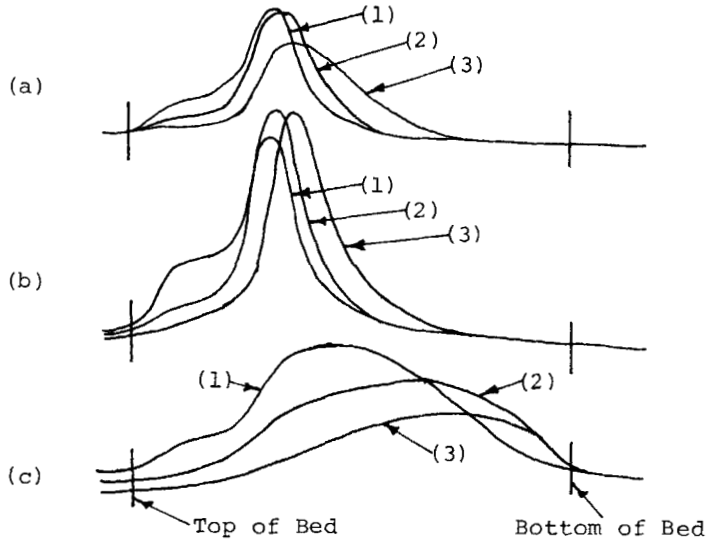

Figure 1: Mo-99 Column profiles for (a) 0.22 Ci, (b) 2.22 Ci, and (c) 20 Ci simulated generators. Overlay curves represent column scans after elution volumes of: (1) 5 cc, (2) 25 cc, and (3) 55 cc.

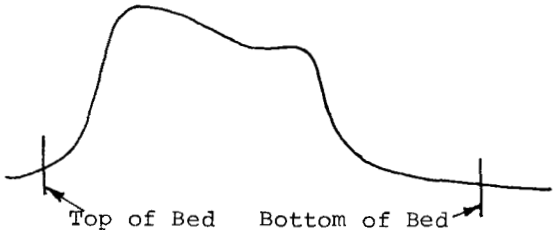

Figure 2: Mo-99 Column profile for generator confirming dispersion of Mo-99 activity.

THE REACTION MECHANISM OF THE TECHNETIUM 99-m COMPLEX FORMATION WITH THE LIGANDS USED FOR THE PREPARATION OF RADIOPHARMACEUTICAL MEDICINES

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The rapid development of technetium-99m application is drawing attention of a great number of researchers to unsolved problems of its chemistry and radiochemistry.

It's well-known that in most of pharmaceutical medicines based on technetium-99m, the latter is contained in a reduced form.

Evidently extraction is the most appropriate method of investigating complex formations of reduced technetium-99m in solutions. We have carried out the extraction of technetium (IV) using a number of extractants. The results show that the following system suits the researchers: I M of water solution $NaClO_4$- O,I M of TTA (thenoilthreefluoroacetone) in toluene.

Taking into consideration an instant oxidation of technetium (IV) we have worked out special equipment and corresponding methods of work (I).

While studying the extraction of reduced technetium from perchlorate solutions it has been found that only one molecule of TTA takes part in the formation of the complex extracted. The ion of ClO_4 may be present in the composition of the complex extracted, depending on the conditions of the experiment. Both variants of the extraction mechanism lead to the same expression of constant calculation for the complex formations $\varkappa$ (for the formation of the $TcO(OH)L_mH_n$ in particular):

$$\left(\frac{D_o}{D_L} - 1\right)\left(1 + \frac{h}{K_1} + \frac{K_2}{h}\right) = \varkappa \ell^m h^n$$

where: D_o - Tc(IV) equivalent distribution coefficient;
D_L - Tc(IV) distribution coefficient with aqueous ligand;
ℓ - equivalent aqueous ligand concentration;

h - hydrogen ions concentration in aqueous phase;
K_1, K_2 - Tc(IV) hydrolysis constants.

The study of the distribution coefficient dependence on ℓ - and h - values gives the possibility of determination of the composition of the complexes formed. We determined the composition and stability constants of Tc (IV) complexes with DTPA (I).

In the present paper we give the similar investigations in the systems containing β - mercaptoethylamine, pyrophosphate, dimercaptosuccinic acid and oxa-bis-(ethylenamino - N_1, N' - dimethylphosphonic acid under conditions usually used in RFP-preparation (ligand concentration is 10^{-2} - 10^{-4} mol/I, pH 3-8).

It was revealed that:

I) β - mercaptoetilamines form a complex $[TcOSCH_2CH_2NH_2]^+$ with a reduced technetium, $\lg \alpha = 7,68 \pm 0,6I$.

2) In pyrophosphate solutions the formation of two complexes with Tc (IV) is possible: $[TcO(OH)P_2O_7]^{3-}$, $\lg \alpha_1 = 8,24 \pm 0,I3$ (pH > 5,5) and $[TcO \cdot P_2O_7]^{2-}$, $\lg \alpha_2 = I3,92 \pm 0,I0$ (pH < 5,5).

3) Dimercaptosuccinic acid in the conditions investigated also forms two complexes. The former dominates at pH < 3 and is supposed to have the following composition - $TcOL_2H_2$, $\lg \alpha_1 = 27,I9 \pm 0,05$, the latter has the composition of $[TcO(OH)L]^{3-}$ dominating at pH > 3, $\lg \alpha_2 = I7,I6 \pm 0,I5$, (L - anion DMS).

4) In the system Tc (IV)-oxa-bis (ethylenamino-N, N'-dimetilphosphonic) acid the complex of $TcOH_8L$-type is formed with the logarithm of the stability constant 25,00±0,80 (L -ligand anion). The possible structures of the complexes formed have been discussed.

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LONG TERM STUDY OF HIGH LEVEL Ge-68 GENERATORS

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Separation of Ga-68 from its radionuclidic parent for subsequent use in radiopharmaceutical preparations has been accomplished by numerous methods (1-21, 23). Basically the methods can be divided into substrate adsorption type generators and/or solvent extraction. The former, by far the majority, are composed of four groups defined by the permutation of the column material (organic or inorganic) with the nature of the eluant (complexing or "ionic").

The combination currently most popular is based on the alumina substrate eluted with 0.005M EDTA. Despite its popularity, this combination can have disadvantages which become increasingly evident with generators containing the larger number of millicuries associated with ones currently available commercially (6, 17). These disadvantages include presence of EDTA and aluminum in the eluate, low initial elution efficiencies, and deterioration of elution efficiencies with time.

For a number of years, the Nuclides and Sources Division of N.E.N. has provided a radiochemical generator based on Al_2O_3/EDTA which many customers convert for radiopharmaceutical use. As a consequence, considerable data has been accumulated on this system. Our research group has investigated both variations on the commercially available unit and some alternatives to Al_2O_3/EDTA. A promising alternative is based on a separation using SnO_2/1M HCl (22, 23). It produces ionic Ga-68, has relatively high initial elution efficiencies, stable long-term performance, low parent leakage, and low levels of tin leakage. Separation by this method has recently been reported for its suitability for generating ionic Ga-68 for radiopharmaceutical use (24, 25, 26).

N.E.N. has been collaborating with Donner Laboratory and Washington University in an ongoing long-term investigation of two high level SnO_2/1M HCl generators. The radiochemical generators were assembled and extensively evaluated at N.E.N., then shipped to the other laboratories for further evaluation of the generators, and of the Ga-68 for radiopharmaceutical use. The performance of the generators has been very reproducible and has shown no deterioration with time. Nominal elution characteristics for these generators are summarized in Table 1.

Table 1. Summary of Elution Characteristics of Two High Level SnO_2/1M HCl Ge-68/Ga-68 Generators

I.D.	Total mCi Ge-68 a)	Elution Eff. b)	Ge-68 Leakage c)	Sn Leakage d)	Age as of 8/23/82 days e)
A	15.2	68.2 ± 0.5	5×10^{-4}	≤ 2	327
B	51.0	67.1 ± 0.5	2×10^{-3}	≤ 2	248

a) The total Ge-68 absorbed on the SnO_2 at time of fabrication.

b) Each generator was eluted with 10 ml 1M HCl at least once daily and frequently twice per day. The elution efficiency was determined both by difference and by assaying the eluate. Periodic histograms showed > 95% of the activity was in the first 5 ml.

c) Percentage Ge-68 (on the generator at time of elution) found in eluate.

d) Ppm Sn in eluate.

e) Generator A is currently at Donner, and B is at Washington Univ..

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PREPARATION OF ^{45}Ti-LABELED COMPOUNDS AND THEIR MEDICAL APPLICATION

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^{45}Ti has a half-life of 3.09 h and a high positron branching ratio (85 %) and can be produced by an in-house cyclotron. The ^{45}Ti-labeled compounds are expected for the positron emitting radiopharmaceuticals in nuclear medicine(1). We prepared some ^{45}Ti-compounds and investigated their behaviors in animals.

^{45}Ti was produced by the proton irradiation of about 65 or 130 mg of the scandium foil(0.127 or 0.254 mm thickness, 99.9 % purity, Alfa Division Ventron Corp.) at 11.5 MeV via the ^{45}Sc(p,n)^{45}Ti reaction using the Tohoku University cyclotron. The radionuclidic purity was analyzed by the γ-ray spectrophotometry using the Ge(pure) detector and only the ^{45}Ti was detected as the radioactive nuclide. The ^{45}Ti was obtained with yields of 25±2 and 48±0.5 mCi/μA•saturation for 0.127 and 0.254 mm thick foils, respectively(calculated from the nuclear data in Table of Isotope, 7th Ed.).

