A SIMPLE SYNTHESIS OF RADIOLABELLED BROMOACETIC ACID,

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Bromoacetic acid has been used as a chemical precursor in the synthesis of a large number of biologically active compounds including uracil (1) and malonic acid (2). We required ^{3}H and ^{14}C labelled bromoacetic acid as an intermediate in the preparation of new bifunctional chelating agents for dual label studies with proteins and monoclonal antibodies. Although commercially available, ^{3}H and ^{14}C labelled bromoacetic acid is very expensive in contrast to sodium acetate. Our initial attempts to synthesize bromoacetate via the well documented phosphorus catalyzed bromination of acetic acid were discouraging (3-4). Modification of the published procedures to increase product specific activity resulted in low radiochemical yields and product isolation was cumbersome.

As an alternative, the sulfur catalyzed (5) bromination of acetic acid proved to be a facile synthesis of radiolabelled bromoacetic acid directly from ^{3}H and ^{14}C sodium acetate. This procedure was not well documented and we undertook to optimize the reaction conditions.

The original procedure reacted bromine in the presence of elemental sulfur with refluxing acetic acid. We modified these conditions to utilize sodium acetate as our starting material. The effect of temperature, bromine volume (Figure 1), sulfur concentration (Figure 2) and reaction time (Figure 3) on the chemical yield were investigated. Under optimal conditions, the reaction proceeded without additional solvent. The sulfur (in chloroform) was added to sodium acetate (in methanol) and the solvent removed in vacuo taking care to coat the conical reaction vessel walls uniformly with the mixture. Bromine was then introduced and the reaction heated at 105 C for 60 minutes. The radiochemical and chemical yields, determined by liquid scintillation and HPLC analysis respectively, of a series of trial reactions under optimal conditions are given in Table 1. The major factor contributing to poor reaction yields was determined to be the presence of water in the reaction.

Under the optimum conditions, bromoacetic acid, labelled with either ^{3}H or ^{14}C can be prepared in near quantitative yields. Purification of the product can be accomplished readily using reverse phase HPLC.

TABLE 1. Chemical and Radiochemical Yields of ^{14}C-Bromoacetate*

Number	% Reaction Yield	
	HPLC (214mm)	LSC
1	84.7	98.7
2	35.8	44.1
3	70.2	78.5
4	77.0	94.2
5	99.1	93.3
6	93.0	91.7

*All reactions conducted under optimum conditions: 1.0 mg sodium acetate, 10 uL bromine, 0.1 sulfur, 60 minutes at 105 C.

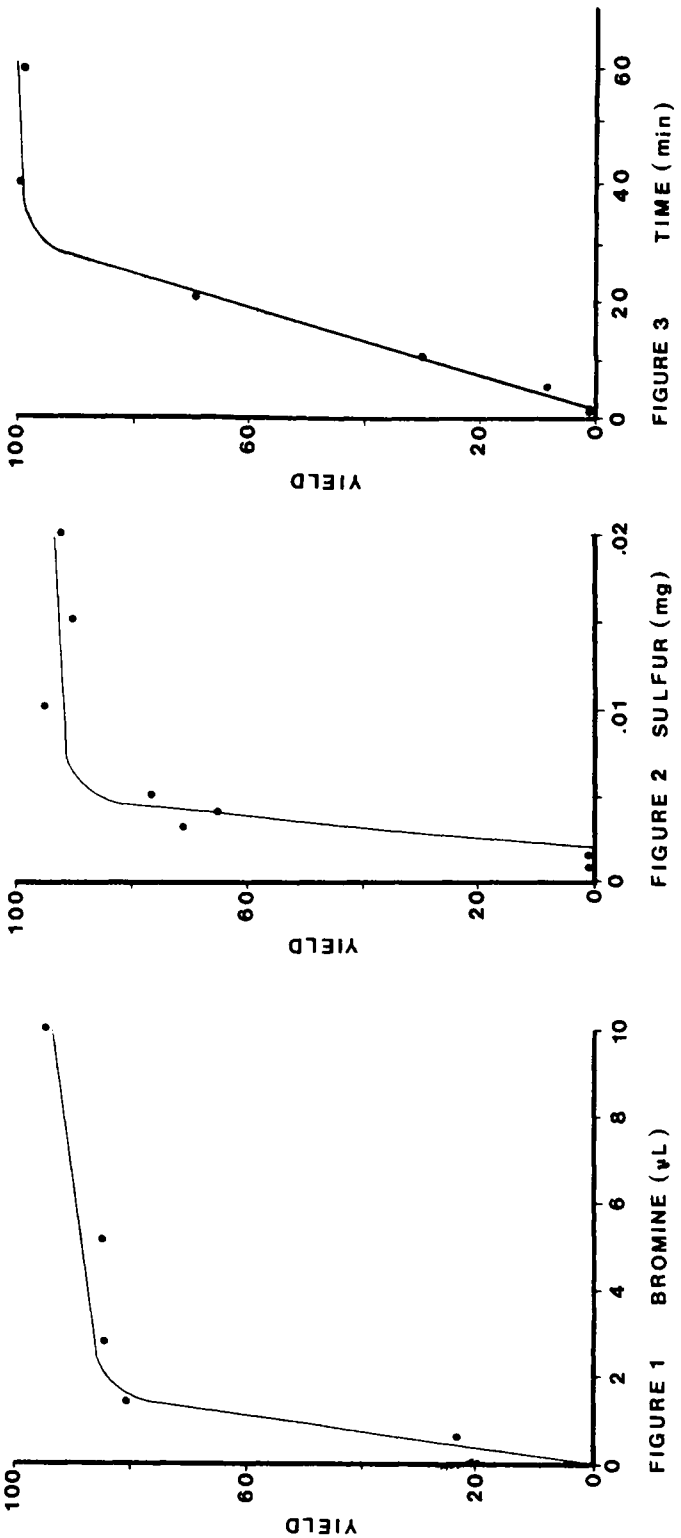

FIGURE 1

FIGURE 2

FIGURE 3

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MULTIVARIATE STRATEGIES IN OPTIMIZATION OF RADIOCHEMICAL YIELD

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Hydromorphone is an opiate ligand that has shown differences in tolerance and physical dependence in two species of macaque monkeys. By using PET, the distribution and the kinetics of hydromorphone in brain of these species will be studied.

N-[methyl-^{11}C]Hydromorphone was prepared by alkylation of the corresponding desmethyl compound with [^{11}C]-methyl iodide (Figure 1).

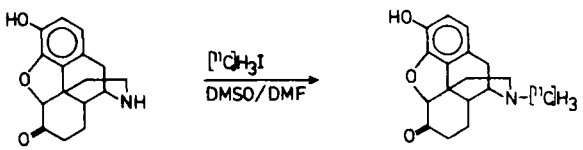

Figure 1. Alkylation of desmethyl hydromorphone

Difficulties were experienced in performing this N-alkylation reaction. The main problems were the large amount of side-products formed and the low solubility of desmethyl hydromorphone in the solvents commonly used (1) in this type of reaction.

To optimize the yield, two response surface methods, Simplex (2,3,4) and the procedure RSREG in SAS (Statistical Analytical System)(5), were used. In these methods, several parameters were varied in the same set of experiments and the yields determined. The parameters that influenced the yield of labeled product to the greatest extent were determined by factorial experiments (4) to be the concentration of the desmethyl compound, the solvent composition and the reaction temperature.
With n variables, n+1 experiments have to be done to create the first simplex. Depending on the yield obtained in the different experiments, a new simplex is created and a new experiment performed with this new setting of the parameters (Figure 2). This process is repeated until no improvement in yield is observed.

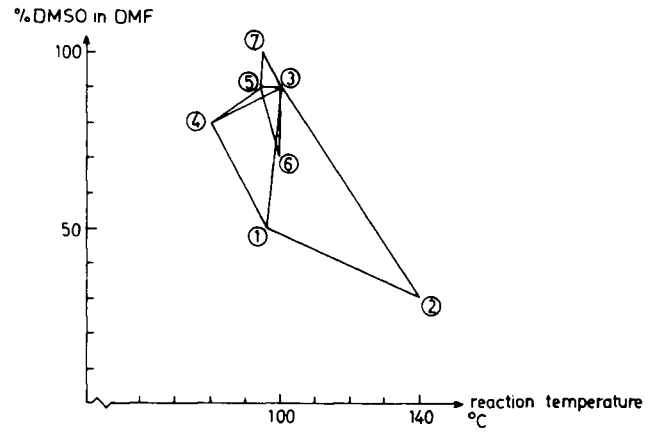

Figure 2. Simplex optimization with two variables

With the Simplex method, the maximum is difficult to determine, but the area where it should be situated can be located. The method gives a good estimation of how the important variables should be set when using the RSREG method. New experiment are performed in this area and RSREG uses the experimental data to create a mathematical model of the response surface. With this mathematical model, the extreme points can be found (Figure 3).
Another advantage of RSREG is that several experiments can be performed at one time.

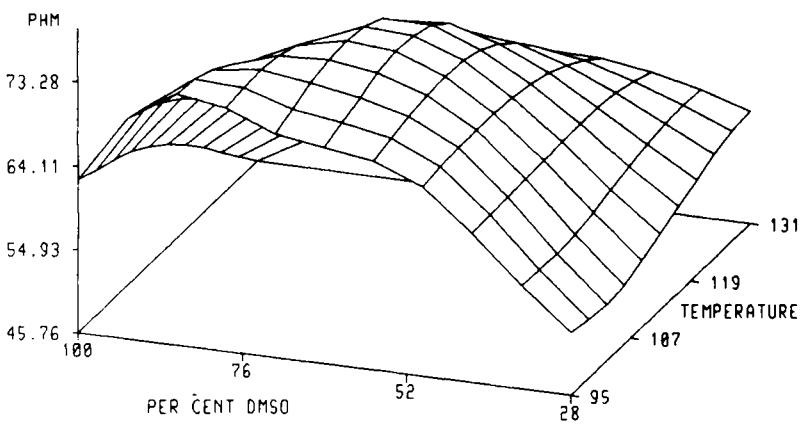

Figure 3. Response surface with three variables

By using the methods mentioned above, we were able to raise the radiochemical yield of labeled ligand from 50 to about 75 percent.

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EFFICIENT HPLC FOR THE DEVELOPMENT OF NEW LABELLING METHODS FOR RADIOPHARMACEUTICALS.

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During the development of an efficient labelling method, it is very important to use a high performing quality control method. We have proven that earlier described chromatographic methods (HPLC, TLC, ...) coupled to so called new or improved labelling methods, are far from optimal and can lead to false interpretation of the results of the labelling involved. An eloquent evidence is our development of the nucleophilic radioiodination of pharmaceuticals resulting in kit-preparation of different radiopharmaceuticals such as 123I-IAMP, 123I-Hippuran, 123I-HIPDM, 123I-MIBG and 131I-MIBG (1). When applying an efficient custom developed HPLC method(RP 18 column. ACN/H2O/MeOH//TMA/HoAC-10/45/45//0.17/0.13) on the reaction products obtained when applying the Cu(II) method described by Carlsen L. et al. (1982), the CIS-kit (3) and the melt method of Kuhl et al. (1982) for the 123I-radioiodination of IAMP ,it is shown that besides of the radiopharmaceutical of interest and the free 123I- (coupled to a loss of 123I2) different side products are generated (5).This is contrary to the two peaks (pseudo free 123I- and the product) obtained with the HPLC conditions of Carlsen L. et al. (1982). The presence of more polar impurities in this kind of radiopharmaceutical preparations can cause a descreased brain to lung activity ratio (5). In case of 123,131I-MIBG, our optimised HPLC separation (RP18-5µ column, methanol/H2O/trimethylamine/acetic acid- 55/45/.05/.05) showed that the labelling methods of Mangner T. et al. (1982) and Eisenhut M. et al. (1984) generated 123I-benzylamine or 123I2. The presence of this impurity must certainly be avoided if therapeutic doses of 131I-MIBG are concerned. When applying the TLC method described by Tramposch K. et al (1983) as a control for their labelling procedure of HIPDM an apparent labelling yield of 95 % was obtained. Efficient HPLC (RP-18 Select B-7µ,methanol/0.01M heptanesulfonic acid in H2O/acetic acid -70/30/1) showed the "product spot" to contain two radioactive side products (14%) coupled to a decreased "real" labelling yield and rendering their method unsuitable for kit preparation.
Severe HPLC control showed that when applying our Cu(I) method only a small amount of free 123I- (< 1 %) was found besides of the radiopharmaceutical of interest and opened the way for labelling kits. The quality control could be reduced to a simple SEP-PAK C 18 test (9).
In case of developping a new radiopharmaceutical for human applications a diversification of the HPLC system is required. For example the semi-preparative HPLC recovery of 15(p-123I-phenyl)-9 methyl-pentadecananoic acid is carried out by means of a EtOH/H2O/HoAC-85/15/.5 mixture on a semi-preparative Hibar RP 18-7 µ column ,as EtOH was the only solvent tolerated in this kind of study. A small part of the recovered sample was analysed on an analytical Hibar RP-18 7 µ column using a ACN/ HoAC-99/1 mixture as quality control ,as the latter method shows a better ortho-para separation than the semi-preparative one.

When labelled receptor antagonists are concerned, generally a "carrier free" recovery of the radiopharmaceutical is required. Some authors tolerate the presence of a small amount of substrate molecule, other use two columns in series to improve the separation. Until now the " recycling procedure" seems to be unknown in radio-HPLC. We have shown that making use of a semi-preparative RP-18 - 7 µ 250 x 10 mm column and a recycling system, p-131I-spiperone could be obtained "carrier free". By cutting the largest part of the cold substrate and recycling the p-131I- spiperone containing 15-20 % of p-Br-spiperone, a perfect separation was obtained.
As a conclusion one can state that the development of a labelling method only can start with an efficient HPLC control and that only efficient HPLC allows to develop a product which can bear the name radiopharmaceutical.

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METABOLISM OF ^{45}Ti-LABELED COMPOUNDS : EFFECT OF ASCORBIC ACID

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Titanium is relatively common element in the earth's crust, but its biochemical properties are not yet clearly understood. Recently, Pais et al.(1) reported the positive effect of titanium-ascorbate(Ti-AsA) in plant cultivation where promotion of plant growth was observed. ^{45}Ti-DTPA is often prepared for medical applications as a positron emitting radiopharmaceutical in our center(2). We prepared ^{45}Ti-AsA and investigated its biochemical behavior in plants and animals, and studied its radiopharmaceutical applications.
^{45}Ti was produced by the reaction of ^{45}Sc(p,n)^{45}Ti with 11.5MeV protons and separated from scandium by an ion exchange technique as previously reported method(3). The ^{45}Ti-titanium ascorbate complex was prepared from no carrier added ^{45}Ti(IV) and an excess amount of ascorbic acid(5mg). A doubly labeled complex was also prepared from ^{14}C-ascorbic acid. Chemical and radiochemical purities of the complexes were checked by HPLC.
In plants(leaf lettuce), we observed the enhanced growth effect under hydroponic circumstance with Ti-AsA. ^{45}Ti was highly absorbed by the roots in the chemical form of Ti-AsA as Ti(IV) (probably $TiOCl_2$). The ascorbic acid supplement (20 ppm) resulted in enhanced uptake of ^{45}Ti(IV). Thus AsA content of plants plays an important role in the uptake and accumulation of titanium.

The distribution of ^{45}Ti(IV) and ^{45}Ti-AsA in Wistar rats are shown in Fig. 1. The liver, spleen and blood showed a high uptake of ^{45}Ti-AsA similar to that of ^{45}Ti-DTPA. ^{45}Ti(IV) was gradually cleared from the blood. Animals, such as guinea pig and osteogenic-disorder rat(OD-rat)(4), unable to synthesize AsA, may be strongly affected in their mechanism of accumulation of trace metals.

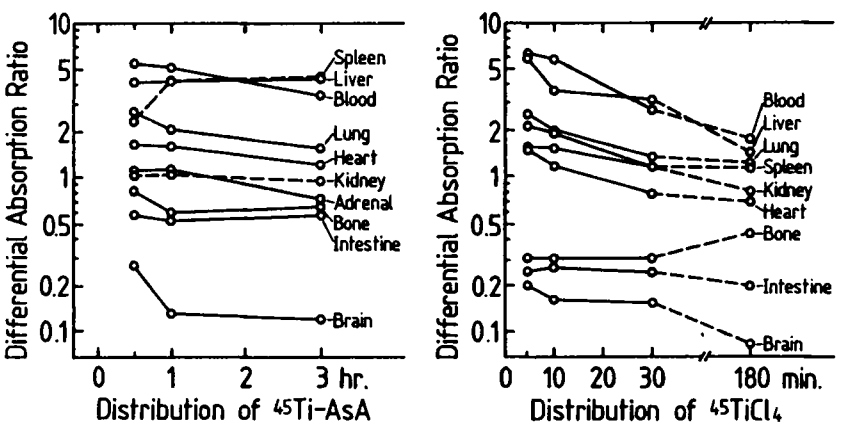

Fig. 1 Tissue Distributions of ^{45}Ti-AsA and $^{45}TiCl_4$ in Wistar Rats.

Autoradiograms of OD-rat 30 min after the injection of doubly labeled Ti-AsA (^{45}Ti; ^{14}C) are shown in Fig. 2. The image obtained for ^{45}Ti-AsA did not coincide with that obtained for the ^{14}C-AsA. The ^{14}C-AsA uptake was higher in the adrenal glands, gut epithelium and liver. The ^{45}Ti-AsA uptake was higher in the heart muscle, lung, spleen, liver and blood. Radioactivity maps of kidney with ^{45}Ti and ^{14}C are greatly different. The influence of marginal ascorbic acid deficiency and excessive ascorbic acid consumption on the metabolism of ^{45}Ti-AsA in guinea pigs were investigated. Guinea pigs fed experimental diets (low AsA: 28mg/100g diet, high AsA: 4000mg/100g diet) for

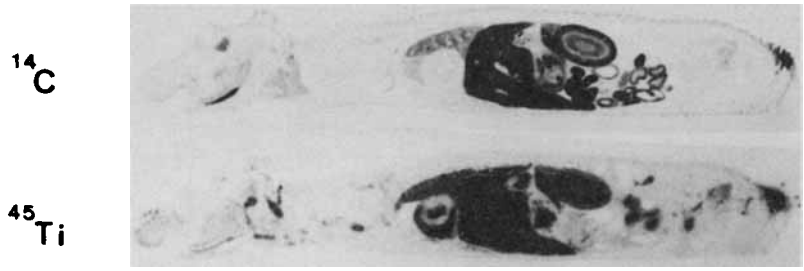

Fig. 2 Double Nuclide — Autoradiograms of Ti-Ascorbate in Rat (^{45}Ti and ^{14}C) with ^{45}Ti and ^{14}C-Ascorbic Acid.

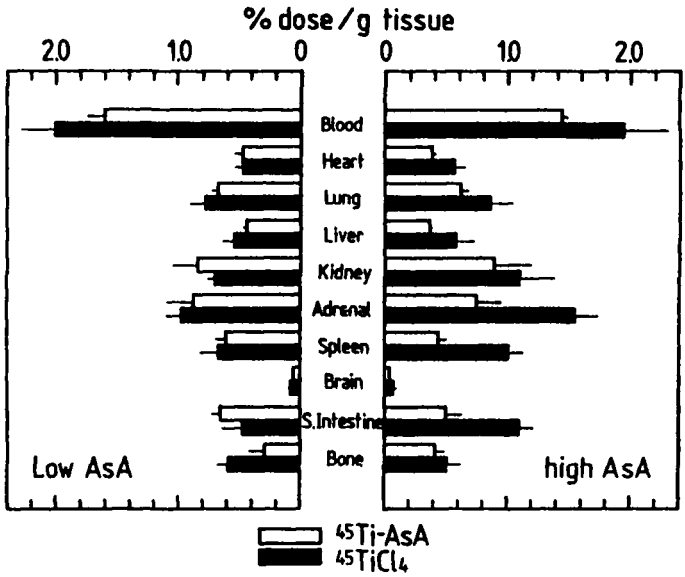

Fig. 3 Tissue Distribution of $^{45}TiCl_4$ and ^{45}Ti-AsA in Guinea Pigs with Low or High Ascorbic Acid Intake.

two weeks, were then administered with ^{45}Ti(IV) or ^{45}Ti-AsA. The uptake of ^{45}Ti(IV) was accompanied by a parallel rise of AsA level in the diet, while the uptake of ^{45}Ti-AsA was independent of AsA level in the diet(Fig. 3). Metabolites of ^{45}Ti-AsA in the plasma of guinea pig and OD-rat were analyzed by HPLC(Fig. 4). Peaks B and C were identified Ti-AsA-albumin and AsA, respectively. Peak A may be Ti-conjugated with lipoprotein. These results show that ^{45}Ti is mainly present as a ^{45}Ti-AsA-albumin complex, but ^{14}C-AsA is present in the form of unbound AsA. This tendency is independent of the concentration of ascorbate in plasma. This observation may well explain the non-coincident patterns of the autoradiograms of ^{45}Ti and ^{14}C.

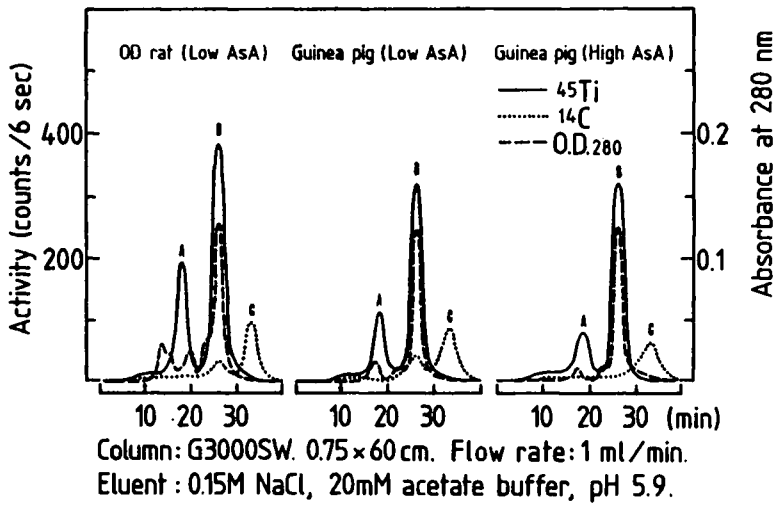

Fig. 4 HPLC of Rat and Guinea Pig Plasma after 10 Minute Incubation with ^{45}Ti-^{14}C-AsA *in vitro*.

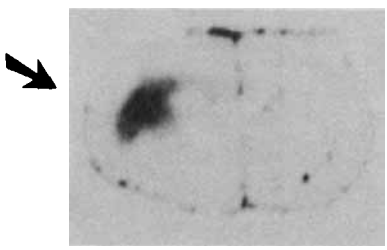

Fig. 5 Autoradiogram of Rat Glioma with ^{45}Ti-Ascorbate.

The autoradiographic image of a glioma bearing rat brain(target to nontarget ratio; 26.7) was obtained by administration of ^{45}Ti-AsA (Fig. 5). ^{45}Ti-AsA may be a useful radiopharmaceutical for studying the B.B.B. in medical applications.

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NEW APPROACH FOR THE SEARCH OF EXPLOITABLE FUNCTION OF AMINO ACID AS FOR PANCREATIC RADIOPHARMACEUTICAL.

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Various natural or synthetic amino acids (AAs) have been labeled with positron emitter as for pancreatic studies, based on the high AA metabolic activities of this organ. Nevertheless, the ideal AA, exploitable as for externally administered pancreatic radiopharmaceutical is still unknown. Being aware about the great complexity involved in the selection of proper AA, one objective of the present work was to search for a simplified working methodology.

In this study, the twenty natural AAs found as fundamental units of protein were classified according to their frequency of occurrence in pancreatic digestive proteins (% AA in protein pool) and the content in the free AA pool in pancreas (AA in free pool) (Table 1)[1-5]. Also the ratio of AA in protein pool to AA in free pool (AA requirement index :ARI) was considered (Table 1). In order to survey and select the AA to satisfy the requirement of a pancreatic radiopharmaceutical among the twenty natural AAs, the following strategy was necessary: classificaton of AA in three groups and selection of the most representative of each group (with easy and simple synthetic method) as follows: (1)AA most frequently found in the free pool (Glu), (2)AA most abundantly found as protein constituent (Ser), (3)AA with high ARI (Phe, Met and Leu).

Using those AAs labeled with C-14, their pancreatic utilization was surveyed by in-vitro (rat tissue slices) as well as in-vivo (mice biodistribution) studies. Then, their participation in the active transport and the protein synthesis was tested.

The in-vitro screening showed the representatives of group(3) (Met, Phe) as contributing to high value in tissue slice accumulation, while those of group(1) and (2) (Glu, Ser) displayed low value (Table 2). Also in the in-vivo screening, the AAs of group(3) (Phe, Met) showed very high accumulation in the target organ (pancreas, Table 3). Thus, ARI might be seen as a practical parameter for the screening of natural AAs. Also good correlation of ARI with the active AA transport and metabolic retention such as protein synthesis (found in Phe study), might as well be satisfactory requirement needed in pancreatic radiopharmaceutical. In other words, the basic criteria, offered for the pancreatic radiopharmaceutical, is the design of an agent with active transport system and fast incorporation into some retention mechanism.

TABLE 1. Amino acids in protein and free pool of pancreas

	PROTEIN POOL (digestive enzyme)							FREE POOL	ARI[d]
	% AA in						% AA in	AA in	
	AMY (2.0	CBP 26.0	CTP 32.0	DRB 1.4	RBN 2.4	TRY 14.0)[a]	PROTEIN POOL[b]	FREE POOL[c] (µmol/100g)	
Val	7.8	5.2	10.2	9.3	7.3	7.9	6.17	45.2	4.5
Leu	4.9	7.5	8.4	9.0	1.6	6.1	5.76	25.2	7.5
Ile	4.4	6.8	4.4	4.3	2.4	6.6	4.32	14.5	9.8
Met	1.7	1.0	0.9	1.6	3.2	0.9	0.79	2.0	13
Phe	4.9	5.2	2.7	4.3	2.4	1.3	2.60	10.9	7.8
Trp	3.2	2.3	3.5	1.2	0	1.8	2.05	4.0	17
Thr	4.9	8.5	1.8	5.8	8.1	4.4	3.75	27.7	4.5
Lys	4.2	4.9	6.2	3.5	8.1	6.6	4.50	35.6	4.2
His	1.9	2.6	0.9	2.3	3.2	1.3	1.29	17.4	2.4
Arg	5.7	3.6	1.8	4.3	3.2	0.9	1.87	14.4	4.3
Gly	11.0	7.5	10.2	3.5	2.4	10.9	7.06	233.1	1.0
Ala	6.3	6.8	9.7	8.6	9.7	6.1	6.23	381.6	0.54
Pro	4.4	3.3	4.0	3.5	3.2	3.5	2.83	37.4	2.5
Gln	--	3.6	4.4	3.5	5.7	5.2	3.26	342.0	0.31
Asn	--	5.5	6.2	4.7	8.1	7.4	4.72	36.3	4.3
Ser	6.8	10.4	12.4	11.7	12.1	14.9	9.34	85.6	3.6
Glu	8.0	4.6	2.2	3.9	4.0	0.9	2.33	951.5	0.081
Asp	14.0	3.9	4.0	7.8	4.0	3.9	3.33	138.2	0.80
Tyr	3.8	6.2	1.8	5.8	4.8	4.4	3.06	9.4	11
Cys	2.1	0.7	4.4	1.6	6.5	5.2	2.54	(2.5)	(33)

a = % protein abundance in protein pool.
b = Summ of % AA found in protein x (a).
c = AA content in free AA pool (µmol/100 g wet wt.).
d = amino acid requirement index (ARI)= $(b/c)_{AA} \times (c/b)_{Gly}$.

AMY:amylase, CBP:carboxypeptidase, CTP:chymotrypsinogen, DRB:deoxyribonuclease, RBN:ribonuclease, TRY:trypsinogen.

TABLE 2. Amino acid accumulation into rat pancreas slices[a]

L-Phe	L-Met	L-Leu	L-Ser	L-Glu
140.82 (28.01)	121.90 (20.46)	89.02 (34.85)	79.42 (8.42)	25.24 (9.22)

[a]Accumulation %dose/g slice, mean of 5 experiments (1 s.d.). Incubation performed for 30 min.

TABLE 3. Biodistribution of C-14-amino acid in mice[a]

	L-Phe	L-Met	L-Leu	L-Ser	L-Glu
Pancreas[b]	46.14 (12.41)	33.04 (0.57)	30.77 (4.52)	26.74 (4.53)	8.30 (1.25)

[a]Sacrificed 30 min after i.v. injection.
[b]Accumulation %dose/g tissue, mean of 3 mice (1 s.d.).

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RADIONUCLIDES FOR THERAPY

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With the emergence of new, biological vehicles of great organ specificity (eg. steroid hormones, antibodies) the concept of systemic tumour therapy with the aid of radiotherapeutica has gained new momentum. In order to assess the options open for optimal adaptation of the radiation properties to the pharmacocinetics of a vehicle, a search was done to identify potentially useful therapeutic radionuclids. Main criteria for selection were half live, low γ-yield and stable daughter nuclide.
The reasons for these criterias will be discussed (see table I.) The resulting possibilities fall into 4 categories:
1) α-emitters
2) β^--emitters prepared carrierfree
3) β^--emitters prepared carrier added
4) Electron capture nuclides, emitting Auger-cascades
Among the latest group some well known, diagnostically used nuclides are found. Their therapeutic use necessitates the precise localisation in or very near the genetic material of the cell to be killed, only there the destructive power of the very short range Auger-electrons can be used.

TABLE I: Nuclides Selected for Therapeutical Use

category	nuclides
α-emitter	AT-211
β^--emitters carrierfree	P-32, S-35, As-77, Y-90, Ag-111, Pm-149, Tb-161, Lu-177
β^--emitters carrier added	Pd-109, Pu-142, Gd-159, Er-169, Tm-172, Yb-175, Re-188, Ir-194, Pt-197
electron capture, auger-emitters	Cr-51, Ga-67, Ge-71, Br-77, Ru-97, Sb-119, I-123, Cs-129, Nd-140, Er-165, Ta-177, Hg-197, Tl-201

The list of nuclides selected in this study will be compared with the current used nuclides (see table II). Reasons for discrepancies will be discussed.

TABLE II: Comparison of Nuclides in this study (1) with the current used and proposed ones (2,3)

	nuclides
nuclides in accordance	P-32, S-35, Cr-51, Br-77, Y-90, Ru-97, Pd-109, Sb-119, I-123, Er-169, Lu-177, Re-188, Hg-197, At-211
new nuclides in this study	Ga-67, As-77, Ge-71, Ag-111, Cs-129, Nd-140, Pm-149, Gd-159, Tb-161, Tm-172, Yb-175, Ta-177, Ir-194, Pt-197, Tl-201
current nuclides *not selected in* this study	Sc-46, Cu-67, Br-80m, Sr-89, Pd-100, I-125, Ba-128, I-131, La-140, Sm-153, Dy-165, Au-198, Pb-212, Bi-212, Ra-224, Pu-238, Es-253, Fe-255

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TUMOUR LOCALIZATION OF SAMARIUM-153 CHELATES IN MALIGNANT MELANOMA.

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Tumour affinity of radiolanthanides prompted an investigation of uptake of Sm-153 chelates in B16 murine melanoma. Samarium-153 has favourable characteristics for imaging with a principal gamma emission energy 0.103 MeV (28%). The other 58 gammas are in the energy range 0.041-0.764 MeV and are all below 0.6% abundant. The mean beta emission of Sm-153 is 0.227 MeV (100%). Samarium-153 has a half life of 46.7h and was prepared in the AAEC reactor by the Sm-152 (n, γ) reaction. Samarium-152 enriched to 98.5% as Sm_2O_3 was irradiated for 24h in a flux of 5×10^{13} n cm^{-2} sec^{-1} which typically yielded 10 GBq Sm-153 from 1 mg Sm_2O_3.

Samarium-153 chelates of DTPA, HEDTA, IDA, HIDA, PIH (pyridoxal isonicotinoyl hydrazone), PBH (pyridoxal benzoyl hydrazone) and BZ (benzimidazole) were prepared and their behaviour was compared with that of Sm-153 chloride and citrate. In general, compounds of Sm-153 were prepared by the addition of a slight stoichiometric excess of Na_2CO_3 to a solution of $SmCl_3$. The complexing agents were then added in at least a 20:1 molar excess, usually as a solid, and NaOH was used to adjust to pH 6-7.

After filtration through a 0.22 μm membrane filter, in vitro evaluation was performed using TLC. The sample was applied to a cellulose plate (Eastman) and developed using the mixed solvent system pyridine : ethanol : water (1:2:4). The chromatographic behaviour of Sm-153 chelates of DTPA, HIDA and PIH is compared with that of Sm-153 chloride in Figure 1. The chloride was immobile whilst DTPA moved at the solvent front. PIH and HIDA exhibited intermediate mobility and broad bands.

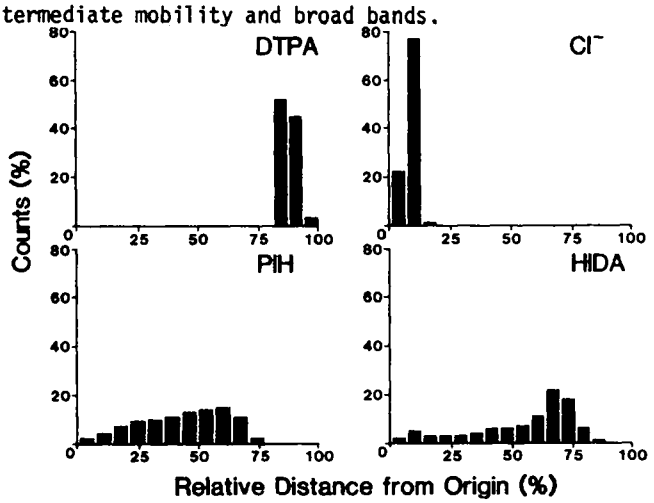

Figure 1. Chromatographic behaviour of Sm-153 chelates of DTPA, HIDA and PIH compared with that of Sm-153 chloride.

In vivo evaluation of Sm-153 chloride and chelates in control animals showed three major organ distribution patterns:

The chloride was almost exclusively taken up by liver, DTPA was rapidly excreted by kidneys whilst HIDA and HEDTA uptake appeared to be evenly divided between liver and kidney.

The in vivo hepatic accumulation of Sm-153 chloride can be attributed to colloid formation (1). In vitro studies of the products of samarium hydrolysis under conditions comparable with those used for the preparations whose chromatography is illustrated in Figure 1, led to the isolation of a colloid containing both samarium and sodium (Sm 49%, Na 6.9%) with Sm:Na ratio of 1:1.