The ^{45}Ti was separated by the method of Nelson et al.(2) as shown in Fig.1. Following irradiation, the scandium foil was dissolved in 2 ml of 6 M HCl and one drop of conc. HNO_3 was added to oxidize the titanium to the +4 state. The solution was evaporated up and redissolved in 6 M HCl. Dryness and redissolving were repeated three times. Finally the ^{45}Ti activity was dissolved in 2 ml of 6 M HCl and loaded on a column of AG 50Wx8(1.9x13cm, 100-200 mesh) prewashed with 6 M HCl. The ^{45}Ti activity was eluted with 6 M HCl and the scandium was eluted with 0.1 M HF/4 M HCl. The fraction of ^{45}Ti activity was collected, evaporated up, dissolved in 1-2 ml of 1 M HCl and sterilized by a membrane filtration(0.22μm). Separation was completed in about 2 h from the end of bombardment with a radiochemical yield of 75-90 %. Further purification of the ^{45}Ti activity was carried out in a state of an anion complex on a AG 1x8 column by the method of Walter(3). A small amount of $^{45}TiCl_4$ solution was added to 0.2 ml of the cherating reagents dissolved in saline:0.6 mg/ml phytate, 5 mg/ml of human serum albumine(HSA), 10 mg/ml diethylenetriaminepenta-acetic acid(DTPA) and 21 mg/ml citric acid, and the solution were adjusted to pH4-6 with 0.1 M NaOH.

The DTPA and citrate complexes were ascertained by paper electrophoresis and thin-layer chromatography(TLC). These complexes were eluted faster than $^{45}TiCl_4$ on Sephadex G-10 column with 0.1 M acetate butter pH4.7. In Sephacryl S-200 column chromatography of the ^{45}Ti-HSA, the radioactivity and protein showed the same elution profiles. The ^{45}Ti-phytate did not migrate on paper electrophoresis and TLC and was adsorbed on Sephadex G-10. Except at the strongly acidic condition (above 0.05 M HCl), the ^{45}Ti-phytate formed a macro size colloid and was heterogeneous in molecular size, because most of ^{45}Ti-phytate did not pass through a membrane filter(0.45 μm). The $^{45}TiCl_4$ itself was found to be stable in 1 M HCl solution, but to form a colloid, probably $^{45}TiO_2$(1), relatively easily at near neutral pH region, because the $^{45}TiCl_4$ behaved in a similar manner as the ^{45}Ti-phytate. Whether this phenomenon was due to a small amount of contamination in the ^{45}Ti preparation was not investigated in detail.

Donryu rats were injected with ^{45}Ti-DTPA intravenously. The ^{45}Ti-DTPA was found to be present in the blood at the highest level and to be distributed at constant levels for 30 min in all tissues except for the heart. An autoradiogram of the rats brain showed the ^{45}Ti-DTPA passing the blood-brain barrier which was broken by freezing with liquid nitrogen(Fig.2) or the administration of mannitol intraarterialy. The ^{45}Ti-complexes with citrate and HSA showed the similar tissue distribution patterns as the ^{45}Ti-DTPA. The whole body distribution of ^{45}Ti-phytate in rabbits was obserbed with a positron tomograph using the ECAT II (Ortec). In Fig.3 a positron tomograph provided distinct images of the lung, the liver and the spleen. The accumuration of ^{45}Ti-phytate was dependent on the colloidal size. The tissue distribution study also showed the similer accumuration.

Although some problems remain unsolved in the preparation of ^{45}Ti-labeled compounds because of the unstability of $^{45}TiCl_4$, these results indicated that the ^{45}Ti-compounds would become available as a series of the positron emitting radiopharmaceuticals.

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Sc foil (12mmx12mm10.127mm)
↓ proton, 11.5 MeV, 5 μA, 60 min
↓ 2 ml 6 M HCl, 1 drop HNO_3
↓ evaporated up
↓ 1 ml 6 M HCl
↓ AG 50W column chromatography
$^{45}Ti^{4+}$ Fr.
↓ evaporated up
↓ 1 ml 1 M HCl
↓ chelate reagents
↓ 0.1 M NaOH
^{45}Ti-compounds/pH4-6

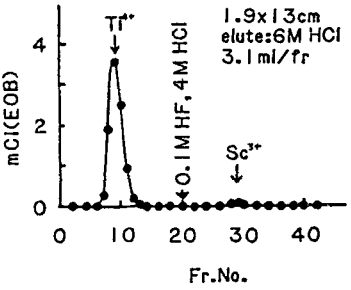

Fig. 1. Preparation of the ^{45}Ti-labeled compounds.

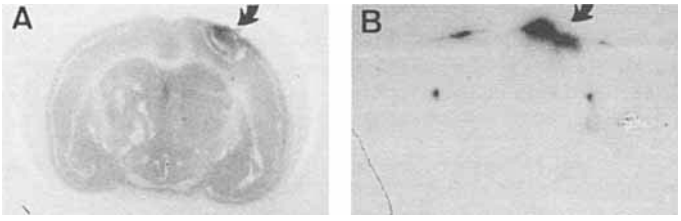

Fig. 2. Autoradiogram of the brain in rat injected with ^{45}Ti-DTPA. The section of the brain is shown in the photograph (A) and the autoradiogram (B). The arrow indicates the damaged position with liquid nitrogen.

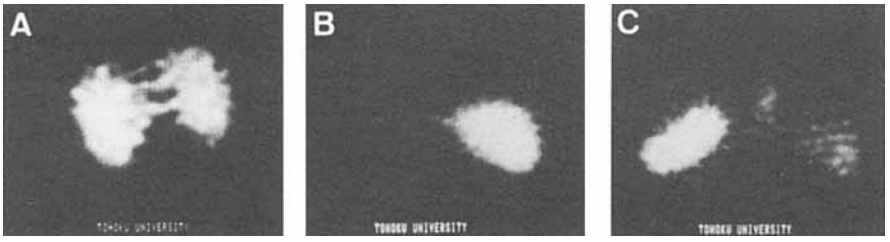

Fig. 3. Positron tomographic images of ^{45}Ti-phytate in rabbits. A, B and C show the trance axial sections of the lung, the liver and the spleen, respectively.

A NEW METHOD FOR STANNOUS TIN LEVEL DETERMINATION IN KIT REAGENTS FOR Tc-99m RADIOPHARMACEUTICALS USING Sn-Re REDOX COUPLING

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Accurate determination of stannous tin level is one of the important items in the quality control of kit reagents for Tc-99m radiopharmaceuticals, especially at the production sites of the kit reagents. Although some methods have been reported for this purpose (1-5), none of them provides satisfactory accuracy under the coexistence of ascorbic acid (a stabilizer), strong chelating agents (e.g. DTPA) and/or undissolved particles (e.g. MAA). The need for an accurate and universal method for stannous tin level determination, therefore, has been strongly pointed out.

Currently, a new method was developed in our laboratory using Sn-Re redox coupling; the stannous species [Sn(II)] to be tested was reacted with an excess amount of potassium perrhenate [$KReO_4$, Re(VII)] in 2-3 N HCl solution which contains excess amount of ascorbic acid and sodium thiocyanate [NaSCN]. Under the condition, a redox reaction "Re(VII)$\rightarrow$Re(IV)/Sn(II)$\rightarrow$Sn(IV)" takes place, and thus formed Re(IV) is immediately co-ordinated with thiocyanate ion to give Re(IV)-SCN complex. The Re(IV)-SCN complex was then extracted into diisopropyl ether (DIPE) and the concentration was determined spectrophotometrically at the absorption maximum (363 nm) against the blank DIPE solution as the reference.

Above principle is illustrated in FIG. 1, and the established standard procedure (TABLE 1) provides the conditions for the quantitative progress of the reaction: the absorbance value for the Re(IV)-SCN complex was found to be proportional to the amount of added Sn(II) over a wide range of stannous level (FIG. 2). When stannous species was oxidized by air bubbling or hydrogen peroxide, the test gave negative results indicating that the method is specific for the di-valent tin [Sn(II)] as illustrated in FIG. 1.

This new method for stannous tin level determination bears several advantages:

1) Ascorbic acid in the sample stannous solution showed no inhibitory effect since a large excess of ascorbic acid is added in advance into the Re(VII) solution to ensure the quantitative reaction of Sn(II) with Re(VII).

2) Various chelating agents currently used in Tc-99m radiopharmaceuticals (e.g. DTPA, PYP, HEDP, HMDP, DMSA, HIDA, EHIDA, PG, PI) showed no masking effect since the reaction proceeds in 2-3 N HCl solution.

3) Even MAA (macro-aggregated human serum albumin) showed no inhibitory effect since the highly acidic medium completely strips the stannous ion from the aggregated particle to react with Re(VII).

4) The pH deviation in the sample solution showed no effect since minimum volume (0.1-1.5 ml) of the sample is directly added into about 50 ml of 2-3 N HCl solution.

5) The procedure is simple enough. Strict volumetric accuracy is required only for the step of the addition of stannous sample and that of DIPE. The total procedure requires about 25 min including the photometric measurement.

6) The results were highly reproducible; only two parallel handlings (one for sample, the other for blank) are needed, and the Sn(II) level in the sample is easily obtained using the pre-determined calibration line or equation (FIG. 2).

7) The DIPE extraction step enables us to apply this method for the sample containing substances (e.g. pyridoxal) whose absorption spectrum is overlapped

with that of Re(IV)-SCN complex. Furthermore, Re(IV)-SCN complex is more stable in DIPE than in aqueous solution (6).

The results obtained by applying this method for various aqueous or freeze-dried kit reagents will be also presented.

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TABLE 1. Procedure for determining stannous tin concentration.

1. Charge 10 ml of hydrochloric acid (12 N) in a 100 ml extraction flask.
2. Add 35 ml of water.
3. Add 3 ml of potassium perrhenate ($KReO_4$, Puratronic grade, Johnson Matthey Chemical Ltd., England) aqueous solution (160 mg $KReO_4$/100 ml water).
4. Add 1 ml of 20 wv% aqueous ascorbic acid solution.
5. Add 1 ml of 20 wv% aqueous sodium thiocyanate (NaSCN) solution.
6. Add stannous sample (0.1-1.5 ml) which contains 0-1.5 μmol (0-180 μg) of Sn(II), and mix well.
7. Stand at room temperature for 15 min.
8. Add 20 ml of diisopropyl ether (DIPE)* and shake vigorously for 2 min to extract Re(IV)-SCN complex.
9. Discard the aqueous phase.
10. Take a portion of the DIPE phase into a quartz cell of 1 cm optical path.
11. Measure absorbance at 363 nm against the blank DIPE solution (same volume of water is added in step 6. as the replacement for the stannous sample) as the reference.
12. The unknown stannous concentration in the sample solution is determined using a calibration line or equation (FIG. 2).