The infra red spectrum of this material shows absorption bands indicative of the presence of water (broad absorption 3200-3700 cm^{-1}) and CO_3^{--} (complex of peaks near 1400 cm^{-1}). Quantitative determinations using thermal gravimetric analysis and x-ray diffraction indicate that this colloid differs from samarium sodium carbonate hydrate as previously reported (2).
This colloidal preparation is related to those formed in vivo after intravenous administration, attributable to the relatively high blood concentrations of Na^+ and HCO_3^- /CO_3^{--}. The competition between hydrolysis of samarium compounds leading to colloid formation and exchange reactions with serum components such as albumin and transferrin is currently under investigation.
Tumour localization studies of Sm-153 chelates were performed in C57 black mice bearing both melanotic (MEL) and amelanotic (AMEL) B16 melanoma and results were compared with Sm-153 chloride, Sm-153 citrate, Ga-67 citrate and Se-75 methionine. All samarium compounds were evaluated at 1, 6, 24 and 48h after intravenous administration using 5 animals for each data point. Evaluation was by gamma counting of organs and by external gamma camera imaging and results were compared with those obtained 24h and 48h after intravenous administration of Ga-67 citrate. At the time of study both melanotic and amelanotic tumours had grown to approximately 1cc in size and

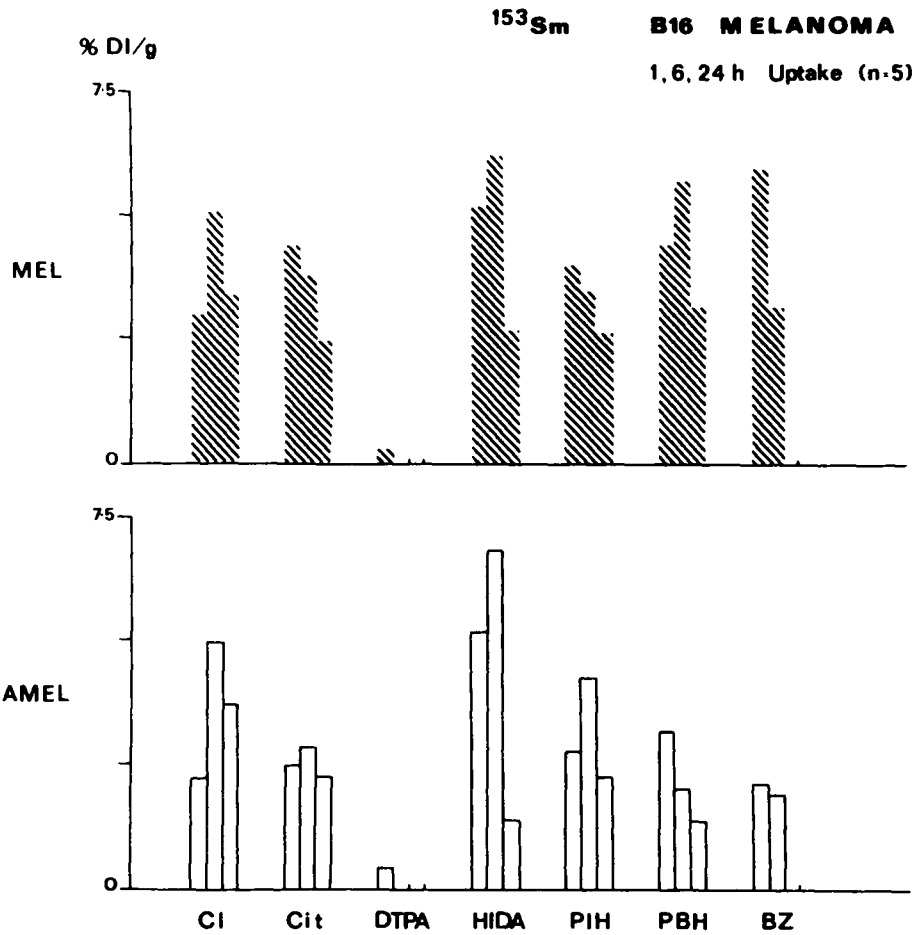

Figure 2. Tumour uptake of Sm-153 chelates compared with that of Sm-153 chloride and citrate in melanotic and amelanotic B16 murine melanoma.

histopathological studies showed a constant degree of melaninization and confirmed the absence of focal necrosis.

Relative tumour uptake of Sm-153 chelates is shown in Figure 2.

Sm-153 DTPA was rapidly excreted by the kidneys and tumour uptake was negligible. Sm-153 HIDA uptake by the liver made tumour imaging difficult despite relatively high affinity (AMEL 6.82±1.00% DI/gm at 6h, MEL 6.24±0.8±% DI/gm at 6h). Sm-153 PBH peak tumour uptake (AMEL 3.14±0.76% DI/gm at 1h, MEL 5.69±1.20% DI/gm at 6h, and Sm-153 PIH (AMEL 4.23±0.90% DI/gm at 6h, MEL 4.03±0.90% DI/gm at 1h) compared unfavourably with that of Ga-67 citrate (AMEL 12.71±1.98% DI/gm at 24h, MEL 41.45±12.21% DI/gm at 24h) and Se-75 methionine (AMEL 11.07±1.55% DI/gm at 24h, MEL 10.55±1.48% DI/gm at 24h). Imaging studies demonstrated delineation of the B16 melanoma tumour by Sm-153 PBH, PIB and HEDTA.

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PRODUCTION OF Sm-153 FOR RADIOTHERAPEUTIC APPLICATIONS

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Production of Sm-153 by neutron capture has been investigated using the University of Missouri Research Reactor (MURR) in the course of the development of a new radiotherapeutic bone agent, Sm-153 Ethylenediaminetetramethylenephosphonate (Sm-153-EDTMP). In this application high specific activity and high radionuclidic purity are necessary; 1 mg samples of 99.06% enriched Sm-152 samarium oxide irradiated for times up to one week at high flux ($\sim 3 \times 10^{14}$ thermal neutrons $cm^{-2}sec^{-1}$) uniformly produced about 62% of the theoretical yield of Sm-153, while an irradiation at lower flux (8×10^{13} $cm^{-2}sec^{-1}$) produced about 74% of the theoretical activity expected, some samples exceeding 5500 Ci/g Sm.

A liquid sample of Sm-153 was prepared for standardization by the National Bureau of Standards, who reported an improved value for the half-life of Sm-153 of 46.27 hours (compared with the old value of 46.8 hours) and a new value for the abundance of the 103.2 keV gamma-ray of Sm-153 of 29.8% (compared with the earlier value of 28.3%).

Impurities observed in both low and high flux irradiations included Eu-152, -154, -155, and -156, with the Eu-154 and Eu-156 double capture products disproportionately increased in the higher flux irradiations, as expected. It is possible to minimize their presence using low flux irradiations or very brief irradiations at high fluxes. The total amount of these radionuclidic impurities was less than 2 ppm when ~ 1 Ci Sm-153 was produced. Thus, Sm-153 can be produced in high purity and high specific activity for use as a radiotherapeutic label.

Rh-105 AS A POTENTIAL RADIOTHERAPEUTIC AGENT

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There is now considerable interest in the development of moderate-energy beta emitters which can be used as radiotherapeutic agents by incorporation in conventional radiopharmaceuticals such as phosphonates or by conjugation to antibodies. The latter application demands high specific activity radionuclides. If the application is to move from research to clinical use large scale quantities must be available. For example, if only 1000 patients per week were to be treated with 100 mCi doses, a source of at least 200 Ci (E.O.B.) per week would be needed. One radionuclide which is now widely available is ^{131}I. It can be produced routinely in multicurie quantities from nuclear fission or by neutron capture on ^{130}Te followed by beta decay of ^{131}Te to ^{131}I. Both of these production methods are based at nuclear reactors which are widely available throughout the world and which have proven to be dependable sources of radionuclides on a commercial scale. For some applications, however, the ^{131}I 8-day half-life may be too long and it may dehalogenate in vivo. An alternate, and successful, approach to labeling has been the use of bifunctional chelates with ^{67}Cu. That radionuclide has a shorter half-life, lower beta energy, lower gamma energy, and lower gamma yield than ^{131}I (1). Reported yields for its production, however, are far below the quantities which may be needed (2,3).

A radionuclide which possesses nuclear properties similar to ^{67}Cu and production possibilities similar to ^{131}I is ^{105}Rh. It emits betas (70% 560 keV, 30% 247 keV) and gammas (5% 306 keV, 19% 319 keV) with a half-life of 35.5 hrs. It can be produced by $^{104}Ru(n,\gamma)^{105}Ru$ (ab = 18.7%, σ = ~0.5 b, I = ~ 6.5 b) followed by ^{105}Ru decaying with a half-life of 4.4 hrs to ^{105}Rh. The only major radionuclide contaminants are 2.9-d ^{97}Ru and 39-d ^{103}Ru. The only stable isotope of rhodium is ^{103}Rh and its production is limited to the small amount resulting from the beta decay of the 39-d ^{103}Ru. Ruthenium metal containing only 1 ppm rhodium is commercially available. The only disappointing nuclear property is that ^{105}Rh itself has a high capture cross section (~16,000 b) so that yields at high fluxes are somewhat decreased. The nuclear parameters for ^{105}Rh production are very similar to those for ^{99}Mo or ^{131}I by neutron capture so that existing technology can be adapted. It is also a fission product and could be recovered as a by product of production of other fission products.

Investigations are underway in this laboratory to adapt reported procedures (4) for the chemical separation of ruthenium and rhodium to the production of ^{105}Rh at the 10-100 mCi level for research studies and to provide

information for its ultimate production at the 100 Ci level. Samples of 1-100 mg of high purity natural ruthenium (rhodium <1ppm) as the metal have been irradiated for times of 3 to 30 minutes in the University of Missouri Research Reactor (MURR) at a flux of $8x10^{13}$ n/cm^2 sec. These samples have been used for studies of the radiochemical separations. A sample of 99%-enriched ^{104}Ru (rhodium<0.01 %) was irradiated at the same flux for 72 hrs and counted after several half-lives decay without chemical separation to determine the amount which could be produced at that flux. The chemical separation consists of bubbling Cl_2 through a slurry of ruthenium metal in 10-ml KOH solution. The Cl_2 oxidizes the Ru to RuO_4 and sweeps it from the solution to a series of CCl_4 and NaOH traps. Traces of remaining RuO_4 are extracted from the reaction solution by CCl_4 and the ^{105}Rh product is concentrated by coprecipitation from NH_4OH solution with Fe^{3+}. The resulting $Fe(OH)_3$ ppt is redissolved in HCl and the ^{105}Rh product, probably as $RhCl_6^{3-}$, eluted from a Dowex 1-x8 anion exchange column. Preliminary results show ~65% yield of ^{105}Rh and a $2x10^4$ separation factor from Ru. There is ~1% Fe breakthrough from the column. In addition, ICP atomic absorption analysis of the reaction mixture shows about 4 μg of cobalt which must have come from the reagents or handling since it could not be accounted for by the reported purity of the sample or by ^{60}Co lines in the Ge(Li) spectrum.

The results of these experiments are summarized in Table 1. Three columns are shown; I, the results from these experiments, II, predicted results for 1 gram of natural ruthenium in the same position, and III, predicted yields from a production reactor. Yields are calculated as Ci/g target, Ci/mmole rhodium assuming <1 ppm of Rh in natural-Ru targets and <100 ppm in enriched targets, and Ci/mmole total Rh and Ru assuming the separation factor above. Higher absolute yields could be obtained by recovering ^{105}Rh from fission products.

TABLE 1. Yields of ^{105}Rh at E.O.B. for 72-hr irradiation

	I	II	III
Target	99% ^{104}Rh	nat Ru	nat Ru
Flux (n/cm^2 sec)	$8x10^{13}$	$8x10^{13}$	$2x10^{14}$
Ci/g Ru Target	4[a]	(~0.8)[c]	(~1.5)[c]
Ci/mmole Rh product	>4,000[b]	(>42,000)[c]	(>56,000)[c]
Ci/mmole (Rh product +Ru contaminant)	>2,700[b]	(>1,500)[c]	(>3,000)[c]

a) This work
b) This work assuming ^{104}Ru target <0.01% Rh
c) Predicted from this work assuming nat Ru target <1 ppm Rh

These results indicate that ^{105}Rh can be produced in the quantities and high specific activity necessary for antibody labeling for radiotherapy. Work is underway to improve the separation from the Ru target and from reagent impurities.

This work was supported in part by DOE Grant No. DE-FG02-86ER60400.

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DEVELOPMENT OF TECHNIQUES FOR RAPID ANALYSIS OF ^{90}Sr BREAKTHROUGH AND ^{90}Y ACTIVITY FROM A ^{90}Sr-^{90}Y GENERATOR.

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Recently there has been interest in labelling monoclonal antibodies with therapeutic amounts of a pure beta emitting isotope. ^{90}Y offers many desirable features and may be conveniently and economically obtained from a ^{90}Sr-^{90}Y generator. It has a short half life of 64 hours, emits a single energetic beta (2.27 Mev maximum), has no gamma associated with it, and decays to an inocuous stable isotope ^{90}Z. When obtained from ^{90}Sr-^{90}Y generator it is carrier free which is essential for labeling antibodies to high specific activity using bifunctional chelates. A potential hazard with this system is breakthrough of the parent ^{90}Sr which has a half life of 28 years and is an extremely toxic bone seeking isotope. Therefore it is essential that the daughter be completely separated from its parent. Two uCi of ^{90}Sr fixed in bone is the lifetime maximum permissible dose[1]. We have devised two simple techniques which allow us to monitor rapidly the amount of ^{90}Sr breakthrough and quantitate the ^{90}Y activity over a 5 log range. A ^{90}Sr-^{90}Y generator was constructed according to the method of Hnatowich[2] using 7.7 mCi ^{90}Sr purchased from ICN. The ^{90}Sr was spiked with 355 uCi of ^{85}Sr which produces a monoenergentic gamma of 514 keV, behaves chemically like ^{90}Sr and binds to the Dowex 50w x 8 cation exchange resin. The ^{90}Y was eluted from the generator with 0.03 M EDTA leaving the ^{90}Sr bound to the resin. Thus, the amount of gamma emitting ^{85}Sr appearing in the milk was a measure of the amount of breakthrough of ^{90}Sr. A shield was constructed which absorbed the beta emission and reduced the bremstrahlung and so that it could be counted in a gamma well counter (Figure 1).

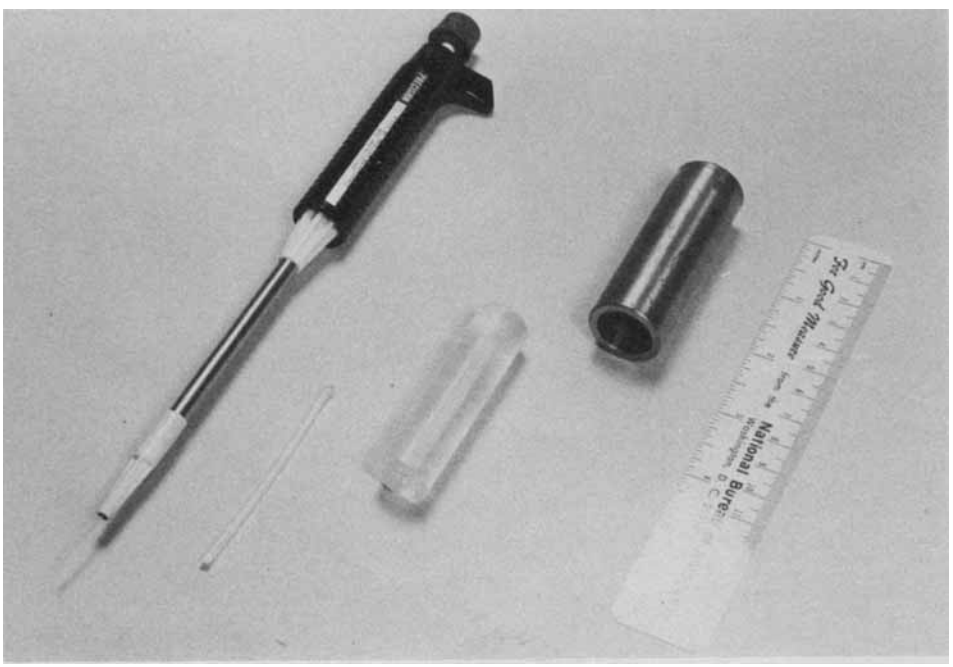
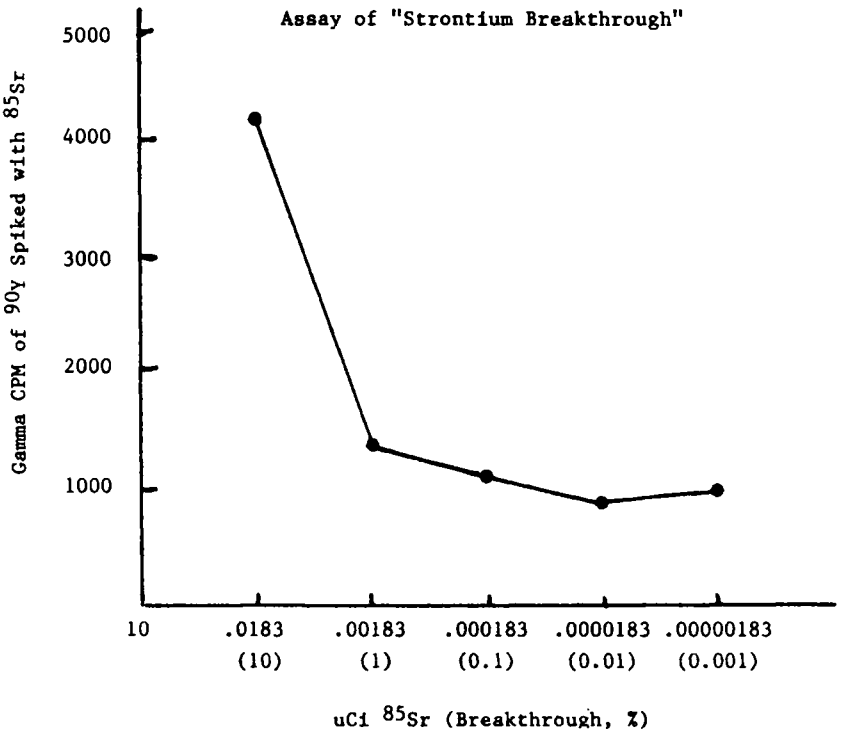
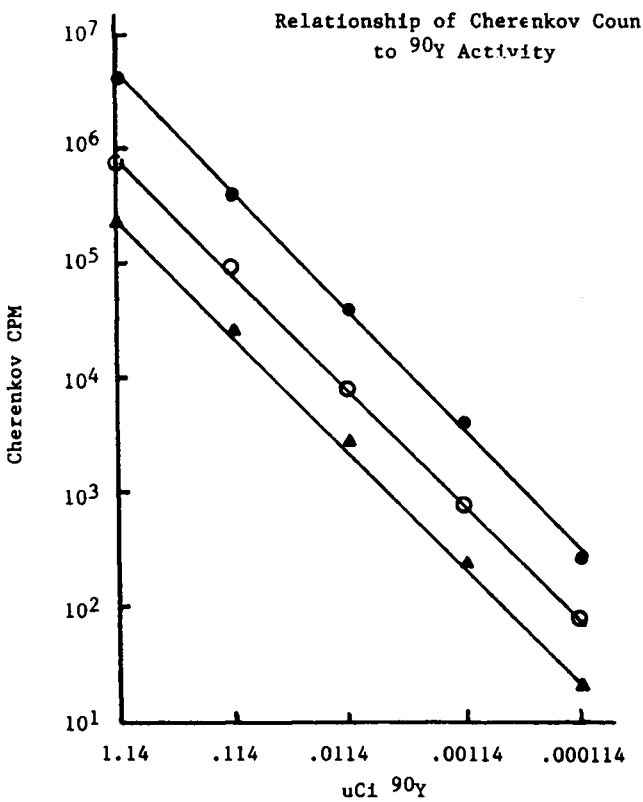


Figure 1. Micropipette, with tip, 2.5 x 80 mm polystyrene sample carrier, Lucite beta shield, and lead shield.