* DIPE was pre-treated with 2 wv% ascorbic acid-2NHCl solution.

FIG. 1 Principle of the method.

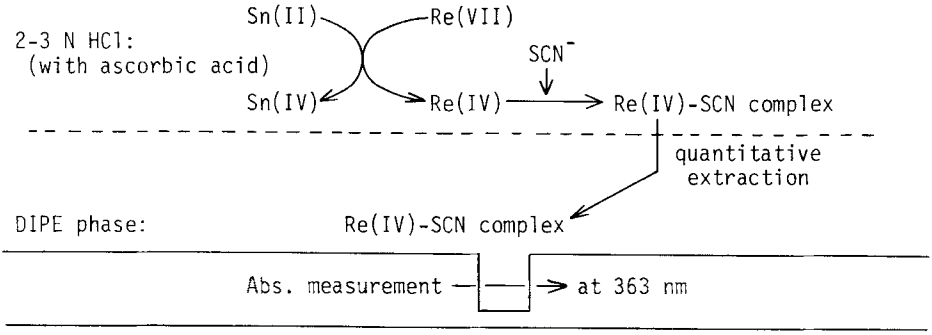
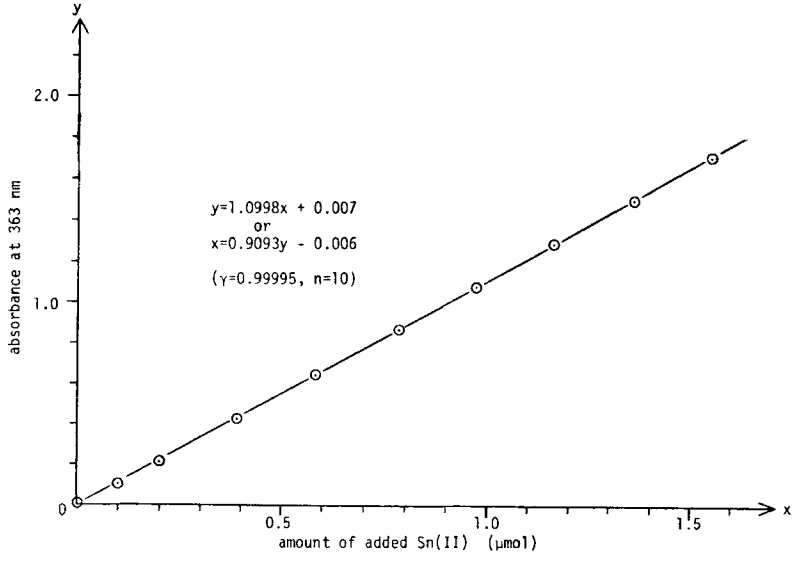


FIG. 2 Standard calibration line associated with the Sn-Re redox method.



EFFECTS OF RADIOCHEMICAL IMPURITY ON "IN VIVO" KINETICS OF ^{99m}Tc - PHOSPHATE AND ^{99m}Tc PHOSPHONATE AGENTS

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This study was undertaken in order to show that besides the chemical structure of phosphate and phosphonate compounds also the radiochemical impurities present have considerable influence on "in vivo" kinetics.

In the experimental work the ^{99m}Tc- pyrophosphate (^{99m}Tc-PyP) was used as the representative of a group of compounds bonded by P-O-P and ^{99m}Tc-methylendiphosphonate (^{99m}Tc-MDP) by organic P-C-P bond (1).

For radiopharmaceuticals studied the partition coefficient (PC) chloroform/water at various pH values was determined. The PC results obtained for pH ranging from 6.1 to 6.3 are 0.19 and 0.01 for ^{99m}Tc-PyP and ^{99m}Tc-MDP, respectively.

For the separation of radiochemical impurities the Sephadex chromatography was used. The pure fraction of radiopharmaceuticals was eluted with $Na_4P_2O_7$ solution in maximum 4 ml, $^{99m}TcO_4^-$ in 20 ml, while ^{99m}Tc-hydrolyzate remains bonded to the column and it elutes after ^{99m}Tc oxidation with 0.15% H_2O_2 in 0, N HCl.

In addition, the pure fractions of ^{99m}Tc-PyP and ^{99m}Tc-MDP and the commercial products were studied comparatively. Also, the degree of diffusion in erythrocytes and their bonding to plasma protein were determined. The ^{99m}Tc-PyP both, purified and commercial, have the higher percent of bonding, 9.5 and 5.8 in comparison with ^{99m}Tc-MDP with the percents of 5.1 and 3.5. These results show the higher liophility of ^{99m}Tc-PyP, what is also confirmed by PC (2).

The method applied has a considerable influence on the results of binding blood plasma proteins. The better reproducibility of results is gained by precipitation method (3-chloroacetic acid and $(NH_4)_2 SO_4$ for commercial agents and much lower values for the purified fractions. The dialysis method gives lower values, 16 to 18 percents of protein binding.

The results of biodistribution obtain on white rats are presented in the Table. The purified fractions obtained from commercial preparations give lower uptake in liver, the reason being the derived ^{99m}Tc-hydrolizate on the Sephadex column. The radioactivity in kidneys was decreased. However, unexpectedly, we obtained lower values with the purified agent deposited in femurs compared to the commercial ones. The values bone/liver are increased while the values bone/muscle for the injected purified fraction were decreased. A biological half time excretion from blood with commercial agent ^{99m}Tc-PyP was $T_{a/2}$ 30 min, $T_{b/2}$ 90 min, and the blood clearence 0.39 ml/min. The commercial ^{99m}Tc-MDP agent values were $T_{a/2}$ 20 min, $T_{b/2}$ 89 min and the blood clearence 0.41 ml/min.

The radiochemical purity was determined at TLC on silicagel. We used the polar solvent 10% $MgSO_4$ by aim of which the hydrophile radiopharmaceuticals migrate and nonpolar molecule ^{99m}Tc-hydrolizate remains at the start line.

TABLE

Biodistribution of commercial and purified fractions of ^{99m}Tc-PyP and ^{99m}Tc-MDP, two hours after i/v application

Organs	Fixation %/g			
	^{99m}Tc-PyP		^{99m}Tc-MDP	
	Commercial	Purified	Commercial	Purified
Thiroidea	0.8	0.5	0.6	0.4
Liver	3.3	0.2	0.3	0.1
Kidneys	1.0	9.5	0.5	2.0
Femur	4.6	2.0	6.8	5.1
Muscle	0.08	0.07	0.03	0.03
Intestine	1.2	6.0	0.5	1.4
Bone/liver	1.4	10.0	22.7	51.0
Bone/muscle	57.5	28.5	226.6	170.0

Animals were injected the same quantity of agent in order to avoid the carrier effect.

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TC-COMPLEXES WITH AMINOPOLYCARBOXYLIC AND HYDROXYCARBOXYLIC ACIDS AND THEIR KIDNEY UPTAKE

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Both by reducing pertechnetate with 3 val Sn(II) in aqueous aminopolycarboxylate solutions (diethylenetriaminepentaacetate (DTPA), ethylenediaminetetraacetate (EDTA), hydroxyethylethylenediaminetriacetate (HEDTA), nitrilotriacetate (NTA), iminodiacetate (IDA) and N(2.6-diethylacetanilide)- iminodiacetate (EHIDA)) and by ligand exchange reaction between K_2TcBr_6 and the complexonates the same Tc(IV)-complexes were formed.

The analytical properties of the dissolved products depended on the nature of the ligands and their concentration, the pH-value, the Tc-concentration and the age of the solution. The reaction course of the products of Tc(IV) with aminopolycarboxylic acids (tridentate and tetradentate ligands) and polyaminopolycarboxylic acids (with two or three nitrogen atoms) followed different pathways as demonstrated in the reaction scheme for DTPA and NTA as representatives of both groups of ligands.

The yellow Tc-DTPA-complex ($H_3\left[TcOC_{14}H_{18}N_3O_{10}\right] \cdot 3\ H_2O$) with a Tc/ligand ratio of 1:1 is a cation at pH-value < 1.8 and behaves as a tribasic acid. (The yellow Tc-EDTA complex ($H_2\left[TcOC_{10}H_{12}N_2O_8\right] \cdot 3\ H_2O$) does not form a cation, the maximum anion charge is -2.)

The temperature independent magnetic moment of 2.4 B.M. and the absence of Tc-O-Tc-bridging vibration indicates a monomeric structure. The oligomeric brown Tc-DTPA-complex is obtained by a condensation reaction in aqueous solution from the yellow Tc-DTPA-complex. The magnetic moment is 1.2 B.M. The band in ir-spectrum at 810 cm^{-1} is assigned to the Tc-O-Tc bridging vibration.

The red Tc-NTA-complex ($K_2\left[Tc(OH)_2C_6H_6NO_6\right]_2$) is a dimeric compound with a Tc/ligand ratio of 1:1 (1). Both Tc-atoms are connected via hydroxy bridges. The vibrations of the $Tc\langle^{O}_{O}\rangle Tc$-bridging system (715 cm^{-1}, 555 cm^{-1} were determined by preparation of the complex from $H_2{}^{18}O$.

In order to study relationships between the chemical structure of technetium chelates and their biodistribution the chemical state of the species of the $^{99}TcO_4^-$ reduction by Sn(II) in solutions of citric, malic, tartaric, gluconic, and -hydroxyisobutanoic acid has been investigated using polarographic, electrophoretic, spectrophotometric methods, and diffusion measurements.

A rapid reduction of $^{99}TcO_4^-$ into the corresponding anionic Tc(V)-bis hydroxycarboxylate complexes was observed. This species were subject to reversible reduction to the 1:2 Tc(IV)- and Tc(III) complexes at the dropping mercury electrode. In these processes deprotonation took place (2).

Table 1

Ox.state	citric	tartaric	malic
V	$TcO\ cit_2^{-3}$	$TcOOH\ tart_2^{-2}$	$TcOOH\ mal_2^{-2}$
IV	$TcOH\ cit_2^{-3}$	$Tc(OH)_2 tart_2^{-2}$	$Tc(OH)_2 mal_2^{-2}$

Ox.state	gluconic	-hydroxiisobutanoic	
V	$TcO\ glu_2^{-1}$	$TcO_2\ hyd_2^{-1}$	
IV	$TcOH\ glu_2^{-1}$	$TcOOH\ hyd_2^{-1}$	

The compounds having the same ligands but different oxidation states are very similar with regard of size, ionic charge and ligand environment but proved to be very different in their biodistribution (table 2). On the other hand the complexes of the same oxidation state but different ligand are very different with regard to those parameters but similar in their biodistribution pattern.