Assay of "Strontium Breakthrough"
Gamma CPM of 90Y Spiked with 85Sr
5000
4000
3000
2000
1000
10
.0183
(10)
.00183
(1)
.000183
(0.1)
.0000183
(0.01)
.00000183
(0.001)
uCi 85Sr (Breakthrough, %)


Relationship of Cherenkov Counts
to 90Y Activity
Cherenkov CPM
10^7
10^6
10^5
10^4
10^3
10^2
10^1
1.14
.114
.0114
.00114
.000114
uCi 90Y

The carrier (shield) diameter was limited to 2.4 cm in order to fit in the Picker stationary gamma scintillation counter and the length was 7 cm. The shield consisted of a lead cylinder 3.5 mm thick (bottom also 3.5 mm thick, not shown) and a lucite cylinder 14.2 mm in diameter with a 2.8 mm diameter hole in the center into which the 2.5 x 80 mm sample container could be inserted. Two microliter volume aliquot samples were placed in a low z material pipet tip and inserted into a low z polyethylene tube sample carrier. The lucite carrier was thick enough to absorb almost all beta particles and the low z material mimimized bremsstrahlung production. The layer of lead surrounding the lucite markedly reduced low energy bremsstrahlung by 99%, but permitted most of the high energy ^{85}Sr gamma to penetrate to the crystal (Table 1).

Table 1. EFFECT OF SHIELDS ON ^{90}Y AND ^{85}Sr COUNTS IN GAMMA COUNTER

	NET CPM (% OF UNSHIELDED)		
ISOTOPE/WINDOW	UNSHIELDED	IN LUCITE	IN LUCITE LEAD
^{90}Y, 50-2000	1,308,010 (100)	218,298 (16.69)	13,156 (1.006)
^{90}Y, 464-564	114,793 (100)	1,751 (1.525)	1,051 (0.916)
^{85}Sr, 50-2000	63,632 (100)	58,511 (91.95)	35,520 (55.82)
^{85}Sr, 464-564	24,338 (100)	20,305 (83.43)	11,481 (47.17)

In this gamma counter the ^{85}Sr spectrum revealed a peak at 514 keV and the bremsstrahlung spectrum diminished at higher energies but still comprised a significant amount of the background noise. A window setting of 464-564 keV was found to be optimal with our counter. As a test of the system, pure ^{90}Y was purchased from Oak Ridge National Labortories (ORNL) and was spiked with ^{85}Sr at different concentrations to simulate breakthrough of 10, 1, .1, .01, and .001 percent of ^{90}Sr from the column (Figure 2). The sensitivity of the carrier-counter technique can indicate that breakthrough of ^{90}Sr is less than .01 percent when the Sr^{85}/Sr^{90} on the column = 0.5.

The activity of the ^{90}Y milk was determined by assaying the Cerenkov radiation using a liquid scintillation counter without need for scintillation cocktails or phase shifters[3]. Cerenkov radiation is linear over an activity range covering at least 5 logs (Figure 3). Raising the baseline reduces the number of counts per minute in a linear fashion, and ^{85}Sr and ^{90}Sr do not generate significant Cerenkov radiation. Quantitating the amount of ^{90}Y using Cerenkov radiation was accomplished by assaying a diluted aliquot of milk and dividing the net cpm by the cpm/uCi (1.3×10^6) determined in this system by the reference material from ORNL and multiplying by the dilution factor. Sr^{90} breakthrough was calculated in a few minutes from the net cpm in the gamma counter using the 464-564 keV window and the lead lucite shield and was < .1% This system allows immediate and accurate determination of Sr^{90} breakthrough which will facilitate the clinical use of ^{90}Y labeled antibodies by allowing their safe injection into humans within minutes of their preparation.

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AN IMPROVED GENERATOR FOR THE PRODUCTION AND USE OF ^{212}PB AND ^{212}BI.

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A new generator has been designed for the production of ^{212}Pb and its daughters. The previous design (1) utilized ^{228}Th as the parent. A stream of water was passed over the support to dissolve and carry away the ^{220}Rn daughter. ^{212}Pb and its daughters were retained on a cation exchange resin downstream. This design had the two major drawbacks: first, the system was cumbersome, requiring a large source of deionized water; and second, the long half life of ^{228}Th, 1.9 years, raised concern over the potential hazards if the generator was breached and the thorium was lost.

In order to eliminate these problems, a new generator was designed which used ^{224}Ra as the parent in the generator. ^{224}Ra has a 3.6 day half life which is better suited to use in a hospital. Any catastrophic breach of the system can be handled by isolating the spill and allowing it to decay to stability. Radium is separated from thorium by isolating the thorium on an anion exchange resin in nitric acid. The radium is passed through a second column to insure little breakthrough of the thorium parent. Finally, the radium is taken to dryness and put on the generator in dilute HCl.

The generator consists of a small column of cation exchange resin with a total volume of 300 microliters. The ^{212}Bi daughter can be eluted in 0.5 N HCl or in 0.15 N HI with approximately 50 percent yield. The ^{212}Pb-Bi can be eluted in 2.0 N HCl or in 0.5 N HI with approximately 70 percent yield. Breakthrough of the parent is less than one part per million.

This system has been in use for over one year and has operated without any major problems. A lead shield was designed to contain the generator with minimum weight and maximum shielding surrounding the generator.

Current studies have focused on the use of ^{212}Bi attached to monoclonal antibodies (2). The bismuth can be eluted directly, or the lead can be eluted and placed on a second, smaller, column. The bismuth then can be eluted when needed. The advantage of the second column is its virtual elimination of any radium contaminants.

Bismuth as the iodide is neutralized by a buffer to maintain pH 4.5. The protein which contains a DTPA chelate is added and incubated at 37° for 15 minutes. The protein-^{212}Bi is purified by passage through an HPLC system equipped with a size exclusion column to eliminate aggregates and free radionuclides. In spite of the high radiation dose to the protein, competition assays show no loss of activity by the protein after the ^{212}Bi decays to stability.

Thus we have developed a system that enables one to utilize the high LET alpha emitting radionuclides without the necessity of cyclotron production and tedious manipulation to attach the radionuclide to a protein.

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*Deceased

A $^{82}Sr/^{82}Rb$ generator suitable for continuous infusion

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A ^{82}Rb generator suitable for continuous elution and intravenous infusion into man has been developed at Hammersmith over the past five years [1] and the final design is the result of operational experience over this period. Three injection moulded polypropylene columns were used in series (see Fig. 1). These were identically packed with α form tin dioxide of particle size 0.5 - 0.1 mm (Applied Research, Belgium) [2] between two sinters and pretreated by washing with 0.3M HCl followed by exhaustive washing with water for injection to neutral pH. The first column acted as the primary adsorption site for the ^{82}Sr. The purpose of the second column was to conveniently monitor the slow movement of the ^{82}Sr as it spread from the first column to the second column. The third column which acted as a final guard column, was initially packed with Bio-Rad Chelex-100 resin [1]. Subsequent experience had shown that it was impossible to maintain a sterile (and pyrogen free) system using this material, so currently all three columns are packed using tin dioxide.

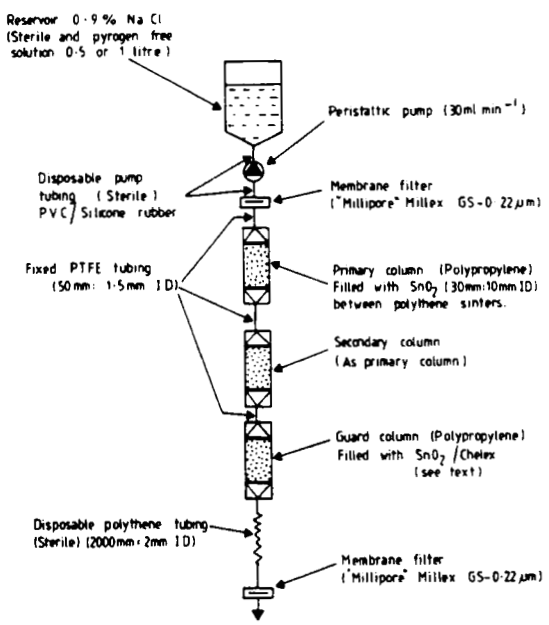

Figure 1.

Diagram of a $^{82}Sr/^{82}Rb$ generator

The stock solution of ^{82}Sr was obtained from Los Alamos [3] and adjusted to pH 9 - 10 with NaOH. It was loaded on to the first SnO_2 generator column and the column washed with between 250 - 500 mls of 0.9% saline at 30 ml min^{-1} until the breakthrough of $^{82}Sr/^{85}Sr$ and other contaminating radionuclides (^{51}Cr, ^{54}Mn, $^{56,57,58}Co$, ^{88}Y and ^{88}Zr) were at a minimum. The generator system at this stage was kept as clean as possible by the use of sterile components and solutions. A number of different methods of sterilising the generator were attempted, such as gamma sterilisation of components before and after construction, loading through membrane filters etc. but these were found unsatisfactory. The only method which was suitable for routine use and consistently gave a sterile eluate even after 70 days, involved the overnight disinfecting of the generator system using an isotonic solution of sodium hypochlorite. This was found to be extremely successful in keeping the system clean and gave no observable long-term effects on the breakthrough of the strontium. Following the

setting up procedure and before the generator could be released for a clinical study, samples of the eluate had to pass a strontium breakthrough test (gamma-ray analysis using a Ge(Li) detector) and a limulus amoebocyte lysate pyrogen test. When the generator was initially prepared the gamma-ray spectrum of the stock $^{82}Sr/^{85}Sr$ solution was examined and the levels of ^{82}Sr, ^{85}Sr and other radionuclides were calculated. The problem in distinguishing the 511 keV and 514 keV gamma photo peaks of ^{82}Sr and ^{85}Sr was overcome by using the curve stripping procedure previously described [4]. By carefully handling this data the $^{82}Sr/^{85}Sr$ ratio was calculated to the time of the generator's initial preparation.

For routine use of the generator, 25 ml breakthrough samples of the eluate were examined using the Ge(Li) detector. Knowing the $^{82}Sr/^{85}Sr$ ratio decay corrected to the day of measurement and the total counts in the unresolved 511 + 514 keV region, the separate activities due to ^{82}Sr and ^{85}Sr were calculated.

Other metal radionuclide contaminants were also detected in the generator eluate. These all occurred at unpredictable times during the use of the routine generators that had been prepared from Los Alamos stock solutions. Fortunately the concentrations of these radionuclides were well within acceptable whole body burdens.

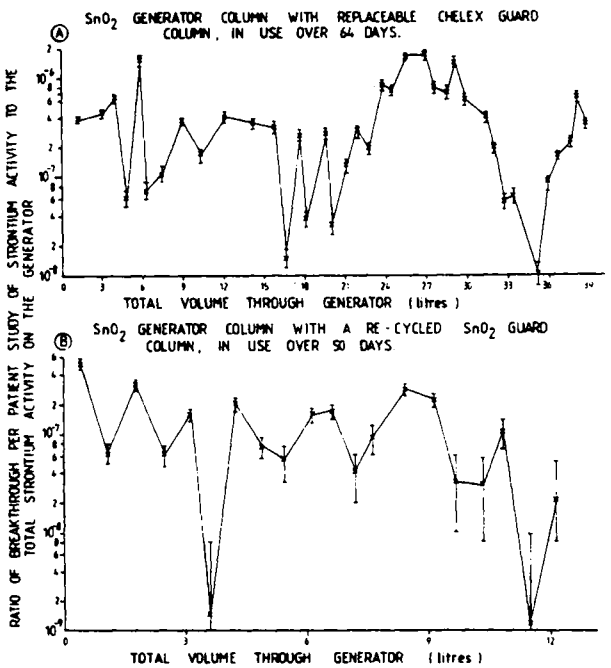

Figure 2.

Breakthrough of strontium activity

The breakthrough of strontium radionuclides from two typical generators is illustrated in Fig. 2. The upper plot (A) is for a generator with two tin dioxide columns and a replaceable chelex guard column, whilst the lower plot (B) is for a similar generator with a tin dioxide guard column. Although there is a fluctuation in the breakthrough for both of these generators it shows no tendency to rise towards the end of the useful life of the generator at around 70 days. It is contained within a narrow range at an extremely low level (i.e. 2% of the whole body burden of 18.5 kBq for ^{82}Sr [5]). The chelex guard column has the advantage over the tin

dioxide guard column in that it traps the other metal contaminants (Cr, Co, Mn etc.) but has the disadvantage that it offers a favourable medium for the growth of bacterial contamination and is difficult to sterilise. The tin dioxide guard column on the other hand appears to release less strontium breakthrough but more importantly is relatively easy to maintain sterile using sodium hypochlorite.

A summary of the sterility and pyrogen tests on elution samples taken over an extended period of two months are shown below. This table clearly demonstrates the advantages of the tin dioxide guard column, in obtaining a "clean" generator over this period.

	Chelex Guard Column		Tin Dioxide Guard Column	
	Sterility Test	Pyrogen Test	Sterility Test	Pyrogen test
Total Number of Samples	19	6	17	17
Number of failures	19	1	0	0

In conclusion, however safe this generator may appear from the foregoing we strongly believe that because of the potential dangers of the eluate, a quality assurance programme is still necessary to keep a careful check on the operation of the generator.

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HYDROXYLAPATITE-BASED $^{82}Sr/^{82}Rb$ GENERATOR

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By virtue of their stability towards ionizing radiation, inorganic ion exchangers have long been favored over organic resins as the supports for radionuclide generators. Notable applications to date have been aluminum oxide ($^{99}Mo/^{99m}Tc$); tin dioxide ($^{68}Ge/^{68}Ga$); silica/zinc sulfide ($^{195m}Hg/^{195m}Au$); and zirconium phosphate ($^{81}Rb/^{81m}Kr$).

Hydroxylapatite (a modified crystalline calcium phosphate) is a complex inorganic matrix with extensively documented applications in purification and fractionation of proteins and nucleic acids (1), though rarely cited for ionic separations (2). We have evaluated several commercial hydroxylapatite preparations as supports for a $^{82}Sr/^{82}Rb$ generator suitable for chemical &/or biomedical applications.

The $^{82}Sr/^{82}Rb$ separation potential for hydroxylapatite (Calbiochem; Fast Flow) was determined by measurement of distribution coefficients in phosphate buffered saline over a wide pH range [Figure 1] and appears similar to hydrous stannic oxide (3). A separation potential of approximately 10,000 was also evident in water and 5% dextrose.

Dramatic differences in Sr(II) adsorptivity were evident among seven adsorbents tested [Table 1]. Items 6 & 7 were identified as octacalcium phosphate and whitlockite, respectively, (4) and corresponded to published photomicrographs for those modified calcium phosphates (1).

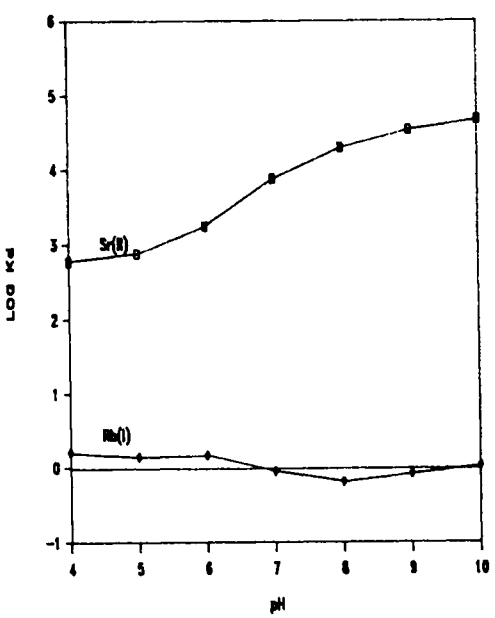

Figure 1. pH Dependence of Sr(II) and Rb(I) distribution coefficients on synthetic hydroxylapatite.

TABLE 1. Sr(II) Adsorptivity of Commercial Hydroxylapatite

Source; Type	Log Kd (ml/g)	pH
1. Calbiochem; Fast Flow	4.5	8.0
2. Fluka; Fast Flow	4.4	8.0
3. Calbiochem; Type B	4.2	7.1
4. BDH; Hydroxylapatite	4.0	6.8
5. Bio-Rad; Bio-Gel HTP	3.7	6.3
6. Clarkson; Hypatite C	2.4	5.9
7. BDH; Spheroidal Hydroxylapatite	2.0	6.5

A model generator (0.7 cm x 5 cm) was prepared with 100 uCi of ^{82}Sr. Over 99.9% of the activity was retained on the column. For elutions with water at approximately 5 ml/min, ^{82}Sr breakthrough was $<2.5 \times 10^{-6}$ ml^{-1}. The ^{82}Rb bolus was eluted in less than 5 ml although the eluate activity yield was less than 30%.

Attempts to prepare equivalent hydroxylapatites of other group II metals, Ca, Sr, and Ba, (5) yielded amorphous granules, possibly not true hydroxylapatites, whose distribution coefficients for Sr(II) were in the order Ba>Sr>Ca.

The features of this generator may be useful in limited applications where the presence of sodium in the generator eluate may be undesirable, as in the preparation of ^{82}Rb cryptate complexes (6). Synthetic hydroxylapatite is proposed as a worthy candidate for further study in the field of radionuclide separations.

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The production of ^{43}K using an Argon gas target

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At the present time potassium-43 is not widely used in nuclear medicine. There appear to be many reasons for this. The emitted energies of its decay; $E\beta$- 825 keV, $E\gamma$ 373 keV (88%), 618 keV (81%); make it less than ideal for applications involving a gamma camera. However, its half-life of 22.3 h has been found to be useful in certain studies. A number of medical applications have been reported, such as body space and potassium transport measurements [1,2], as well as studies of myocardial function [3,4]. Unfortunately it is difficult to produce, especially in a pure form free from ^{42}K, $t_{\frac{1}{2}}$ 12.4h $E\beta$- 3520 keV, $E\gamma$ 1524 keV (18%). Of the production methods reported in the literature there are only three methods which can be considered to give useful quantities of ^{43}K. Qaim and Probst [5] have examined the ^{51}V (d,2α pn)^{43}K reaction, the Los Alamos group have proposed a spallation route using vanadium targets [6,7], while Clark et al. [8] reported on the ^{40}Ar (α,p)^{43}K route using an argon gas target (see Table 1). The work outlined here is an extension of this latter approach and may help towards a better understanding of the performance of other inert gas targets and the subsequent recovery of the alkali metal and halogen products. For example Ne - ^{18}F; Kr - ^{75}Br ^{77}Br, ^{81}Rb; and Xe - ^{123}I.

Table 1 The yield of ^{43}K and ^{42}K impurity.

Production Route	Beam Energy (MeV)	Yield ^{43}K at end of irradiation	Relative yield of ^{42}K (%)	Reference
^{51}V (d,2α pn)	45-55	590 kBq (16 μCi) μAh^{-1}	10	5
^{nat}V(p,spall.)	800	(18 GBq per target)	8.5	6,7
$^{40}Ar(\alpha,p)$	0-19.3	2.5 MBq (68 μCi) μAh^{-1}	6.6	8 & this work

Our earlier work described by Clark et al. [8] involved the irradiation of Argon gas with alpha particles in a recirculating system using oil free diaphragm pumps. These experiments showed that the potassium radionuclides produced during the irradiation could be swept out in the gas phase and caught by a glass fibre filter paper. This method was found to be satisfactory in capturing 70 - 80% of the total activity produced, and provided an extremely convenient method of recovery free from other possible contaminants present in the system (e.g. Al). Unfortunately the efficiency of this process slowly dropped to 20% over a period of some years due to the increasing absorption of the potassium by the aluminium target walls. Numerous factors were subsequently investigated in an attempt to raise the amount of potassium retained in the gas phase, but none were successful.

Parallel studies of the target parameters needed for the ^{nat}Kr (p,xn)^{81}Rb production route, initiated a change in approach and a target was designed for irradiation of non-circulating Argon. The target which was made of aluminium was basically uncooled. It was 400 mm long; 70 mm diameter and filled with Ar to 2 atmospheres. By using this size of target and pressure of argon, it was not expected that the alpha particle beam would strike the walls of the target from multiple scattering effects [9]. The beam line and water cooled entrance window (40 mm by 21 mm) contained a mixture of absorber foils (e.g; 0.125 mm Cu/2% Be; 0.025 mm "Havar") to degrade the incident alpha beam available to 19 MeV in order to reduce the ^{42}K contamination. In addition, an aluminium foil (0.025 mm) was retained on the inside of the target window to capture the recoil products (e.g.

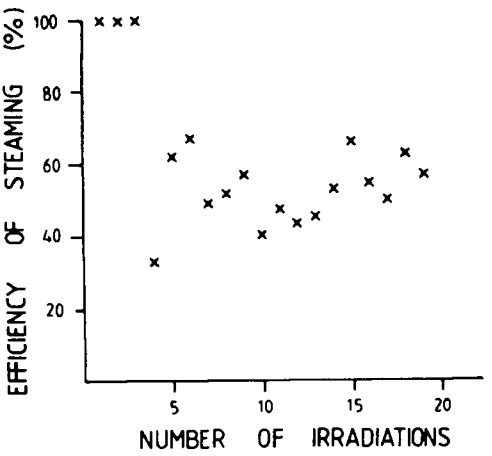

Figure 1 RECOVERY OF ^{43}K FROM AN ALUMINIUM TARGET BY STEAMING

^{57}Co; ^{58}Co) from the energy degrading foils. These long lived radionuclides might otherwise have been recovered along with the ^{43}K.

After irradiation the target was removed from the beam line and vented of argon through a membrane filter. This indicated that no potassium was present in the gas phase. The potassium activity absorbed by the target walls was recovered by steaming the target in a similar way to the recovery of ^{81}Rb reported elsewhere [10]. The efficiency of the steaming is presented in Fig. 1 and clearly shows a decrease with subsequent irradiations and steaming. In considering the use of other materials for the target (including the window) which would be more suitable for this form of recovery it was important to remember that the recovered solution was ultimately destined for intravenous injection. Thus, care had to be taken to ensure the radionuclidic purity of the product by the selection of window foils to give a minimum of ^{42}K and recoil products. In addition, the amount of stable metals in the solution had to be monitored to prevent the possible injection of toxic levels.

An alternative target was considered which was constructed of stainless steel (Type 315 and 347) with a 0.025 mm Havar window. Though the recovery of potassium was 100% efficient, the recoils from the window (^{57}Co, ^{58}Co) presented a contamination problem. It was also observed that the purity of the stainless steel could not be guaranteed and there soon appeared to be signs of oxidation resulting in milligrams of iron in the recovered solution. The possible long-term activation of the target body (^{57}Co and ^{58}Co) was also considered a disadvantage.

A target machined from solid nickel with nickel welded front and back plates gave similar problems, although the recoiling of induced activity from nickel foil windows was undetectable in the final solution. The reliability of steaming was better than aluminium but the levels of stable Ni in solution gave cause for concern. Initially this was 12 mg but reduced to a total of 200 μg for each steaming. If injected, this amount of nickel has to be compared to the level of stable nickel in human blood of only 0.04 μg ml^{-1} [11] (i.e. a total of around 200 μg).

The use of electrochemical nickel [12] is being investigated in the hope that some of these problems will be resolved [13]. Such a target would then be applicable to the production of ^{81}Rb from the krypton route as well as the production of ^{43}K described here.

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BLIP II: A NEW SPALLATION RADIONUCLIDE RESEARCH AND PRODUCTION FACILITY.

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The Brookhaven Linac Isotope Producer (BLIP) was the world's first facility to demonstrate the capability of a large linac for efficient medical radionuclide production by proton spallation and lower energy reactions (1,2). It utilized the excess beam capacity of a proton linac that injects 200 MeV protons into the 33 GeV Alternating Gradient Synchrotron. The significant impact of the BLIP and its continuing usefulness to the nuclear medicine community has been amply proven in its 12 years of operation. However, several serious technical problems coupled with a recent overall decline in reliability made a renovation imperative.

Years of operational experience at high current and high radiation levels has suggested many desirable improvements to the facility. Thus, it was decided not to simply replace aged assemblies but to incorporate extensive new design features. The new system provides more reliable operations due to new components and simplified design, better repairability, much faster target handling, a reduced personnel radiation exposure, and a new capability to explore the use of high energy neutrons for producing neutron rich species. The major changes are that a) sand provides the bulk of the neutron shielding, not water, b) the target holders are moved in and out vertically on motorized chain and sprocket drives, c) the target holders are immersed in water not helium and this water provides both cooling and shielding functions, d) two small transfer caves of stacked lead bricks are replaced by one large hot cell of poured lead with master-slave manipulators, and e) the beam line is improved with the addition of a vertical steering magnet, and the installation of two newly designed multiwire chambers for beam focusing.

Shielding is required to protect operations personnel against very penetrating high energy neutrons which are formed by the direct interaction of the proton beam and target nuclei. Secondary charged particles and gamma rays are of minimal concern as they can be more easily absorbed. In BLIP I, this neutron shielding was provided by 13,000 gallons of water contained in a stainless steel tank 2.44 m diameter by 10.2 m deep. Although the water shield was inexpensive, offered some visibility of the irradiation chamber, and could be removed by pumping, it caused many problems. This large volume of water has been replaced with sand. Monte Carlo neutron transport calculations (3) and neutron flux measurements (4) were used to guide the design of the sand shield. The volume of sand, 2.44 m diameter by 6.9 m is supported by a table placed inside the existing BLIP tank. The 1.2 m high ventilated air space under the table is a void volume for water should a cooling water leak occur. This sand barrier is pierced by two shafts. The first is a 40.6 cm diameter by 10.4 m stainless steel shaft filled with water. The targets, drive assembly, and cooling water plumbing are contained in this shaft. The water supplies neutron shielding within the shaft. To allow repair of the shaft should a window leak, it is designed to be removable in sections into the hot cell. A second concentric steel shaft of 45.7 cm O.D., which is fixed in place by the sand, allows the inner shaft to be lifted out. The second penetration of the sand is a 32.4 cm diameter carbon steel shaft which can be used as an inspection port or for pump-out of the bottom of the main tank in the event of a significant leak of the target containment shaft. Shielding in this shaft is provided by 4.6 m of removable concrete plugs supported so as to be flush with the top of the shaft. At the operating floor personnel are shielded from gamma rays emitted during target handling by a hot cell facility with master/slave manipulators. The overall arrangement of BLIP II is schematically depicted in Figure 1.

The high energy deposition of the beam (10 kW) necessitates active target cooling. Individually encapsulated targets (from 2-4) are positioned in slots inside seven independently moveable target holders in which cooling water flows

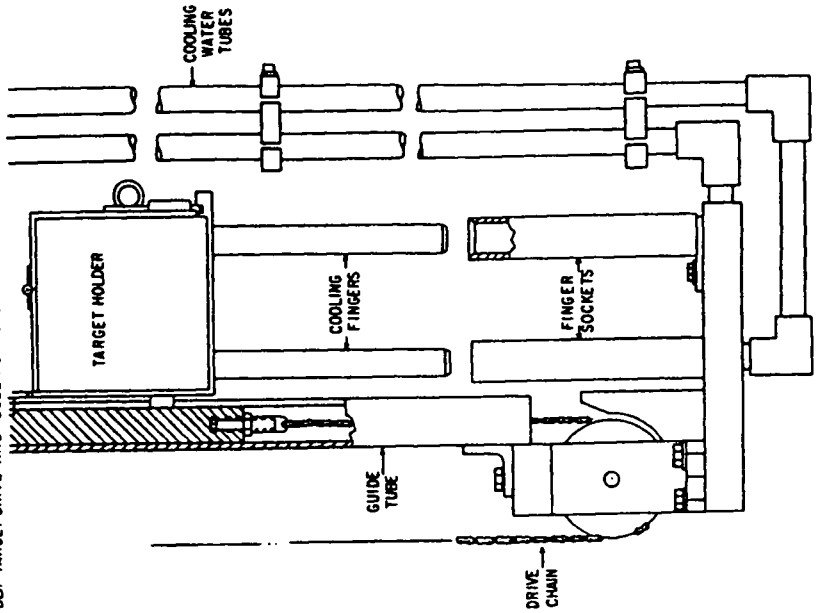

FIG. 2.
BLIP TARGET DRIVE AND COOLING ASSEMBLY (FRONT VIEW)

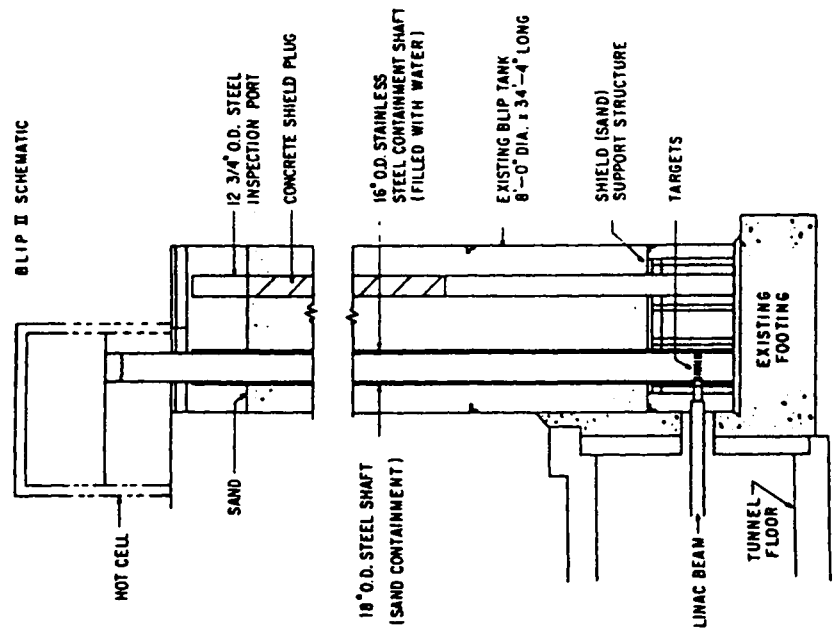

FIG. 1. TARGET AND SHIELDING ASSEMBLIES OF BLIP II.

past the target faces in the gaps between targets. Heat transport calculations guide the trade-off between target thickness and flow velocity. The entire beam is absorbed in the target array. Targets are positioned in the array to receive the proper proton energy to maximize production of desired radionuclides and minimize unwanted impurities. Instead of a traveling hose to supply water, the new target holders plug into inlet and outlet water sockets when in beam. Individual cooling sockets can be replaced remotely if necessary or the entire assembly can be brought up into the hot cell for more extensive repair. The lines are individually routed to flow and pressure sensors inside the hot cell and then sent back to empty at the bottom of the shaft. The target holders are themselves immersed in this water which provides extra cooling. This design is forgiving, because small leaks in the target holders can be tolerated. The pump suction is near the top of the shaft so that water deposited at the bottom slowly rises in the shaft, requiring 8-10 minutes to reach the pump. This designed delay in the water flow path allows time for radiation levels from short-lived radionuclides produced in the water (^{15}O, ^{13}N, ^{11}C) to decrease before reaching the pump and heat exchanger on the operating floor.

Each target holder is attached to a guide bar which is motor driven vertically inside a slotted, square guide tube with a chain and sprocket system. All structural materials are stainless steel. The stainless steel bicycle chain provides ample strength and permits target retrieval in about 30 seconds. It is very flexible, allowing the assembly to remain small. The target and cooling assembly is shown in Figure 2. Alignment is very critical as target holder horizontal spacing is only 0.16 cm and the cooling water fingers must mate with their respective sockets (tolerance 0.005 cm) after 10 meters of travel.

The compactness of the target assembly is an important feature for ease of installation and removal for repair but it also provides an important new capability that was not practical with the old BLIP design. The high intensity of 200 MeV protons impinging on targets produces copious spallation neutrons, mostly emitted into a cone about the beam axis. These high energy neutrons (En $\leq$160 MeV) can be utilized themselves to irradiate targets, yielding a considerable improvement in the ratio of neutron rich to neutron deficient isotopes induced in the targets (5,6). However, the neutron exposed targets must be as close as possible to the back of the proton target stack to achieve maximum neutron flux. In effect, this capability duplicates that of the Medium Energy Intense Neutron Facility (MEIN) (5) at BNL but larger targets and longer irradiations are possible at BLIP.

Improvements to the beam line were implemented as well. An additional vertical pitching magnet was inserted so the 12.7 m beam line now contains two large bending magnets, 4 vertical pitching magnets and 4 quadrupole focusing magnets. Two new multiwire secondary emission monitors were installed to provide accurate beam profile information continuously. These secondary emission monitors operate in vacuum and are more reliable than the preexisting gas flow multiwire ionization chambers. An electronic control system containing sensors of such parameters as radiation levels, water pressure, temperature and flow, ventilation rate, and shielding is interlocked with the beam and allows unattended operations for extended periods.

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A MODEL FOR BEAM PENETRATION IN GAS TARGETS

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The penetration of charged particles in gas targets has been studied by several groups concerned with radionuclide production (1-3). An increase in the particle beam penetration with increasing beam current on the target has thus been observed. This is caused by a reduction of the target gas density in the beam.

A conical target chamber, previously described (4), equipped with a glass window was used in a photographic study of the proton beam penetration in nitrogen gas targets (5). The light emitted by nitrogen atoms in the beam volume of the target gas was photographed in both the vertical and the horizontal plane (5). The proton beam penetration (r) was measured from the photographs and the penetration increase ($\Delta r = r - r_0$) in relation to the tabulated proton range (r_0, Ref. 6) was determined. The target pressure was observed with a Kulite XTM-190 pressure transducer with the exception of one study, where a pressure gauge was used.

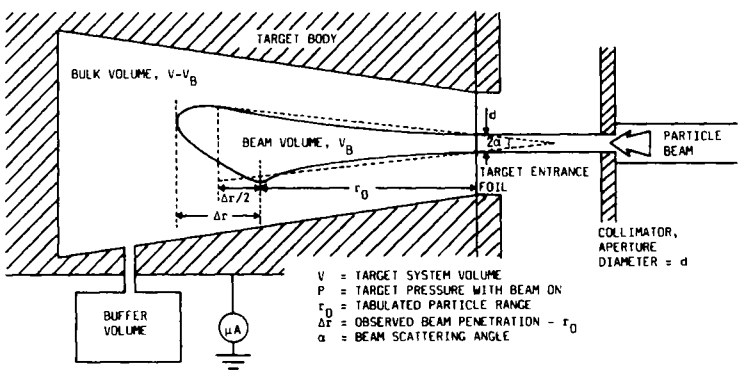

Figure 1. Illustration of parameters for derivation of model for beam penetration in gas targets (not to scale).

The proton beam penetration in nitrogen gas targets under static condition was studied using four different target system volumes (56, 88, 150 and 1060 cm^3). The target system volume was varied by connecting different buffer volumes to the target chamber (Fig. 1). Using the four different target system volumes the beam penetration was studied for a 10.4 MeV proton beam incident on nitrogen gas as a function of the beam current on the target. The initial target pressure was 1.088 MPa in all runs. An increased proton beam penetration in the target was observed when the beam current was increased due to the reduction of the gas density in the beam volume of the target. The range of the lowest part of the proton beam, most clearly observed in the horizontal views for a low beam current on the target (e.g. 1 μA, Refs 4 and 5), corresponded within the experimental errors ($\lesssim 1.5$ %) to the tabulated proton range r_0 (6) at the initial target pressure and temperature. The increase in beam penetration related to the tabulated particle range ($\Delta r/r_0$) is shown as a function of beam current in Fig.2 for the four target system volumes. As can be seen, the proton beam penetration in nitrogen was strongly dependent on the target system volume. The relative increase in proton beam penetration for the 150 cm^3 target system volume was 50 % for a 7.5 μA beam current on the target, whereas that for the 56 cm^3 target system volume and the same beam current was 31 % (5).

Using the 150 cm^3 target system, the proton beam penetration in the nitrogen-gas target was studied under flow-through conditions for the gas flow rates 100, 200, 300, 400 and 500 cm^3/min. The calculated $\Delta r/r_0$ values are shown in Fig. 2.