Table 2

	kidney uptake %/g	
Tc-chelate	Tc V chelate	Tc IV chelate
citric	8,6	2,9
tartaric	13,2	1,7
malic	13,3	2,7
-hydroxi-isobutanoic	8,3	1,5

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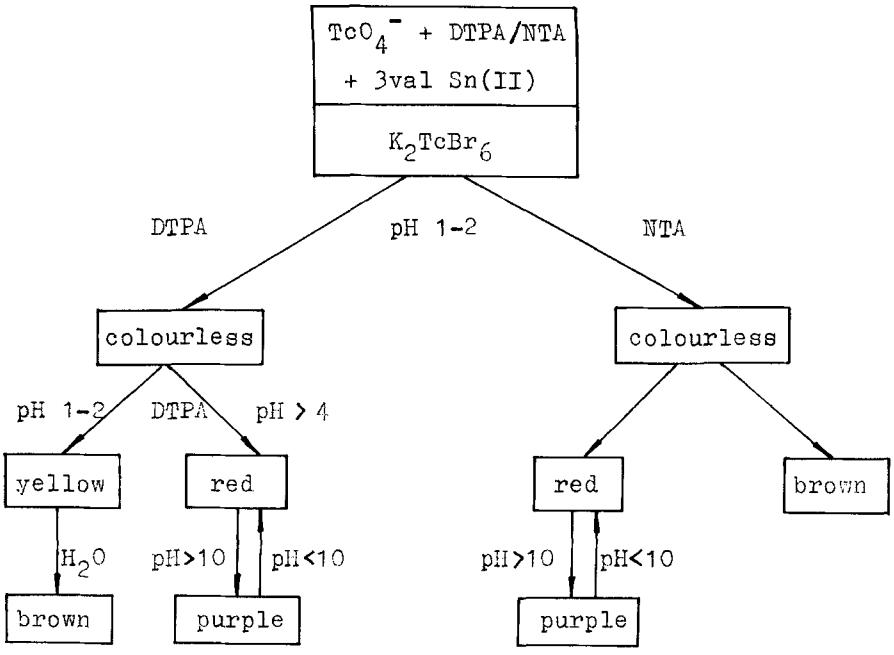
TcO_4^- + DTPA/NTA
+ 3val Sn(II)
K_2TcBr_6
DTPA
pH 1-2
NTA
colourless
colourless
pH 1-2
DTPA
pH > 4
yellow
red
red
brown
H_2O
pH>10
pH<10
pH>10
pH<10
brown
purple
purple

[^{153}Sm]SAMARIUM COMPLEXES AS RADIOPHARMACEUTICALS

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Samarium is a lanthanide rare earth with low toxicity (1,2). Of the stable nuclides of samarium, ^{152}Sm, with a natural abundance of 27% and a thermal neutron capture cross section of 208 barns (3), is a suitable target nuclide for the production of ^{153}Sm. ^{153}Sm, with its 46.8 hour half-life and high abundance (28%) of 103 KeV gamma radiation (3), is the most appropriate samarium radionuclide for use in nuclear medicine. ^{153}Sm chelates and salts have been used as tumor imaging agents (4,5). Recent studies have shown ^{153}Sm citrate to be tumor avid in a rabbit tumor model (6). The synthesis, *in vitro* screening and *in vivo* testing of several ^{153}Sm complexes are now reported.

^{153}Sm was obtained by irradiation of $^{152}Sm_2O_3$ (98.2% enriched ^{152}Sm) at 10^{12}n.cm^{-2}.sec^{-1} using the University of Alberta SLOWPOKE nuclear reactor. The irradiated product was dissolved in N HCl prior to conversion into other chemical forms. Seventeen complexes were prepared for testing, including ^{153}Sm citrate and ^{153}Sm transferrin. Initial screening involved chromatographic (ascending paper) and high-speed centrifugal analysis of ^{153}Sm complexes to eliminate forms exhibiting characteristics of colloidal particles. Promising compounds were then tested for uptake by human melanoma 2AB or EMT-6 cells in suspension. The Dunning 3327 prostatic tumor (Copenhagen X Fisher rats) and the Lewis Lung Tumor (BDF_1 mice) models were used for *in vivo* testing. ^{67}Ga citrate and ^{67}Ga transferrin were used as reference radiopharmaceuticals.

All formulations tested were found to contain components which exhibited properties expected of colloidal materials. Only the citrate and transferrin complexes were suitable for biological studies but they developed colloidal properties when they came in contact with growth medium or physiological buffers, either almost immediately in cell uptake studies, more slowly *in vivo*. Final whole-body distributions could be rationalized on the basis of *in situ* colloid formation.

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Percent apparent colloidal content of ^{153}Sm citrate and ^{153}Sm transferrin under various test conditions

Samarium COMPLEX	Percent apparent colloid						
	centrifugation/chromatography			cell culture		liver, in vivo	
	pH 6.5	RPMI medium	MEM medium	15 min	60 min	1 hr	24 hr
citrate	4.2	98.3	0.9	78.7	90.8	19.1	87.4
transferrin	3.7	87.6	0.6	84.8	87.8	25.7	79.9

A SIMPLE APPROACH FOR SINGLE AND DOUBLE COLUMN FABRICATION FOR RADIONUCLIDE GENERATORS

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When a reactor-, cyclotron-, and a natural- product nuclide of long half life decays to a daughter with ideal nuclear characteristics for medical applications, this system is called a medical radionuclide generator. There are single column systems: $^{99}Mo \rightarrow {}^{99m}Tc$ (1), $^{113}Sn \rightarrow {}^{113m}In$ (2), $^{81}Rb \rightarrow {}^{81m}Kr$ (3),.... and double column systems: $^{191}Os \rightarrow {}^{191m}Ir$ (4), $^{82}Sr \rightarrow {}^{82}Rb$ (5,6). The purpose of this communication is to devise a simple, inexpensive and versatile single and double column systems for medical radionuclide generator systems by making use of commercially available, sterile and disposable 1cc Burron syringes (Burron Products, Bethlehem, PA.) and porous polypropylene discs.

Single column: As described earlier(3), the fabrication of column consists of pushing porous polypropylene discs into 1cc syringe with the help of plunger and then loading with pretreated resin or adsorbent (Al_2O_3 for $^{99}Mo \rightarrow {}^{99m}Tc$; SiO_2 or Al_2O_3 or ZrO_2 for $^{113}Sn \rightarrow {}^{113m}In$; Dowex-50 for $^{81}Rb \rightarrow {}^{81m}Kr$) to a required volume and repeatedly washing with the desired eluant. Luer Lock fittings at both ends will aid in quick and reliable connection, as shown in figures 1 & 2.

Dual columns: Whenever there is a need for dual column elution in sequence such as Dowex-1, Dowex-2 for $^{191}Os \rightarrow {}^{191m}Ir$(4) to be eluted with 0.9% saline at 1 pH; Al_2O_3, Chelex 70 for $^{82}Sr \rightarrow {}^{82}Rb$ to elute with 2% saline(5), two columns can be independently loaded with respective column supporting materials and the two columns can be attached to each other instantly with a twist. This feature is convenient especially if the second column is being replaced several times. Usually, the first column is for retaining the parent nuclide very effectively and the second column can be used as a secondary purification step and sometimes works as a trap for the breakthrough parent. Typical system is presented in figure 3.

Thus we have successfully fabricated sterile, simple and inexpensive single and double column systems using Burron syringes and porous (1.5 micron) polypropylene discs and tested satisfactorily: $^{81}Rb \rightarrow {}^{81m}Kr$, eluted with 5% dextrose solution for ventilation studies; $^{191}Os \rightarrow {}^{191m}Ir$, eluted with 0.9% saline at 1 pH for first pass cardiac studies in dogs.

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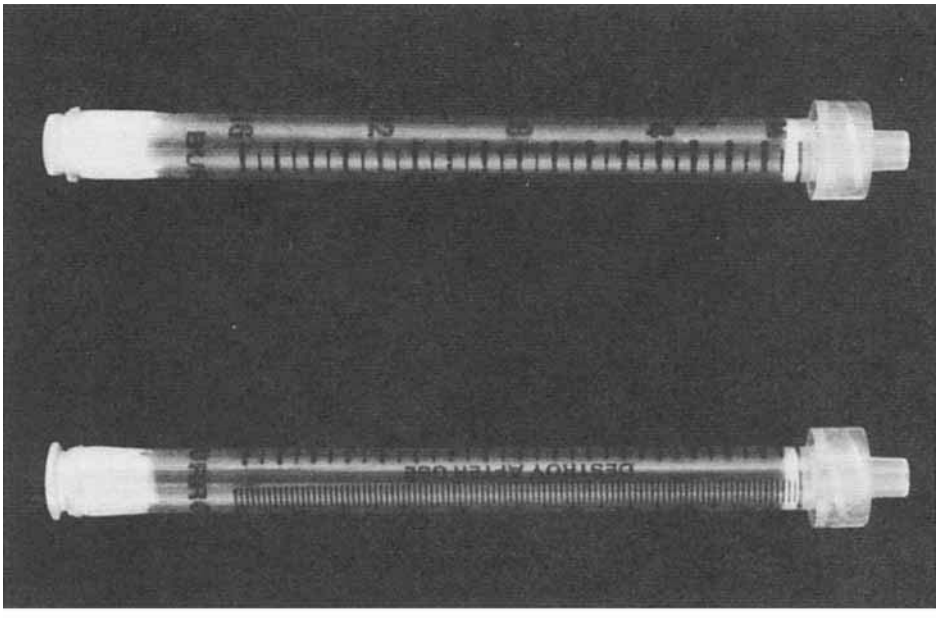