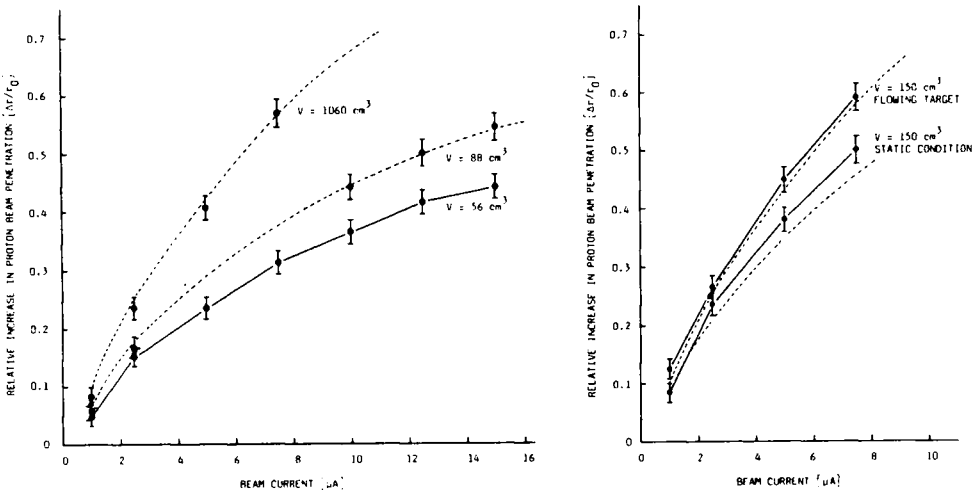

Figure 2. Relative increase in beam penetration ($\Delta r/r_0$) for 10.4 MeV protons on nitrogen gas under static (left) and flow-through conditions as function of beam current on target. V = target system volume. Gas flow rates = 100 - 500 cm^3/min for flowing condition. Initial target pressure P_0 = 1.088 MPa. The dashed lines represent the values for $\Delta r/r_0$ predicted by the model (Eq. (3), $\alpha = 2^o$).

A small variation in the $\Delta r/r_0$-values was observed at the flow rates used. This was within the limits of the experimental uncertainty (Fig. 2). No target pressure increase as compared to the initial target pressure (1.088 MPa) was seen at steady state conditions with beam on the target. As can be seen in Fig. 2, the relative increase in beam penetration was higher in the 150 cm^3 gas target under flow-through conditions than in the 150 cm^3 target system under static condition. As expected the cooling effect of the flowing target gas does not reduce the density reduction in the beam strike at the low gas flow rates used (100-500 cm^3/min, Ref. 5).

A model for estimation of the beam penetration in a gas target was developed. The model requires knowledge of the penetration of the beam in a reference target. The target pressure with beam on must also be known, both for the reference system and the system to be evaluated. Both targets must be irradiated with the same beam parameters (energy, intensity, collimation and focusing). The model also requires that the scattered beam does not reach the target chamber walls.

The average material thickness required to fully stop a charged particle of a certain energy is given by the mean range value. The range value given in mg/cm^2 is density independent. Thus, for the same target gas, the average number of atoms in the volume of a beam fully stopped in the target is constant and independent of the density reduction in the beam, but defined by the incident beam energy and intensity.

The target system volume can be considered as being composed of two partial volumes. The beam volume (V_B, Fig. 1) is the volume containing the primary charged particles still having kinetic energy. The rest of the target system can be considered as a bulk volume (V_b).

Next, consider two target systems of different volumes (the reference system = 1, the system to be studied = 2). The beam volumes are V_{B1} and V_{B2}, respectively. The equation of state, PV =[m/M]RT, where P, V, m, M, R and T have their usual meaning, gives for the number of target atoms N, in the beam volumes (N_a = Avogadros's Number):

$$N = \frac{m}{M} N_a = \frac{PV}{RT} N_a \text{ , and thus, } \frac{P_1 V_{B1}}{T_1} = \frac{P_2 V_{B2}}{T_2} . \quad (1)$$

The power dissipated by the particle beam in the target gas is constant for the two target systems. For systems with volumes of the same magnitude, the transfer of heat from the beam volume can in a first approximation be considered equal. This means that the average temperatures, T_1 and T_2, for the two beam volumes are approximately equal. Equation (1) then reduces to

$$V_{B2} = \frac{P_1}{P_2} V_{B1} . \quad (2)$$

The beam volume is simulated by the volume of a truncated cone with the height $r_0 + \Delta r/2$. Using Eq. (2) and the symbols outlined in Fig. 1, and assuming a small scattering angle α, i.e. $tg\alpha \approx \alpha$, the following expression for the relative increase in particle beam penetration can be derived:

$$\frac{\Delta r}{r_0} = \frac{2}{r_0}\sqrt[3]{\frac{P_1}{P_2}\left[\left(r_0 + \frac{\Delta r_1}{2} + \frac{d}{2\alpha}\right)^3 - \frac{d^3}{8\alpha^3}\right] + \frac{d^3}{8\alpha^3}} - \frac{d}{2\alpha} - r_0 . \quad (3)$$

The values for the increase in the particle beam penetration Δr_1, were determined, as a function of beam current, with the help of the horizontal views of the beam in the 56 cm^3 reference target system. The collimator diameter d was 0.5 cm. For the 10.4 MeV proton beam incident on nitrogen gas the tabulated range r_0 is 154.81 mg/cm^2 (6), corresponding to 11.7 cm at the initial target pressure 1.088 MPa and 278 K (cooling water temperature). The scattering angle for the fully stopped beam was determined from the photographs and was found to be 2.0 ± 0.3°. The values for the relative increase in beam penetration, $\Delta r_2/r_0$, were calculated according to Eq. (3) for the target systems with the volumes 88 cm^3, 150 cm^3 and 1060 cm^3. As can be seen in Fig. 2, the calculated values for the relative increase in beam penetration were in good agreement, or within the experimental uncertainty, with the experimentally determined values, except for the 150 cm^3 target system under static condition. For this the calculated values agreed with the experimental values within 11 %. This was partly caused by an uncertainty in the target pressure. For the 150 cm^3 system this was determined with a pressure gauge.

The photographic view reflects the shape of the beam in the gas target and approximately gives the maximum penetration of the beam. A strongly volume-dependent beam penetration in nitrogen gas targets was observed. A large bulk volume in the target system allows the beam volume to expand. The relative increase in beam penetration was found to be higher in a gas target irradiated under flow-through condition than in the target under static condition. The volume of the target system in the flow-through condition can be considered as infinitely large.

The model gives an estimate of the particle beam penetration in a gas target. The approximation concerning the average temperature ($T_1 = T_2$) in the beam volumes of the target systems, is rough. The large amount of bulk gas in a target system with a large volume has a negative effect on the heat transfer from the beam volume as compared to the small volume system. In this study the difference in heat transfer between the two target systems was partly eliminated by the use of external buffer tanks for varying of the target system volume. This made the modelling easier but, at the same time, the experimental results underestimated the beam penetration in the large volume target.

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A REPORT ON THE HEIDELBERG TARGETRY WORKSHOP

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In October 1985 a workshop was held in Heidelberg, FRG to address the problems associated with the production of radionuclides used in biology and medicine. There were nearly 70 participants representing nearly every continent.

This workshop had its genesis in 1979 when two of the authors met at an accelerator applications meeting and recognized the need to share problems and ideas as related to the production of radionuclides and their target systems that are not suited for presentation, in general, at scientific meetings. Over the next several years this concept was discussed with other targetry colleagues and finally after the Fifth Radiopharmaceutical chemistry meeting in Tokyo the time looked right for bringing together the international community to conduct a workshop on the "nuts and bolts" of targetry.

The format of the workshop (Table 1) was designed to have as much open discussion and exchange as possible. For the most part this was accomplished by having a brief overview of what was in the literature and/or was known as folklore. This presentation was followed by an open discussion and question and answer period. These questions and their answers were not directed to any one in particular but were generally shared among those present.

The first session covered Material Sciences. Within this session there were four topics - windows, target bodies, transfer lines, and target materials.

One of the more interesting and somewhat surprising discussions centered around the inconsistent recovery of ^{11}C as produced by the $^{14}N(p,\alpha)^{11}C$ reaction. Some groups reported good yields with 99.9999% N_2 while others had to add a trace of O_2 to achieve good recoveries.

The second session dealt with beam transport and interaction with matter. Through the careful work at BNL and Turku we have a better understanding as to what is happening in thick gas targets. However, there is still work

TABLE 1. Outline of the Topics in Workshop Sessions

I. Material Sciences
   A. Windows - thermal and physical properties, radiation damage, seals, cooling
   B. Target bodies - corrosion, activation, contaminants, cooling
   C. Transfer lines - metals, plastics, fittings, tuning
   D. Target materials - gases, liquids, solids, purity, carriers enriched

II. Beam transport and interaction with matter
   A. Beam transport - optics, monitoring, diagnostics
   B. Interaction with matter - density reduction, scattering, hot atom chemistry

III. Nuclear data and cyclotron parameters - reliability of cross sections, thick target yields, small/big machines

IV. Scheduling - optimizing production, target sequencing, multipurpose and tandem targets processing

V. Reports from participating labs

VI. Future Direction

needed in dealing with higher energy reactions where the cross section is optimum over only a few MeV at 20-30 MeV. How does one achieve "thick target" when using for example highly enriched ^{124}Xe.

Iain Trevena of AECL shared a story of how they lost and then recovered their target charge of ^{124}Xe through window failure.

The third session dealt with nuclear data. While most people were reluctant to express the need for more or new data for most radionuclides of interest there were a few candidates that needed review. Yves Jongen of Louvain made a presentation of their proposed cyclotron. The discussion centered around whether it should be a proton/deuteron machine or accelerate only protons.

In the fourth session we discussed scheduling and the optimization of radionuclide production. Because there were representatives of groups just embarking in this field there was a lengthy question and answer period. Also some established groups were beginning new phases, i.e. from mainly research to a more clinically orientated program which required new approaches.

The next session was a potpourri of presentations from the various groups represented at the meeting. In the final session possible future topics were raised one of which was how to reduce and minimize radiation exposure to the personnel involved in radionuclide production and the maintenance of these systems.

While everyone expressed interest in having another workshop the time and place were not decided upon. The major concern was to not interfere with the Radiopharmaceutical Chemistry meeting and also not to be so frequent as to be ineffective.

The authors wish to thank the participants for the very frank and open manner with which they shared their ideas. Also a great deal of gratitude is expressed to the DKFZ in Heidelberg for their hospitality and for making the workshop possible through their sponsorship.

THE DEVELOPMENT OF FLUOROANDROGENS AND FLUOROPROGESTINS AS POTENTIAL IMAGING AGENTS FOR RECEPTOR-POSITIVE PROSTATE AND BREAST TUMORS

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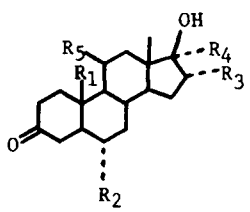
The assay of progesterone receptor (PR) concentration in breast tumors and androgen receptor (AR) concentration in prostate tumors enables hormone responsive neoplasms to be distinguished from those that are non-responsive (1). In principle, a positron-emitting progestin or androgen with suitably high affinity and selectivity for PR and AR, respectively, and an adequately high specific activity might provide a means for imaging receptor-positive tumors and quantifying their receptor content in vivo. The use of fluorine-18 as a radiolabel, coupled with the use of positron emission transaxial tomography, appears to be a most favorable approach in the development of receptor binding radiopharmacueticals for in vivo imaging. Therefore, we have begun a systematic investigation of the development of fluorine-substituted androgens and progestins that might be prepared in F-18 labeled form as probes for AR and PR.

TABLE 1. Relative Binding Affinities of Fluoroprogestins and Fluoroandrogens for Progesterone-, Androgen-, and Estrogen Receptors



Compound	R_1	R_2	R_3	R_4	R_5	Unsaturation at C	PR[a]	AR[b]	ER[c]
1	H	H	H	CH_2F	H	4	43	8	~0
2	-	H	H	CH_2F	H	5(10)	4	~0	~0
3	H	H	H	CH_2F	H	-	16	5	~0
4	CH_3	H	H	CH_2F	H	4	1	2	~0
5	CH_3	H	H	C≡CH	F	4	~0	~0	~0
6	CH_3	H	H	H	F	4	~0	3	~0
7	CH_3	H	H	H	F	-	2	62	~0
8	H	H	H	H	F	-	1	130	-
9	CH_3	F	H	H	H	4	1	10	~0
10	CH_3	F	H	C≡CH	H	4	32	2	-
11	H	H	CH_2CHFCH_3	H	H	4	29	1	~0
12	H	H	H	$C{\equiv}CCH_2F$	H	4	66	~0	-

[a]Progesterone=100. [b]Dihydrotestosterone=100. [c]Estradiol=100.

In designing these fluorine-substituted ligands for AR and PR, we considered those features reported to enhance the binding affinity and selectivity of the ligands to their respective receptors and decrease their affinity for other receptor systems (maximize homologous and minimize heterologous binding), and reduce their non-specific binding. Thus, AR-selective ligands might include a 17α-hydrogen, 4,5-dihydro moiety or the absence of 19-methyl (2), and PR-selective ligands might have an ethynyl (3,4), another larger, unsaturated alkyl group, or a halomethyl (5) in the 17α-position, an alkyl group in the 16α-position (4,6) and/or 10β-hydrogen (4). From the limited number of reports concerning the binding of fluorine-substituted androgens and progestins to their respective receptors, it was apparent that fluorine is well-tolerated at numerous

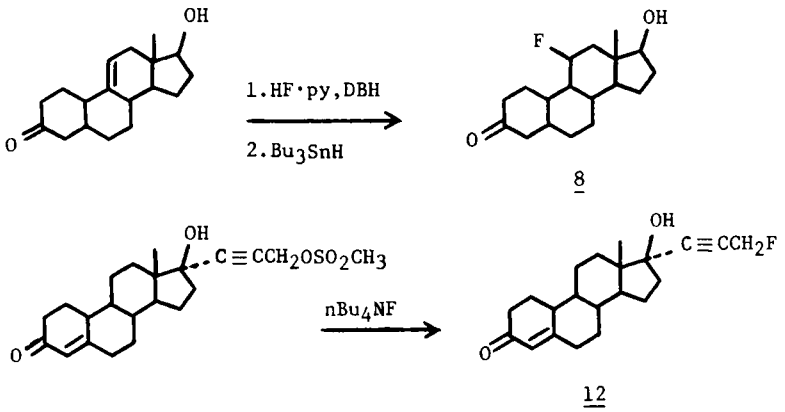


Figure 1: Synthetic schemes for the preparation of compounds 8 and 12.

sites (7). We therefore prepared androgens and progestins substituted with fluorine at positions where F-18 might ultimately be introduced in a facile manner. The structures of these compounds and their affinities for progesterone, androgen and estrogen receptors, obtained from in vitro radiometric competitive binding assays using cytosol from estrogen-induced female rats, rat ventral prostate and lamb uterus, are listed in Table 1. Fluoroandrogen 8 and fluoroprogestin 12 bind with the greatest affinity and with high selectivity to AR and PR respectively.

We have modified the synthesis of 8 and 12 so that trace amounts of fluoride (ultimately F-18) may potentially be incorporated quickly and efficiently (Figure 1). Compound 8 can be prepared by bromofluorination of 9(11)-estren-3-on-17β-ol to give 9α-bromo-11β-fluoroestran-3-on-17β-ol, followed by reductive debromination. Both the halofluorination and debromination reactions are rapid (15-30 min) and yields are high. The halofluorination reaction has been found to be a suitable method for the incorporation of radioactive fluoride: bromofluorination of allylbenzene using fluorine-18 results in radiochemical yields exceeding 50% without carrier addition (8). The direct precursor to 12 is the 17α-propargyl methanesulfonate of 19-nortestosterone, which is rapidly displaced in high yields by fluoride. Similar displacements of trifluoromethanesulfonate esters of steroidal systems using fluorine-18 proceed rapidly with radiochemical yields of 20-40% (decay-corrected) (9).

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SYNTHESIS AND DIRECT ESTROGEN RECEPTOR BINDING AFFINITY OF ^{80m}Br-LABELED 1,1-BIS(p-HYDROXYPHENYL)-2-BROMO-2-PHENYLETHYLENE*

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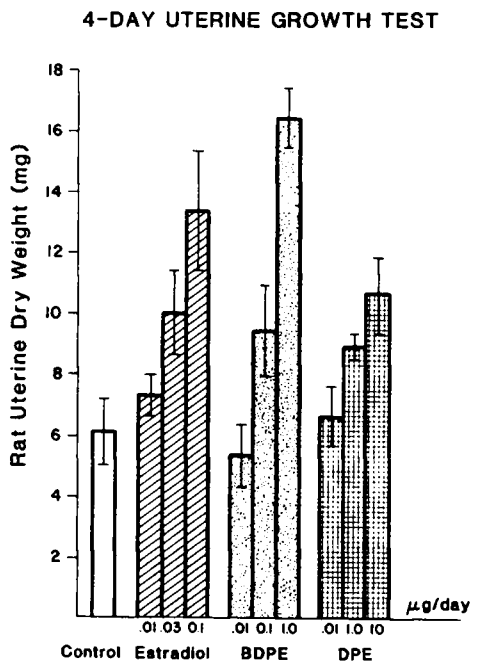
Estrogen receptor (ER) binding radiopharmaceuticals have potential for use in the diagnosis and treatment of cancers of the female reproductive system. A potential means of treating cancers that contain the ER is to use ER binding molecules to carry Auger electron emitting nuclides to those cells that contain the ER. The thymidine analogs, 5-iodo-2'-deoxyuridine and 5-bromo-2'-deoxyuridine when labeled with the Auger electron emitting nuclides ^{125}I and ^{77}Br are readily incorporated into the DNA of dividing cells and are cytotoxic to those cells (1,2). Both 16-α-iodoestradiol, a steroidal estrogen, and iodotamoxifen, an antiestrogen, when labeled with ^{125}I have been shown to be cytotoxic to ER containing cells in culture (3,4).



*This work was performed under the auspices of the U. S. Department of Energy under contract number W-31-109-ENG-38, and the National Cancer Institute under contract number CA-27476.

We have been investigating the use of the Auger electron emitting nuclide ^{80m}Br for use in labeling ER binding compounds. Bromine-80m has a half-life of 4.4 hours and produces an average of 6-7 Auger electrons from each decay. Bromine-80m is produced at the Argonne National Laboratory Cyclotron using a stainless steel target containing ^{83}Kr and the (d,n,α) reaction at 23 MeV. Unlabeled 1,1-bis(p-hydroxyphenyl)-2-bromo-2-phenylethylene (1) (BDPE) has been prepared and in competitive ER binding studies with tritiated estradiol it has been shown to bind strongly to the ER in vitro (5). The relative binding affinity of BDPE is 67 where estradiol is 100 (5). In the four day immature rat uterine growth test (Figure 1), BDPE was shown to be a strong estrogen. The synthetic route utilized in the synthesis of ^{80m}Br labeled BDPE is outlined in Figure 2. First the phenolic groups of cold BDPE were pro-

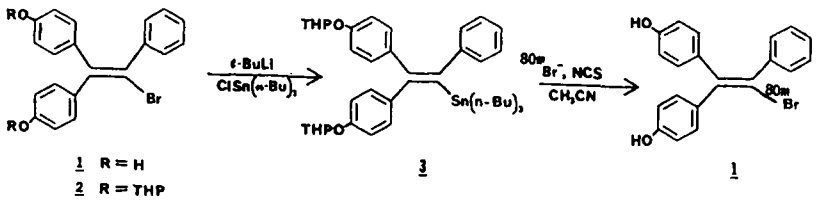
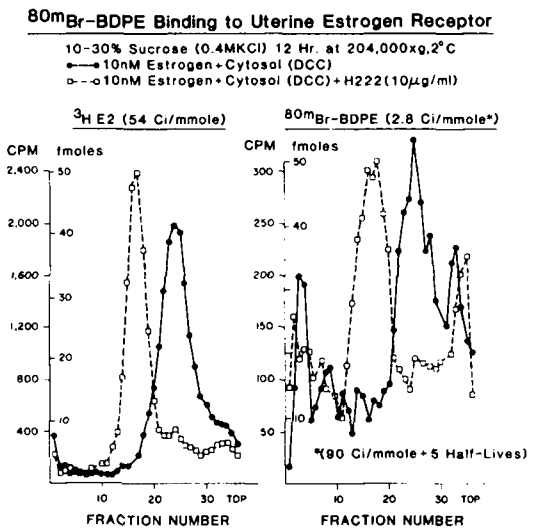
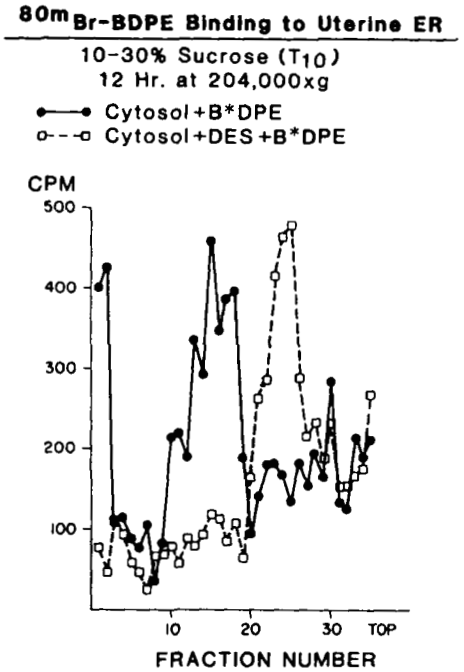


Figure 2. Synthesis of ^{80m}Br labeled BDPE.

tected as tetrahydropyranyl ethers (THP ethers). The vinyl tin compound 3 was prepared from 2 using t-butyl lithium and tri-n-butyl tin chloride. The bromination-destannylation reaction and the hydrolysis of the THP ethers resulting in labeled BDPE occurred in one vial using n-chlorosuccinimide in an acidic acetonitrile/water mixture. In six experiments a radiochemical yield of 65% with a mean specific activity of 150 Ci/mmole has been achieved. At this specific activity ^{80m}Br labeled BDPE was shown by sedimentation analysis to bind strongly to the ER and that the binding was inhibited by excess diethylstilbestrol (Figure 3). Also the monoclonal antibody to the estrogen receptor protein, H222, recognizes the complex of ^{80m}Br labeled BDPE with the ER (Figure 4). Thus BDPE is an excellent ligand to bind the estrogen receptor in culture.





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SYNTHESIS OF ^{11}C-LABELLED TRACERS FOR STUDIES OF FUNCTIONAL MAO ACTIVITY IN BRAIN USING PET.

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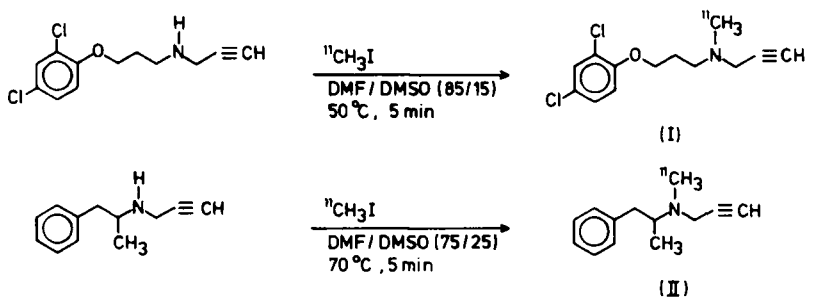
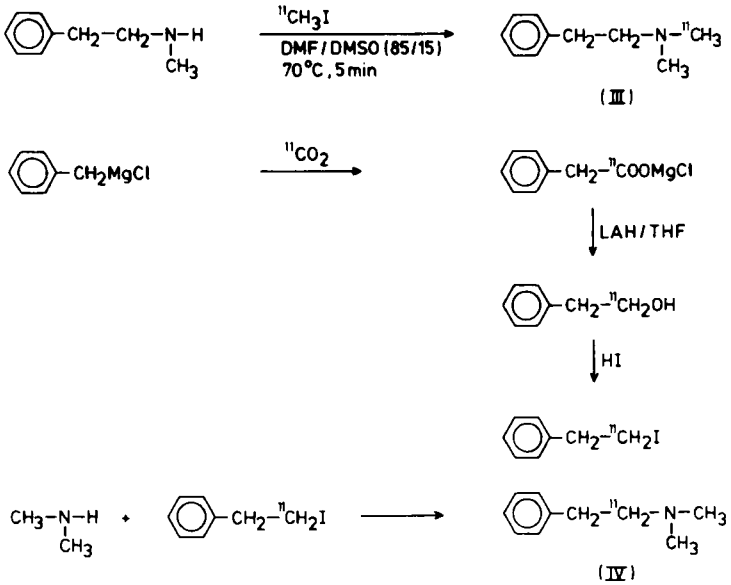
Monoamine oxidase (MAO, EC 1.4.3.4.) oxidatively deaminates endogenous neurotransmitter amines and exogenously administered drugs. The enzyme has been subdivided into two types: MAO-A and MAO-B, characterized by substrate and inhibitor selectivity (1). The A-form is selectively and irreversibly inhibited by clorgyline and the B-form by L-deprenyl. Since both clorgyline and L-deprenyl act as suicide inhibitors and deactivate the enzyme by covalent bonding to its active site, they may in principle be used to label MAO in vivo (2-3). Therefore, to investigate the use of PET in the study of enzyme dynamics and disease, we have synthesized clorgyline and L-deprenyl labelled with ^{11}C (4). Another approach for studies of functional MAO activity is to use substrates for MAO. Dimethylphenethyl amine (DMPA), might be used as a radiotracer for in vivo measurement of MAO-B activity and was therefore labelled with ^{11}C, both in the methyl (5) and the phenethyl group (6). Methylphenethyl amine (MPA) was also labelled with ^{11}C.



Scheme 1

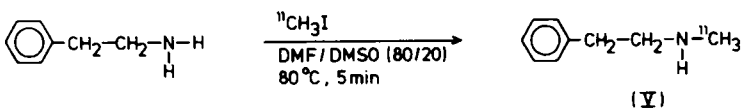
Labelling of clorgyline (I) and L-deprenyl (II) was accomplished by N-alkylation of the free base of the desmethyl compound in a DMF/DMSO solvent mixture, as is shown in Scheme 1. A multivariate optimization method Simplex (7) was used to optimize the incorporation of ^{11}C-methyl iodide. The radiochemical yields were 93 % and 85 % for I and II, respectively, based on ^{11}C-methyl iodide. The total synthesis time was 40 min, including LC-purification, and the specific activity was on the order of 200 Ci/mmol.



Scheme 2

Dimethylphenethyl amine (DMPA), was labelled in the methyl (III) and phenethyl (IV) groups by N-alkylation of the free base of the corresponding norcompounds, as is shown in Scheme 2. The radiochemical yield of III was > 95 % based on ^{11}C-methyl iodide, with a total synthesis time of 40 min. The radiochemical yield of IV was 10-15 % based on ^{11}C-carbon dioxide with a total synthesis time on the order of 50-60 min.

Methylphenethyl amine (MPA,V) was labelled in the methyl group by N-alkylation of the free base of the desmethyl compound, as is shown in Scheme 3. The radiochemical yield of V was 92 % based on ^{11}C-methyl iodide. The total synthesis time was 40 min.



Scheme 3

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POSITRON -EMITTING ANALOGS OF SCH 23390 FOR THE STUDY OF DOPAMINE D1 RECEPTORS

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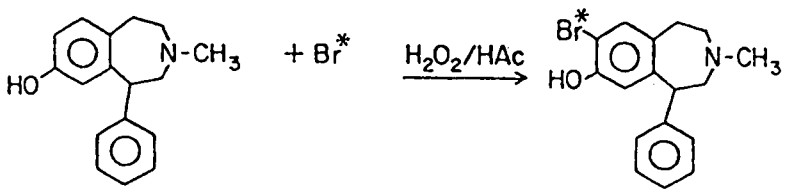
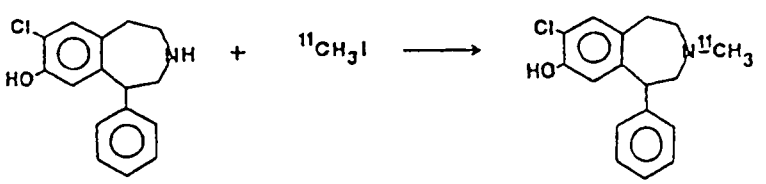
Dopamine (DA) receptors in the CNS have been classified into D1 and D2 receptors based on their linkage to the membrane enzyme adenylate cyclase (1,2). Although the potency of antischizophrenic drugs is highly correlated with their ability to bind D2 receptors in vitro (3,4), the role of D1 receptors still remains to be elaborated. With the discovery of benzazepines as selective D1 ligands either as agonists or antagonists (2), studies on the function of D1 receptors and the functional significance of multiple classes of DA receptors are actively being pursued in several laboratories. We have prepared two positron emitting analogs of SCH 23390 in order to assess its potential as imaging agents in positron emission tomography (PET). To date, PET studies on CNS DA receptors have utilized analogs of butyrophenones which are D2 antagonists. Non-invasive studies of both classes of DA receptors may provide valuable insights into their role in normal and diseased states.

We have prepared an 8-bromo analog of SCH 23390 (BrSCH or SKF 83566) labelled with Br -75 or Br-76 by the reaction of its des-chloro analog with radiobromide in H2O2-glacial HOAc (Scheme 1)(5). After confirming the identity of the product by physical (NMR and mass spectrometry) and pharmacological (inhibition of DA-induced vasodilation of dog renal artery) methods, we followed the time course of the distribution of Br-75-BrSCH in mouse brain. The results showed receptor-mediated uptake, i.e., appropriate regional uptake and saturable uptake in the striatum. The striatum to cerebellum uptake ratio was 25 two hours after injection .



SCHEME 1



SCHEME 2

An anesthesized 8.5 kg male rhesus monkey was given, i.v., ca. 2 mCi Br-75-BrSCH on two occasions and scanned with the University of Chicago PETT VI system (6). Results revealed that the drug localized specifically in the basal ganglia, which provide evidence that D1 receptors, like D2 receptors, are localized in the striatum. The time course of the drug was relatively fast - radioactivity in the brain peaked ca. 30 minutes after injection - consistent with the known short acting effect of this drug. Because of the rapid pharmacokinetics of BrSCH, we synthesized SCH 23390 labelled with C-11 (t 1/2 = 20 min.). C-11-SCH 23390 was prepared by the radio-methylation of des-methyl SCH 23390 using C-11-CH3I (Scheme 2) . Ten minute reaction at 40° C resulted into 60%-80% radiochemical yield based on C-11-CH3I. HPLC purification gave a product which was found by mass spectrometry to have an identical fragmentation pattern as the authentic sample. Administration of C-11-SCH 23390 into several sets of mice showed that the critical organs are the liver, kidney, and the G.I. tract. The distribution of the drug in the mouse brain showed a striatum to cerebellum uptake ratio of 23 one hour post injection. Moreover, the binding in the striatum was saturable.

In experiments involving two anesthesized rhesus monkeys each scanned twice on separate occasions after receiving 2-4 mCi C-11-SCH 23390 (dose = 4-34 ug/kg), this radioligand was found to enter the brain rapidly and taken up, distributed, and cleared in a manner consistent with mediation by DA receptors. Blocking experiments to characterize the localization of Dl receptors in the monkey brain and saturation experiments to assess receptor parameters were done. Results demonstrate that PET involving both C-11 SCH 23390 and current D2 ligands, such as p-bromo-spiroperidol (7), would be a useful tool in differentiating receptor subtypes in the primate brain and may aid in elucidating the functional significance of Dl and D2 receptors.

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SYNTHESIS OF ^{11}C-SCH 23390, A DOPAMINE D-1 RECEPTOR ANTAGONIST, FOR USE IN IN VIVO RECEPTOR BINDING STUDIES WITH PET

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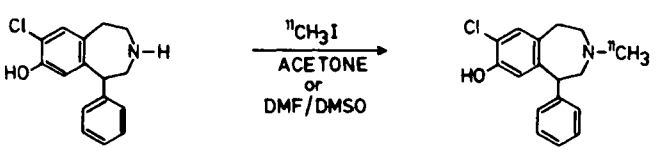
Central dopamine receptors are generally accepted to exist in at least two distinct subtypes: D-1 and D-2. Recently a benzazepine, SCH 23390, was reported (1) to be a selective D-1 dopaminergic antagonist. PET studies of the radio-brominated ^{76}Br-SCH 23390 reported by Friedman, et al. (2) indicated that the analog exhibits specific binding in the striatum of the monkey brain. Here we report the synthesis of ^{11}C-SCH 23390 (3) suitable for the in vivo study of dopamine D-1 receptors in the human brain (4).

Labelling of SCH 23390 ((R)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1 H-3-benzazepin-7-ol) was accomplished by N-alkylation of the desmethyl compound, SCH 24518, as is shown in Scheme 1. A response surface method, Simplex (5), was used to optimize the incorporation of ^{11}C-methyl iodide. Optimal radiochemical yields were obtained in both acetone and DMF/DMSO.



Scheme 1

Both straight- and reversed-phase preparative liquid chromatography were investigated for the purification of ^{11}C-SCH 23390. However, straight-phase LC was found to be superior to reversed-phase since the possibility of contamination by the desmethyl compound is eliminated.

The radiochemical yield was 80% (acetone) based on ^{11}C-methyl iodide and the radiochemical purity was better than 99%. The total synthesis time was 35-40 min and the average specific activity obtained (E.O.S.) was on the order of 11.1 GBq/mmol (300 Ci/mmol).

The ^{11}C-labelled SCH 23390 was used to visualize the dopamine D-1 receptor-rich areas in the monkey (3) and human (4) brain. The present results support the view that SCH 23390 has a high affinity for D-1 receptors in the striatum and that the ^{11}C-labelled compound will be useful as a selective tool for the analysis of a subtype of dopamine receptors in the living human brain.

This work was supported by grants from the Bank of Sweden's Tercentenary Foundation, the Swedish Natural Science Research Council, the Karolinska Institute and the Swedish Medical Research Council, which is gratefully acknowledged. We would also like to thank Dr. Allen Barnett of Schering-Plough Pharmaceutical Corporation for the supply of SCH 23390 and SCH 24518 as well as Mr. Göran Printz and Dr. Petter Malmborg for assistance with the radionuclide production.

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SYNTHESIS OF RADIOTRACERS FOR STUDYING MUSCARINIC CHOLINERGIC RECEPTORS IN THE LIVING HUMAN BRAIN USING POSITRON EMISSION TOMOGRAPHY: [11C]DEXETIMIDE AND [11C]LEVETIMIDE

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The localization and quantitation of muscarinic cholinergic receptors (m-AChR) in the living human brain using a non-invasive method such as positron emission tomography (PET) may provide valuable information about receptor changes which have been observed post mortem in patients with Huntington's chorea and Alzheimer's dementia (1-5), as well as normal brain mechanisms mediated by the m-AChR. Although 3-quinuclidinyl benzilate (known as QNB) has be radioiodinated (6,7) and methylated to give methyl-QNB (8), the former is not useful for PET studies and the latter quaternary homolog does not penetrate the blood-brain barrier and therefore is not useful for studying m-AChR in the brain. Laduron and co-workers demonstrated with [3H]dexetimide and [3H]levetimide that dexetimide (figure 1a) bound to m-AChR *in vitro* and *in vivo* (9,10) with high affinity, low non-specific binding, and slow dissociation rate. The accumulation of dexetimide was stereospecific, saturable, and displaceable while levetimide (figure 1b) did not show preferential uptake or stereospecific displacement in regions known to have high concentrations of m-AChR. Based on these results, we chose to label dexetimide as a radiotracer for studying the m-AChR and levetimide as a radiotracer for assessing non-specific binding associated with the *in vivo* receptor binding studies.