Fig. 1: Burron Syringes fitted with female Luer Locks on top

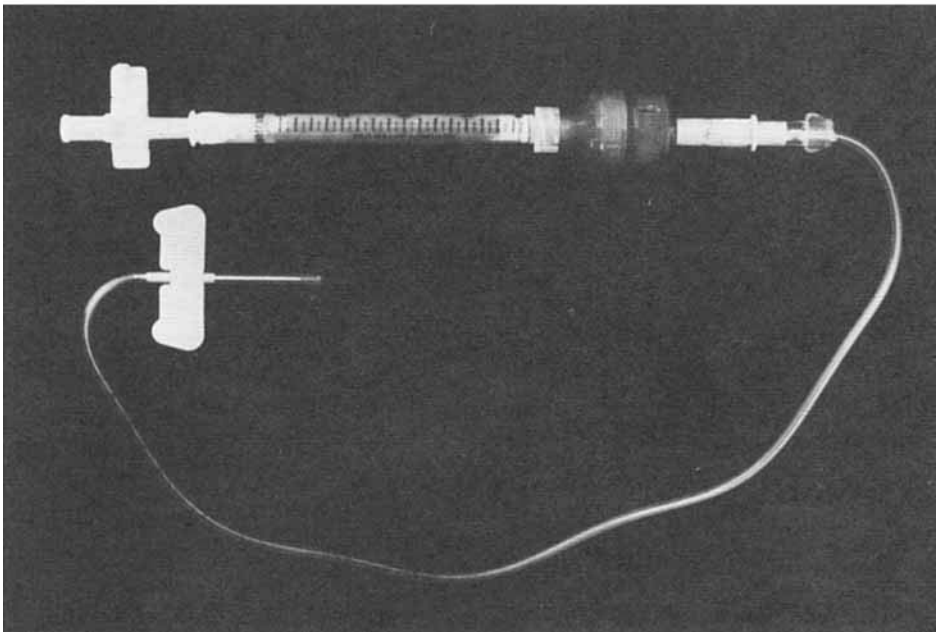

Fig. 2: Single Column System

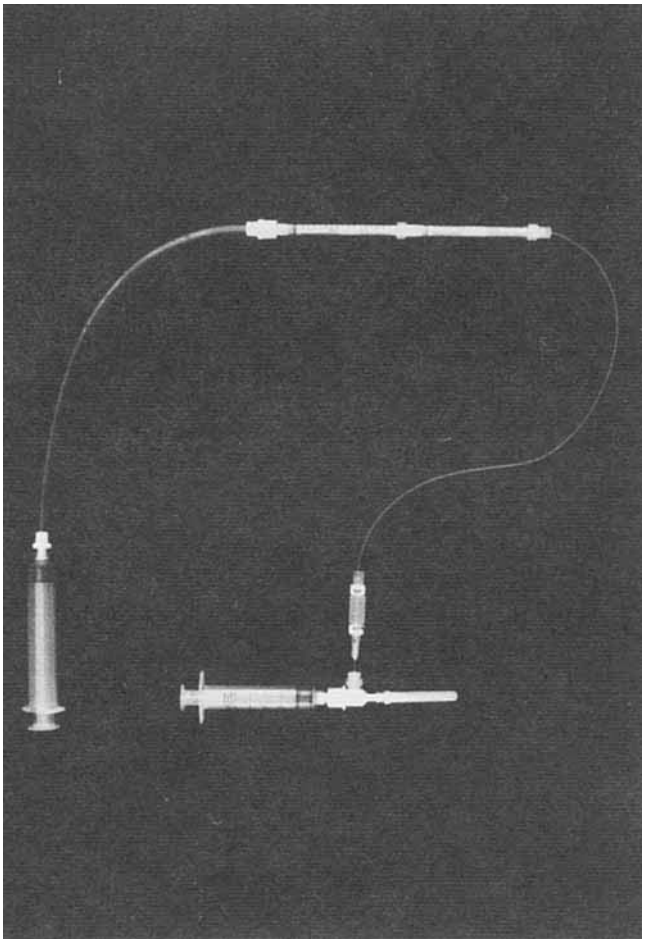

Fig. 3: Double Column System

CHROMATOGRAPHY OF THE BONE IMAGING AGENT TECHNETIUM PYROPHOSPHATE

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Ion-exchange chromatography indicates two principle fractions in commercial tin labeled technetium pyrophosphate preparations (1). To exclude the possibility of a chromatographic artifact, the separation was performed in two dimensions on DEAE cellulose TLC plates. Two discrete spots colinear with the origin were found. There was slight tailing in the direction of second development, extending backward from the leading spot and forward from the trailing spot, suggesting interconversion between the two species at a rate slow compared with the duration of chromatography. Since the sample decomposed on drying, the separation had to be carried out on wet plates in a sandwich chamber. The eluent was 0.1 M $Na_4P_2O_7$ adjusted to pH 7.0 with acetic acid.

Separation by HPLC is shown in Figure 1. Two principal components were found in both administered dose and patients' urine; the urine gave a third peak corresponding to pertechnetate.

Technetium pyrophosphate complexes can be separated by ion-pairing as well as by ion-exchange. Figure 2 shows an example, using conditions similar to those Srivastava and others used to separate Tc-MDP (2). Technetium pyrophosphate preparations from three commercial suppliers gave distinct chromatographic patterns which were consistent for two different lots from each supplier. Despite multiple fractions in the dose, only a single fraction (besides pertechnetate) was found in the urine by this analytical method. This corresponded to the single fraction seen in preparation A of Figure 2.

To resolve the apparent discrepancy between ion-pairing and ion-exchange, fractions were separated by ion-exchange and then applied to the ion-pairing system. The two fractions gave identical results in the ion-pairing system: a single peak, corresponding to that found in preparation A and in patient urine. The single ion-pairing peak represented two species with similar retention time, as confirmed by modifying the eluent composition to achieve partial separation. The additional fractions found by ion-pairing in some technetium pyrophosphate preparations vanish when the sample is passed either through an ion-exchange column or through the patient and therefore appear to represent unstable species. They could also be eliminated by using tin-free labeling methods (substitution and controlled potential electrolysis) or by increasing the pyrophosphate concentration when tin-labelling was used. These findings suggest that the extra fractions are unstable aggregates between tin complexes and technetium complexes.

Preliminary data for methylene diphosphonate complexes similarly showed the administered dose to be a more complex mixture of technetium compounds than found in the urine, although for Tc-MDP the extra fractions in the dose could be identified by ion-exchange as well as by ion-pairing (Fig. 3).

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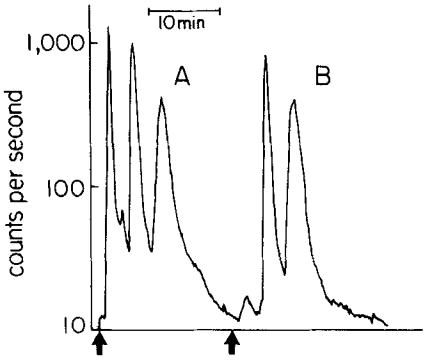

FIGURE 1. Chromatogram of patient urine (A) and administered dose (B) of Tc-pyrophosphate. Column: Partisil 10 SAX, 10 cm. Eluent: 0.1 M $Na_4P_2O_7$, adjusted to pH 7.0 with acetic acid.

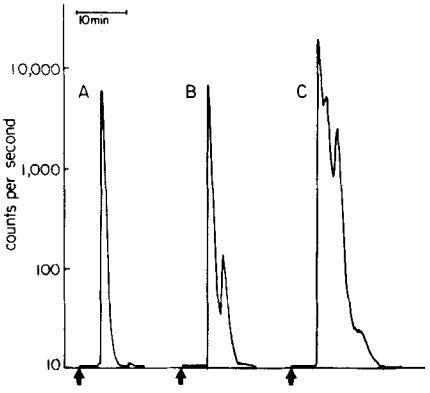

FIGURE 2. Chromatograms of Tc-pyrophosphate from three commercial suppliers. Column: Brownlee C-18, 10 cm. Eluent: 2.5 mM sodium pyrophosphate, 10 mM sodium acetate, 2.5 mM tetrabutylammonium hydroxide, 5% dioxane, 1% n-butanol, adjusted to pH 7.1 with acetic acid.

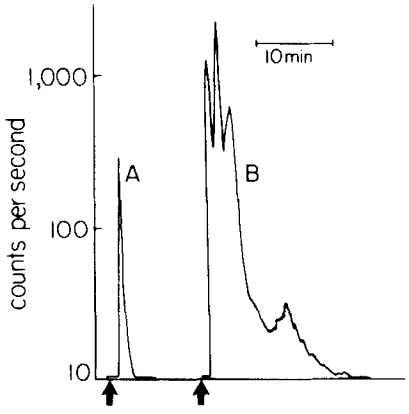

FIGURE 3. Chromatograms of patient urine (A) and administered dose (B) for Tc-MDP. Column: Nucleosil SB, 10 cm. Eluent: 0.1 M $Na_4P_2O_7$, adjusted to pH 7.0 with acetic acid.

CHANGE IN THE CHEMICAL PROPERTIES AND CONSEQUENT BIOLOGICAL BEHAVIOUR OF GALLIUM-67 CITRATE WITH ITS STORAGE TIME

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We have already shown that the chemical formulation of gallium-67 citrate, from different suppliers, used for tumour or inflammation imaging, is unfortunately not the same (1) and that the scintigraphic image quality depends highly on the chemical nature of the radionuclide in the radiopharmaceutical solutions (1-3). Chromatographic and electrophoretic quality control of various gallium-67 citrate preparations from different suppliers showed them to contain $^{67}Ga(OH)_3$, $[^{67}Ga(H_2O)_6]^{3+}$ and citratogallate-67 anion. The amount of these gallium-67 species, which coexist in solution, depends on the concentration of the sodium citrate used in the formulation of the radiopharmaceutical. Our studies further showed (4) that $^{67}Ga(OH)_3$ and citratogallate-67 concentrate in healthy organs, while $[^{67}Ga(H_2O)_6]^{3+}$, which combines easily with transferrin, is transported to the tumour site. Since the amount of sodium citrate in each sample of the radiopharmaceutical is difficult to determine, we developed simple chromatographic quality control method for examining the suitability of gallium-67 citrate solutions for tumour imaging. Gallium-67 citrate preparations containing 43% citratogallate-67 show optimal radionuclide uptake in the tumour and neglegible concentration in healthy organs, like liver, spleen, kidney or bowel.

The radiopharmaceutical suppliers recommend the use of gallium-67 citrate up to two weeks following the calibration date (5). Since the amount of gallium-67 decreases with time due to its decay while that of sodium citrate remains unchanged, we thought it interesting to study how the chemical and biological properties of gallium-67 citrate radiopharmaceuticals vary with time. The nature of gallium-67 species in the solutions was examined chromatographically and electrophoretically at different intervals of time following its receipt. The variation in the biological behaviour of the radionuclide was studied by injecting the radiopharmaceutical at different times of decay of gallium-67 in Morris hepatoma-3924A-bearing rats. The radionuclide distribution was examined scintigraphically, and also by organ and tumour sample radioactivity counting. The behaviour of the optimal gallium-67 formulation was also studied in humans with lung tumour and Hodgkin's disease. The results obtained can be summarized as follows:

1) The gallium-67 citrate preparations for tumour imaging are unfortunately hardly the same.
2) Gallium-67 citrate preparations contain $^{67}Ga(OH)_3$, $[^{67}Ga(H_2O)_6]^{3+}$ and citratogallate-67 ion, which coexist. The relative amount of these gallium-67 species depends on the concentration of sodium citrate in the radiopharmaceutical formulation.
3) $^{67}Ga(OH)_3$ and citratogallate-67 ion concentrate in healthy organs, like liver, spleen, kidney and bowel. $[^{67}Ga(H_2O)_6]^{3+}$ only is taken up by tumours. Gallium-67citrate preparation, having 43% citratogallate-67 at the time of injection, gives high quality scintigrams with neglegible background. With other gallium-67 formulations high uptake of the radionuclide in healthy organs takes place.
4) Since the gallium-67-to-citrate ion ratio decreases with time, due to the decay of gallium-67, the radiopharmaceutical solution shows the formation of increasing amount of citratogallate-67 anion after the calibration date. Due to decrease in the amount of $[^{67}Ga(H_2O)_6]^{3+}$ the uptake of gallium-67 in the tumour is reduced with storage time of the radiopharmaceutical and that in healthy organs is correspondingly increased.

5) In order to obtain optimal and reproducible results with gallium-67 citrate preparations, the concentration of citratogallate-67 should be brought to the value 43% at the time of injection.

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PREPARATION OF CHROMATOGRAPHICALLY PURE TECHNETIUM-99M(IV) FOR THE SYNTHESIS OF ITS RADIOPHARMACEUTICALS

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Technetium-99m is today the most widely used radionuclide for the synthesis of radiopharmaceuticals (1). Sodium pertechnetate-99m is routinely injected for thyroid and brain scintigraphy while its other salts or complexes, in the reduced oxidation state of the element, are employed for the visualization of other organs (2). Technetium-99m has been shown (3) to exist in the oxidation states ranging from 1- to 7+. Although the importance of lower oxidation states of technetium-99m was emphasized as early as 1965 by Harper and coworkers (4), the aqueous chemistry of the radionuclide in these oxidation states is poorly known(2). The usual reducing agent, stannous chloride, commonly used for the preparation of technetium-99m radiotracers has severals undesirable properties: 1) it is a strong reducing agent, 2) it forms metal-metal bond with the element it reduces (5), 3) with the radiopharmaceutical injected tin produces complications for later imaging studies (6) due to its long biological half-time (7). For this reason many attempts have been made to prepare technetium-99m radiotracers free from stannous ions. Deutsch and collaborators (8) have used $NaBH_4$ as the reducing agent. We have tried to reduce the pertechnetate-99m ion by concentrated hydrochloric acid (3,9). Unfortunately the reduction process was very slow. We have extended these studies now to reduce the pertechnetate-99m ion by hydrobromic acid which is a stronger reducing agent.

The reduction was followed by chromatographic and electrophoretic analysis of the reaction mixture.

Unlike the reduction of the pertechnetate-99m by concentrated hydrochloric acid, hydrobromic acid reaction with the pertechnetate-99m ion was found to be temperature dependent. Different technetium-99m(IV) species in the solution were found depending on whether the reduction is carried out at room temperature (20 C) or at 70 C. At the higher temperature polymerized technetiun-99m(IV) was otained. At room temperature hexabromotechnetate-99m(IV) is obtained. The reduction with hydrobromic acid is much faster than that with the hydrochloric acid.

We are studying the reaction of the technetium-99m(IV) with ligands which should give pancreas specific radiotracers. The biodistribution of these radiopharmaceuticals will be described.

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EXTRACTION OF Tc(V) FROM RADIOPHARMACEUTICALS

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The determination by solvent extraction of the oxidation state of $SnCl_2$-reduced pertechnetate in several radiopharmaceutical preparations was carried out at the 10^{-9}M concentration level. This required the formation, by displacement, of a complex of technetium in a known oxidation state and with a high degree of extractability. The work reported here is based on the finding by Rajec and Mikulaj that Tc(V) forms a stable chloroform-soluble complex with 8-hydroxyquinoline (oxine) and that Tc(IV) and Tc(VII) do not (1).

In all cases the concentration of NH_4TcO_4 was $2x10^{-9}$M. $^{99m}TcO_4^-$ was added as a tracer. The ligand solutions included 1% gluconolactone at pH 12,1% citric acid at pH 7,0.2% disodium pyrophosphate at pH 7, 0.013% sodium hydroxyethylidenediphosphonate (HEDP) at pH 4.8, and 0.05% dimercaptosuccinic acid (DMSA) at pH 4. The DMSA solution contained 500ug of $SnCl_2 \cdot 2H_2O$ in 2 ml. The pyrophosphate solution contained 1 mg of stannous chloride, and the other three had 50 ug of the tin compound in 2 ml. After 30 minutes incubation at room temperature, 0.5 ml of each preparation was added to 2.5 ml of saturated Na_2SO_4 solution containing 200 mg of boric acid, followed by 3 ml of 0.4 M oxine in chloroform. The mixtures were shaken in closed vials on a wrist-action shaker for up to 1 hour. All operations up to the closing of the vials were counted in a gamma spectrometer. Some of the results are summarized in Table 1.

Table 1

Oxine Extraction of Tc Complexes Prepared by $SnCl_2$ Reduction

Preparation	Time of Extraction,hrs.	% Tc Extracted
gluconate	1/4	90
citrate	1	96
pyrophosphate	1	4
HEDP	1	1
DMSA	1	1

The high degree of extraction from the gluconate and citrate preparations strongly suggests that the technetium at this low level of concentration is in the +5 oxidation state and that the complexes are readily displaced by oxine. This oxidation state is consistent with that reported for the same complexes but at higher, i.e., 10^{-4}M, technetium concentrations (2,3). In order to show that Tc (IV) complexes (if they exsist) are not extracted by oxine, dilute NH_4TcO_4 was reduced to the $TcCl_6^{2-}$ anion in hot, concentrated HCl (4), neutralized, reacted with the oxine in chloroform.

The citrate solution showed only 4% extraction, and the gluconate showed 25% extraction. The latter result was attributed to an exceptionally facile oxidation by traces of 02 of Tc (IV) to Tc (V) in the presence of gluconate.

Very little technetium was extracted from the pyrophosphate, HEDP, and DMSA preparations. These complexes may contain technetium in lower oxidation states. Alternatively, they may be Tc (V) complexes that are either more stable than the oxinate or are very slow to exchange. Experiments will be presented to distinguish among these possibilities. Some problems associated with displacement and demasking reactions will be discussed. In this connection, it has been reported that calcium gluconate displaces pyrophosphate and HEDP from the corresponding ^{99m}Tc complexes (5). These results were only partly confirmed in the present work.

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PRODUCTION OF TECHNETIUM LABELLED IN VIVO KITS

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In the recent years a steadily increasing interest could be observed also in Hungary concerning the medical application of various in vivo diagnostic kits for labelling with $^{99}Tc^{m}$. So we started some years ago with the development of this sort of clinical diagnostics.

As a results of the last developments we produce at present six kits for various purposes e.g. for imaging of the liver, for kindney and bone scinfigraphy, for labelling of red blood cells etc.

The yearly turnover figures are as follows:

sodium phytate	- about	1000 kits
HEDSPA	- "	800 "
pyrophosphate	- "	400 "
sodium citrate	- "	800 "
sodium gluconate	- "	500 "
HIDA	- "	500 "

Based on the experiences obtained, in this paper we should like to touch upon the problems of production, like

- organisation of the production /synthesis of the raw material, etc./
- effect of radiation on the materials /in the course of sterilization with ionizing radiation/
- quality control /including the comparison of efficiency of various method used/.

UTILISATION POSSIBILITIES OF LOW POWER REACTORS FOR MEDICALLY APPLICABLE TECHNETIUM-99m PREPARATIONS

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Technetium-99m is now the dominating radionuclide in nuclear medicine; chromatographic generators belong to the most convenient ways of gaining it. For producing them in good quality ^{99}Mo of high specific activity (over 37 GBq/g) is needed. This requires either high neutron flux (over 10^{14} n/cm^2 sec) in the case of (n,γ) reaction, or fission produced ^{99}Mo. Both the ways are applicable only at large centers. At smaller centers other Mo-Tc separation methods, such as sublimation or extraction, are to be used. Individual separation methods have been exceedingly described, reviewed and even critically compared (cf. e.g. (1)).

In most medium sized and small countries imported chromatographic generators have been used. However, in a number of those countries import limitations and/or transport restrictions may oppose the broader local utilisation of nuclear medicine methods. The task of exploitation of local reactors becomes immensely important even if the neutron flux is of the order of 10^{13} n/cm^2 sec or sometimes even less. Possibilities of utilising such reactors for producing ^{99m}Tc medically useful preparations have been the subject of efforts in our Institute and they were extensively discussed during an IAEA Consultants' meeting in 1981 (2).

As main approach to the problem, the solvent extraction of ^{99m}Tc by methyl-ethyl-ketone (MEK) from aqueous potassium molybdate ^{99}Mo of low specific activity has been chosen. In estimating the lower limits of the applicable neutron flux in the reactor for various irradiation and cooling times the following assumptions were used. The generator produced should allow to gain even at the end of the 5 days utilisation period at least 3.7 GBq of ^{99m}Tc; the total efficiency was taken as 70 %. The greatest volume of the water phase was taken as 1000 ml, a reasonable limit of usual laboratory handling. For the solubility of MoO_3 the value 100 g/l solution was used. Estimated values are given in the table.

From various technical possibilities for our conditions (3 to $5 \cdot 10^{13}$ n/cm^2 sec, I.T. = 90 hours, C.T. = 72 hours, giving about 5 GBq ^{99}Mo/g Mo) we have chosen the continuous system of extraction in a column without solid phase filament; the organic phase is pumped through a sintered glass at the bottom. Chemical engineering conditions for optimal operation have been solved for columns in diameters 10 to 40 mm and heights 200 to 500 mm. Simultaneously with the extraction the evaporation in an adjacent evaporator takes place. The velocity of evaporation (70 ^{o}C and 60-80 mm Hg) is kept at the level of MEK input (up to 12 ml/min). In such a way the washing out of ^{99m}Tc by a few ml of saline enables high volume activities (1 GBq/ml and higher). The total separation efficiency is 70-75 % and the complete process takes about half an hour. The product is sterilised by millipore filtration. In a central production unit instant technetium for hospitals of the capital is produced daily (processed ^{99}Mo up to 370 GBq); in smaller production units (up to 18 GBq ^{99}Mo) ^{99m}Tc is produced in some remote hospitals from the weekly supplied ^{99}Mo solution. ^{99m}Tc produced by either way is of high quality: radioactive impurity < 10^{-4} %, MEK < 0.05 %, other chemical pollutants undetectable, the product is sterile. The system introduced is running reliably for over 3 years. For the present system doubled values of

neutron intensities given in the table are needed as the maximum volume of columns used has been about 1/2 liter; reaching the tabulated values would need some minor adjustment to enlarge the volume of aqueous phase.

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Table of lowest applicable neutron fluxes (in 10^{12} n/cm^2 sec units).

I.T.→ / C.T. ↓	24	48	72	90	168	336
0	1.8	1.0	0.8	0.7	0.5	0.4
24	2.3	1.3	1.0	0.8	0.6	0.5
48	3.0	1.7	1.3	1.1	0.8	0.7
72	3.8	2.2	1.6	1.4	1.0	0.9

I.T.: irradiation time; C.T.: cooling time; both in hours.

QUALITY CONTROL OF RADIOPHARMACEUTICALS WITH HPLC USING AQUEOUS SIZE EXCLUSION SPHEROGEL COLUMN

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Application of High Pressure Liquid Chromatography (HPLC) for the analysis of Tc-99m radiopharmaceuticals has been reported using a weakly basic anion exchange column (1,2,3) and a reverse-phase ultrasphere octadecylsilyl (ODS) column (2,4). Both of these columns separate various components of Tc-99m radiopharmaceuticals based either on net charge or lipophilicity. A HPLC method for the separation of various components based on molecular size would be useful in the analysis of radiolabeled compounds. It has been recently shown that size exclusion HPLC with μ-Bondagel columns can be used for the detection of free pertechnetate (5). We recently reported (6) the application of HPLC methodology using a new line of aqueous size exclusion spherogel-TSK-SW column (Grade G3000) for the radiochemical analysis of Tc-99m labeled human serum albumin. In this paper, we wish to report further applications of spherogel columns for the analysis and quality control of radiolabeled complexes.

A Beckman HPLC system consisting of a solvent metering pump (model 110A), UV-VIS detector (model 153) and sample injection valve (model 210) was used in an isocratic mode. A spherogel-TSK SW (Grade G2000) column was connected to a sample injection valve through a short precolumn. 10-100μL sample was injected into the column. The column was eluted at a flow rate of one ml per minute with 0.15M NaCl or 0.1M phosphate buffer, pH 7.0. The eluant from the column was monitored by a UV detector and a sodium iodide scintillation detector to simultaneously record the UV absorbance (at 254nm) and the radioactivity. With this system, characteristic retention times (RT) were determined for various radiolabeled compounds. Both free pertechnetate and radioiodide elute from the column as single peaks with RT 28.2 and 28.0 minutes respectively. The results are summarized in Table 1.

Radiochemical analysis of Tc-99m-MDP demonstrated two components in the mixture with RT 21.5 and 22.9 minutes (Figure 1). Less than one percent of free pertechnetate was eluted with a RT of 28 minutes. Tc-99m-MDP obtained from three commercial sources showed similar composition. Upon reinjection of each component, identical retention times were obtained. The two components were collected separately and injected into rabbits. Serial images were obtained in dynamic mode. Very slight differences in the renal excretion between the two components were observed; component with RT 21.5 minutes had a delayed excretion compared to component with RT 22.9 minutes (Figure 2). However, scans performed 2hr post injection did not show any significant difference in the image quality.

Tc-99m-HDP was eluted from the column mainly as a single component with a RT of 21 minutes. Tc-99m DTPA was analysed also using the above column and was eluted as a single peak (RT 22min). Iodinated albumin and glycoproteins have been analysed on this column; free radioiodide is eluted as a single peak (RT, 28min) whereas proteins elute with shorter RT depending upon molecular weight.

When analysis was performed on Tc-99m-HSA with a grade G3000 SW column (exclusion limit: 3×10^5 Daltons), Tc-99m radioactivity was resolved into five different peaks (Figure 3). Less than 2% of the radioactivity was associated with the pertechnetate peak (RT, 28.5 minutes) and 90% of the activity corresponded to albumin peak (RT, 17minutes). 7% of the activity was bound to high molecular weight components with RT 10.2 and 14 minutes.

Routine analysis with HPLC of clinical radiotracer preparations is not currently recommended nor is the significance of these analyses apparent for clinical applications. For example although two components are identified in Tc-99m-MDP preparations, compared to a single component in Tc-99m-HDP, the biologic distribution of these components in rabbits was similar.

This manuscript reports the application of size exclusion HPLC technology modified for the simultaneous detection of UV absorption and radioactivity for the characterization of radiotracer preparations. The method appears to be generally useful and more sensitive for the resolution of components than conventional gel chromatographic techniques.

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Figure 1. Radiochemical analysis of Tc-99m-MDP

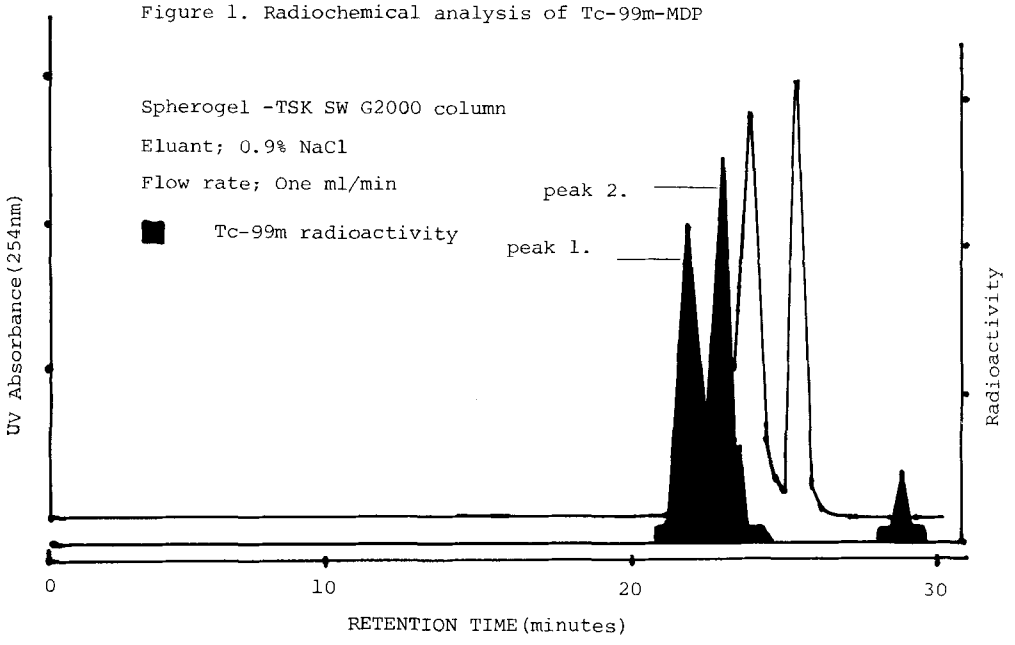

Figure 2. Renal excretion of Tc-99m-MDP components

1. Component, RT 21.5 min
2. Component, RT 22.9 min

——— Kidney activity

----- Bladder activity

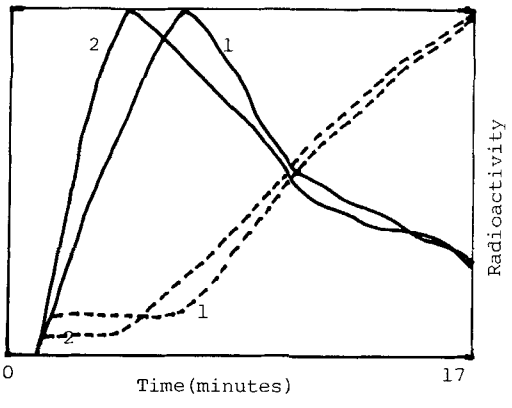

Figure 3. Radiochemical analysis of Tc-99m-HSA

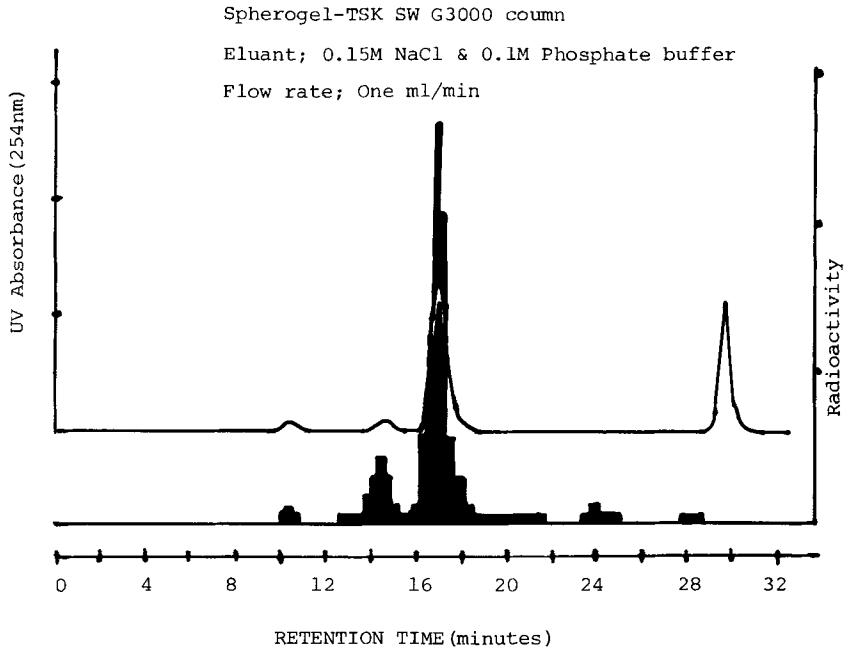

Table 1. Characteristic retention times of proteins and radiochemicals.

Spherogel-TSK SW G2000 column, eluant 0.9% NaCl,

Protein	Mol. wt.	Retention time min	Radiochemical	Retention time min
Fibrinogen	340,000	12.1	Tc-99m-DTPA	22.2
Serum albumin	65,000	16.0	Tc-99m-HDP	21.0
Myoglobin	17,000	20.8	Tc-99m-MDP	21.5
Ribonuclease-A	13,800	21.2		22.9
Alanine(amino acid)	89	25.0	TcO_4^-	28.2
			I-125 NaI	28.0

BINDING OF Tc-99m-MDP COMPLEX BY HUMAN BLOOD SERUM CONSTITUENTS

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In contrast to conventional binding studies on drugs with therapeutic action, there are scarce data about protein binding of diagnostic agents, especially radiopharmaceuticals. However, it is well known that protein binding of a radiopharmaceutical is an important characteristic, influencing its distribution in the body and the rate of elimination.

In the field of technetium-radiopharmaceuticals, investigations of i n v i t r o binding in some model systems were performed (1), including Tc-99m-methylene diphosphonate (MDP), as well as examinations of protein binding of tetracycline and pyrophosphate labelled with Tc-99m (2), Tc-99m-pyridoxylideneglutamate (3) and some derivatives of Tc-99m-HIDA (4). For Tc-99m-MDP as a widely used skeletal imaging agent, there is a lack of evidence concerning its binding and transport in blood after i n v i v o application. The aim of this work was to study interaction of Tc-99m-MDP with human serum proteins i n v i v o and i n v i t r o using the electrophoresis on agarose gel. This electrophoretic method was developed by Johansson (5) and was found to be a valuable analytical tool in protein chemistry.

I n v i v o studies.- Twenty minutes after i.v. administration of Tc-99m-MDP (700-900 MBq) to the healthy volunteers, blood samples were taken and serum was collected by centrifugation. Five different samples were assayed by agarose gel electrophoresis. Electrophoresis was run at a constant current of 160 mA in barbital buffer pH 8,6 for 60 minutes at the potential gradient 20 V/cm, in an apparatus cooled by tap water. Proteins with maximum bound radioactivity were identified comparing radioactive peaks with stained agarose gel bands run in parallel. The results are presented in Fig. 1. As seen, Tc-99m-MDP radioactivity was mostly bound and transported by human serum albumin (HSA) and prealbumin fractions, although all serum proteins fractions contained a certain amount of radioactivity.

I n v i t r o studies.- Total human sera were labelled by the addition of Tc-99m-MDP preparation (10% of the serum volume) and incubation for 60 minutes at room temperature. The above mentioned electrophoretic analysis was performed. After the staining with amido-black and destaining, the plates were scanned on a Berthold apparatus. The obtained scans are presented in Fig. 2.

It can be seen (Fig. 2.) that HSA bound the major part of Tc-99m-MDP, but radioactivity peaks corresponding to alha-2-macroglobulin and beta-1c-globulin fractions were also observed.

On the basis of the results obtained by both experimental approaches it can be concluded that HSA represents the main transporting protein for Tc-99m-MDP radiopharmaceutical.

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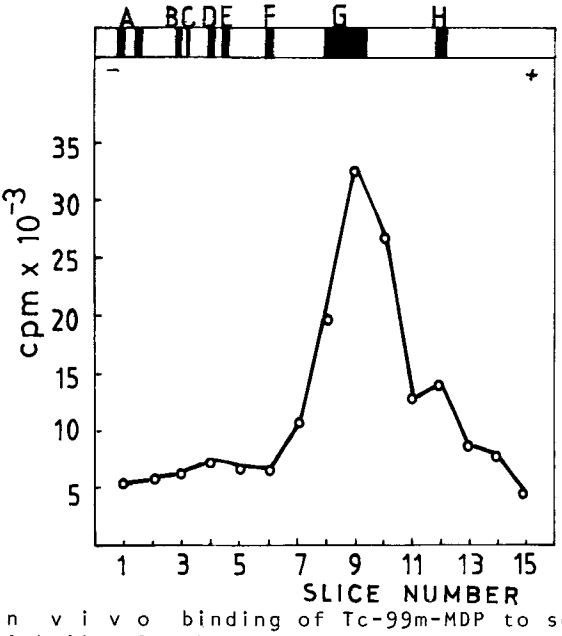

Fig. 1. I n v i v o binding of Tc-99m-MDP to serum proteins
A - immunoglobulin; B - beta-1c-globulin; C - beta-lipoprotein;
D - transferrin; E - alpha-2-macroglobulin; F - alpha-1-antitrypsin;
G - HSA; H - prealbumin.

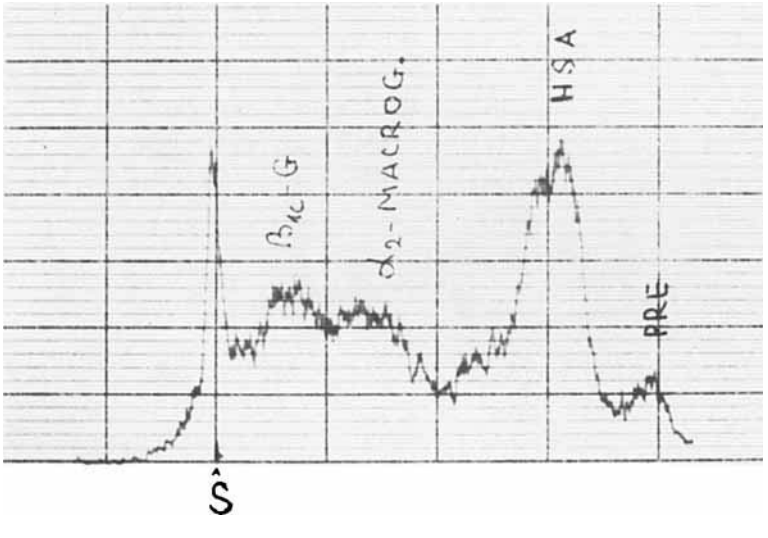

Fig. 2. I n v i t r o binding of Tc-99m-MDP to serum proteins-radioactivity distribution scan

A MODIFIED SORBENT FOR ^{99m}Tc GENERATOR

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^{99m}Tc generators are extensively used in nuclear medicine as the main source for production of medically useful ^{99m}Tc. ^{99m}Tc generator should have a reasonably high elution yield of ^{99m}Tc, good elution profile and the final solution must fulfil all requested criteria for use in medicine. Therefore the ^{99m}Tc generator system is throughly investigated, especially dopped with different oxidation agents.

^{99m}Tc generator of high activity (up to 20 GBq) is based on adsorption of ^{99}Mo from slighthly acid medium on pretreated alumina. ^{99m}Tc in high elution yield is obtained in the sterile solution of 0.9% NaCl. It is well known that the radiation induces certain chemical effects which affect the generator performances. Due to radiation-induced reduction, ^{99m}Tc is changed into non-elutable forms. It is supposed that the hydrated electron is the species which is responsible for the reduction. These effects can be diminished or eliminated by addition of some oxidizing agents to the saline or to the alumina. However both possibilities have drawbacks, especially as these agents could interfere with the subsequent use of the eluate for labelling. We have tried to overcome these problems by the use of carriers (isotopic and non-isotopic) in the ^{99}Mo solution or by spreading the active volume for ^{99}Mo adsorption with the addition of some additives to the alumina. The examination of the efficiency of these options showed that certain positive effects were obtained with the addition of SiO_2 in different ratios to the alumina. The active volume is evidently doubled. Better elution yield and elution profile of ^{99m}Tc were obtained. The work is still in progress.

SYNTHESIS OF ^{103}Ru-LABELLED RUTHENOCENE-DERIVATIVES OF BIOGENIC AMINES AND THEIR ORGAN DISTRIBUTION

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Ruthenocene-derivatives labelled with the γ-emitter ^{103}Ru (1-3) or especially ^{97}Ru (4) are of high interest for the development of radiopharmaceuticals.

In continuation of our earlier research on metallocenes we labelled different amines and biogenic amines with the [^{103}Ru]-ruthenocene-moity (Rc) according to the general formula:

$$Rc\text{-}CH_2\text{-}NH\text{-}R$$

Primarily the amine was condensed with labelled ruthenocene-aldehyde and then the double bond in the resulting Schiff's base was reduced with $Na[BH_4]$. The labelled ruthenocene-aldehyde was synthesized from [^{103}Ru]-ruthenocene by Vilsmeier-Formylation (5,6).

The clearance and organ distribution of the different amine derivatives were studied in mice and rats. Some of these compounds showed a high ^{103}Ru concentration in adrenals with ratios adrenal : muscle up to 500 : 1. The adrenal affinity for these labelled compounds increases if the injected dose was decreasing. But there is a strong variation of the adrenal affinity by the different amine derivatives. As with acetyl ruthenocene - but to a lesser extent - a sexualspecific organ distribution in the adrenals was found which can be modulated by application of sexual hormones.

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