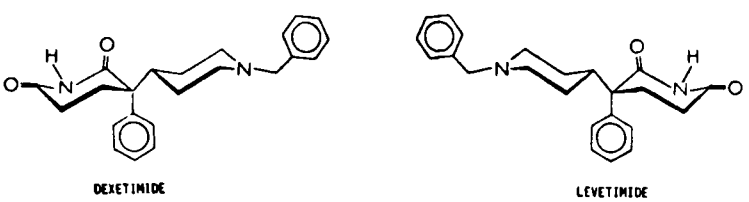
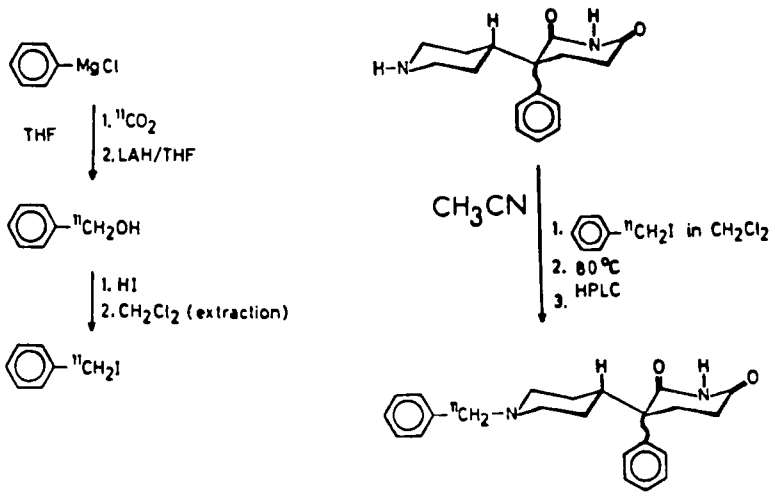


Figure 1.
Chemical structures of (A) dexetimide and (B) levetimide

The synthesis of [11C]dexetimide (or [11C]levetimide) shown in figures 2 and 3 required approximately 35 minutes from end of bombardment and involved five major steps: Grignard reagent carboxylation, hydride reduction, iodination, alkylation, and HPLC purification. The radiochemical yield based on [11C]benzyl iodide was approximately 20 % while the overall radiochemical yield was approximately 8 % based on the initial activity of [11C]carbon dioxide delivered.

Radiochemical purity was confirmed by HPLC. The area of the u.v. absorbance corresponding to carrier product was used to determine the amount of carrier present. Using the measured radioactivity and mass in the aliquot, the average specific activity was determined to be 905 mCi/μmole at the end of synthesis. This corresponds to an average specific activity at the end of bombardment or approximately 3000 mCi/μmole.



Figures 2 and 3.
Synthesis of [11C]dexetimide and [11C]levetimide

Following the intravenous administration (10 μg/Kg) in mice of [11C]dexetimide, high radioactivity concentrations in the striatum and cerebral cortex and a low radioactivity concentration in the cerebellum were observed; this distribution corresponds to the known distribution of m-AChR in the brain. Striatum-to-cerebellum and cortex-to-cerebellum ratios for [11C]dexetimide increase linearly throughout the time of observation with these ratios being 12.7 and 10.5 at 60 minutes post injection. The corresponding labeled enantiomer gave low homogeneous radioactivity concentrations.

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PREPARATION OF CARBON-11 LABELLED PRAZOSIN, A POTENT AND SELECTIVE α1-ADRENORECEPTOR ANTAGONIST

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Several types of receptors for neurotransmitters in living heart have been investigated by positron emission tomography (PET). The muscarinic cholinergic receptor has been characterized by using |^{11}C|MQNB (1) and the peripheral type benzodiazepine receptor by using |^{11}C|PR 11195 (2). |^{11}C|propranolol and |^{11}C|practolol have also been synthetized for studying the beta-adrenergic receptor (3,4).

For in vivo studies of α1-adrenoreceptors using PET we chose to label the high affinity, selective antagonist, prazosin, 2-|4-(2-furoyl)piperazin-1-yl|-4-amino-6,7-dimethoxyquinazoline (5), with carbon-11. Our approach was based on the recently developed technique of preparing a |^{11}C|acid chloride as labelling agent (6). Here we report the preparation of |^{11}C|prazosin by the reaction of |2-^{11}C|furoyl chloride with the appropriate secondary amine.

2-Bromofuran is prepared by the copper-catalysed decarboxylation of 5-bromofuroic acid in quinoline (7) and converted into 2-furylmagnesium bromide by reaction with magnesium in diethyl ether. |^{11}C|Carbon dioxide is produced by the ^{14}N (p,α) ^{11}C nuclear reaction and trapped at a high flow rate in a stainless steel coil cooled by liquid argon. The trap is then warmed and the $^{11}CO_2$ transferred with a slow flow of nitrogen (ca. 2 ml/min) into 2-furylmagnesium bromide (0.02 mmol) in diethyl ether (1 ml) at room temperature. After 2 min the |^{11}C|carbonation is quenched by the addition of phthaloyl dichloride (2 µl, 0.014 mmol) in diethyl ether (0.3 ml). 2,6-Di-t-butylpyridine (100 µl, 0.45 mmol) in diethyl ether (0.3 ml) is then added and the diethyl ether is evaporated at 75°C with a flow of nitrogen (ca. 10 ml/min). The generated |2-^{11}C|furoyl chloride (b.p. 174°C) is then distilled at 225°C with a nitrogen flow of 10-15 ml/min into a solution of the secondary amine, 2-|piperazin-1-yl|-4-amino-6,7-dimethoxyquinazoline (2 mg, 7 µmol) in tetrahydrofuran (1 ml) kept at 0°C. The reaction mixture is then heated at 75°C for 1 min to promote the formation of |^{11}C|prazosin. After evaporation of the solvent, the radioactive residue is taken up in 1.5 ml of mobile phase CH_2Cl_2 containing 2.5 % (V/V) of solution A (EtOH : H_2O : ethylamine 100:2:2, V/V) and injected onto a µporasil column, 300 x 7.8 mm (Waters) which is eluted at 4 ml/min with the same mobile phase. |^{11}C|prazosin which separates from other labelled and non-labelled compounds is collected between 7,5-9 min. This solution is evaporated to dryness and the residue dissolved in 300 µl EtOH, diluted to 10 ml with sterile saline and sterilized by filtration through a Millipore filter, 0.22 µm. The time of synthesis is 40-45 min with an overall radiochemical yield of 25-35 % (decay-corrected from |^{11}C|carbon dioxide). Using a short irradiation time (5 min, 15 µA protons 20 MeV) the specific radioactivity obtained has been about 200 mCi/µmol. In the synthesis of |^{11}C|prazosin for PET-investigations a longer irradiation time will be used (30 min, 30 µA), which means that we will start the synthesis with at least 4-5 times as much activity. Theoretically this means that the specific radioactivity will increase about 4-5 times, giving a specific radioactivity of about 0.8-1.0 Ci/µmol at EOS.

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LABELED LIGANDS FOR THE IN VIVO MEASUREMENT OF THE β -ADRENERGIC RECEPTOR

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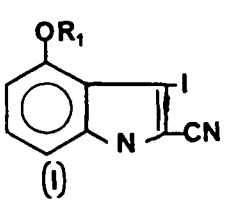
There have been a number of attempts at using labelled ligands for the β-adrenergic receptor to image this receptor in vivo[1,2]. However these attempts were not successful in that the ligand accumulated in receptor rich tissues but this accumulation was not affected by saturating doses of unlabelled ligand. A more recent publication giving limited data on the in vivo behavior of iodine-125 cyanoiodopindolol (I) suggest that ligands of this type would be suitable for imaging in vivo[3].

Experiments with isolated perfussed rat hearts and (I) predict that it will be possible to both detect and quantitate the β -adrenergic receptor in vivo from a single injection of a high specific activity ligand[4]. The method requires that the kinetics of binding of the ligand to the receptor be slow enough to be observable, that the dissociation of the ligand-receptor complex be slow compared to the association and that the half-life of the radionuclide be long enough to observe all these phenomena. For use with positron tomography this requires a ligand for the β -adrenergic receptor with kinetic properties similar to I labelled with fluorine-18.

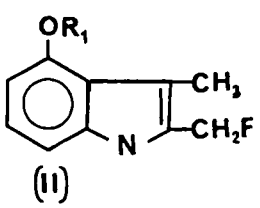
Preliminary experiments have established that the 3-iodine in (I) while stable in vivo is unstable to the chemical conditions necessary to synthesis a fluorinated analogue. A 3-fluoromethyl group on the indole nucleus would have approximately the same size and electronegativety as iodine but would be predicted to be very unstable.

Based upon in vitro data[5,6] there is reason to believe that the derivatives (II-IV) will have similar in vivo properties to (I). The stability of (II) is a little uncertain due to the resonance interactions with the 4-oxygen and thus (III), in which the fluorine is further removed from the aromatic system is also being prepared.

The synthetic strategy for all these compounds is based upon the Fischer indole synthesis, the acid catalysed cyclization of the appropriately substituted aryl hydrazones to give the tosylates V-VII which can then be fluorinated with either fluorine-19 or fluorine-18 and deprotected to give the ligands II-IV for testing.



(I)



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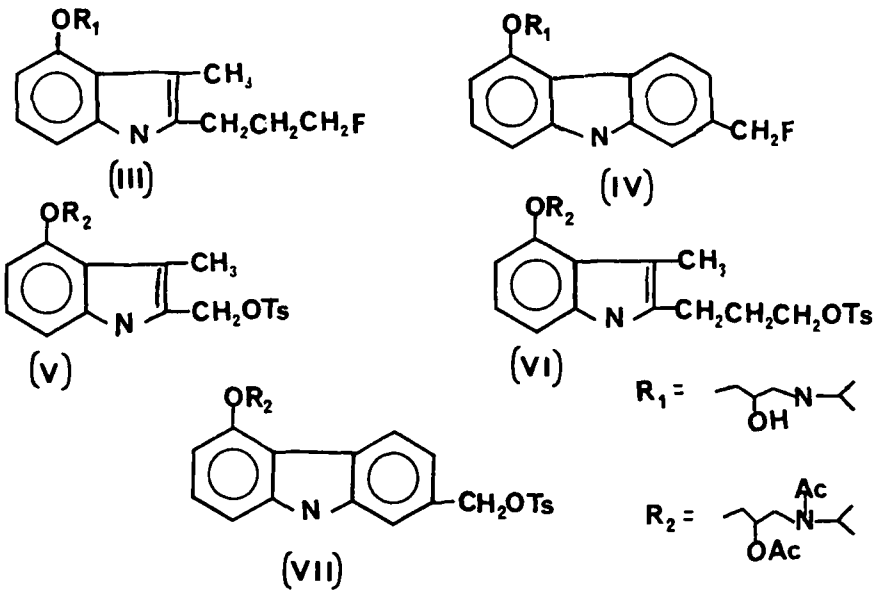

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SYNTHESIS OF 7α-UNDECYL SUBSTITUTED ESTRADIOL DERIVATIVES FOR BREAST TUMOR IMAGING

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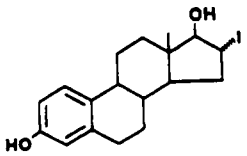
The potential use of estrogen receptor binding radiopharmaceuticals in the management of breast cancer has led to the synthesis of numerous estradiol and hexestrol derivatives labeled with γ-emitting radioisotopes (1,2). Most of these compounds are labeled with radiohalogens since attachment of larger substituents, such as required for labeling with metallo radioisotopes, results in substantial loss of binding affinity for the estrogen receptor. However, Bucourt and others (3) reported that estradiol 7α-derivative attached via a long spacer chain to an insoluble matrix is capable of selectively binding estrogen receptors. One may conjecture that the use of a long linear spacer molecule allows an attached functional group to protrude from the receptor binding pocket. Thus, this type of carrier would be suitable for coupling with bulky groups, such as chelated γ-emittors and cytotoxic moieties, while retaining affinity for the estrogen receptor. On such accounts we prepared a number of 7α-undecyl substituted estradiols and studied their estrogen receptor binding affinity and target tissue uptake.

The reference 16α-[^{125}I]iodo-17β-estradiol (1) was prepared by the method of Hochberg (4). The 7α-(11-hydroxy undecyl)17β-estradiol (2) was prepared from 19-nortestosterone accordingly to the procedure of Bucourt et al. (3). Selective bromination of 2 to yield the corresponding 11-bromo derivative 3 was accomplished with CBr_4 and triphenylphosphine in dry acetonitrile/THF/CH_2Cl_2 (4:1:1) and after 1 h at room temperature pure 3 was obtained via preparative HPLC. Halogen exchange with [^{125}I]NaI in dry MEK (80 °C, 16 h) followed by HPLC gave [^{125}I]4 (30% radiochemical yield). The bromo compound 3 and the iodo compound 4 are well separated by HPLC on an ODS-2 C18 column with methanol/water (89:11). The same exchange reaction with non-radioactive NaI showed the formation of a second product, which was identified as the debrominated product 5. Assigned structures were confirmed by ^{1}H NMR, MS and combustion analyses.

Relative binding affinities (RBA) for the estrogen receptor of the nonradioactive estrogens 1-4 was determined by competition studies using the Dextran-coated charcoal method (5) and the results are presented in Table 1. Prolonged incubation times had little effect whereas added DMF increased RBA values, suggesting the relative chemical stability of the compounds under the incubation conditions and substantially non-specific binding. Decrease of binding affinities for the estrogen receptor for the series of OH-, Br- and I-analogs 2-4 and simultaneous increase in non-specific binding, may reflect the enhanced lipophilicities of the undecyl side chains. The [^{125}I]4 was studied for its direct binding to the calf estrogen receptor using established methods. By comparing the maximum binding with that of the known [^{3}H]estradiol (6), the specific activity of [^{125}I]4 was estimated to be 175 Ci/mmol. The dissociation constant (K_d) for [^{125}I]4 was estimated to be 4.2×10^{-9} M, which is about 20 x higher than the K_d of [^{3}H]estradiol. This K_d ratio is comparable to the RBA values obtained by competition studies (Table 1). Tissue distribution of [^{125}I]4 in immature female Fischer rats showed rapid iodine uptake by the thyroid and no significant uptake in the uterus, either with or without nonlabeled estradiol. Accordingly we conclude that the iodine in 4 is subject to

TABLE I. Estrogen Receptor Binding Affinities



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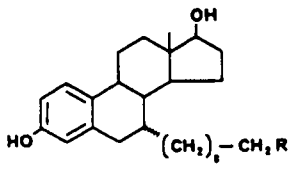

2	R:	$-CH_2CH_2-OH$
3	R:	$-CH_2CH_2-Br$
4	R:	$-CH_2CH_2-I$
5	R:	$-CH=CH_2$
6	R:	$-CH_2CH_2-F$

Compd	RBA[1] 2 h	12 h	20 h	20 h (DMF)	RBA (DMF)[2] RBA
1	26.4	22.1	27.1	45.9	2
2	2.1	2.9	2.0	8.4	4
3	0.05	0.05	0.2	1.6	7
4	0.05	0.04	0.1	1.1	11
5	0.07	0.09	0.09	0.5	5

[1] The binding affinity was determined relative to that of [^{3}H]estradiol, the relative binding affinity (RBA) is 100 times the ratio between the competitor and unlabeled estradiol concentrations required for 50% competition. Incubation times varied from 2-20 h and the 20 h incubation was repeated in the presence of 7% DMF. [2] The ratio between RBA value obtained after 20 h incubation with and without DMF is also presented.

rapid dissociation from the carrier steroid under in vivo conditions. Since in vitro receptor binding properties of the 7α-undecyl substituted estradiols are promising, we choose ^{18}F as a more stable label to evaluate the title compound for its in vivo potential as a selective estrogen receptor based steroid carrier. Synthesis of the nonradioactive 7α-(11-fluoro undecyl) derivative (5) was accomplished through substitution of the bromine in 3 with tetrabutylammonium fluoride in THF. The 3- and 17β-hydroxyl groups of 3 were protected as tetrahydropyran derivatives during this substitution reaction. Synthesis of the analogous [^{18}F]5 using ^{18}F-labeled tetrabutylammonium fluoride (7) to permit biological studies has been projected.

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SYNTHESES AND RECEPTOR BINDING PROPERTIES OF CHLORO DERIVATIVES OF ESTRADIOL AS PRECURSORS OF ^{18}F-LABELED ANALOGS

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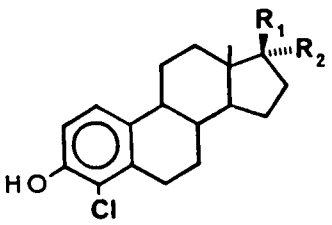
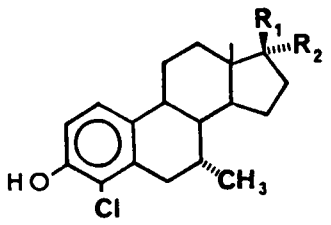
A number of radiohalogenated estrogens have been prepared over the past years as potential receptor imaging agents to be used in conjunction with breast cancer management. Most of the earlier analogs were labeled with radioiodine (1), however, in vivo deiodination and loss of receptor binding properties led to increased attention to bromo and fluoro derivatives (2,3). Particularly, introduction of fluorine into the steroid structure often results in increased hormone potency due to suppression of undesired metabolic reactions and reduced non-specific binding (3). A number of convenient reagents have been developed for the preparation of organo fluoro compounds, and their adaptation for the introduction of positron emitting ^{18}F has been reported (4). Estradiol 4-halogen derivatives are convenient precursors for the synthesis of ^{18}F-estrogens, they are chemically stable compounds and they can be labeled with ^{18}F via established nucleophilic substitution reactions (4). Replacement of chloro- for fluoro-substituents generally improves estrogen receptor binding properties and this allows screening for potential high binding fluoro analogs via the in vitro testing of the chloro precursors. On such accounts we prepared a series of 4-chloroestradiol derivatives with various substituents on the 17α- and 7α-positions and compared their relative binding affinity (RBA) for the estrogen receptor.

The parent compound 4-chloroestradiol (1) was prepared from 19-nortestosterone acetate via chlorination with sulphuryl chloride in pyridine to yield 4-chloro-19-nortestosterone acetate, followed by aromatization of the A-ring with selenium dioxide in t-butanol and hydrolysis of the 17β-acetate with potassium carbonate in methanol. The 4-chloroestrone (2) was obtained by oxidation of 1 with Jone's reagent in acetone. The 17α-substituted analogs of 1, including the 17α-methyl- (3), 17α-ethinyl- (4), and 17α-chloroethinyl-derivative (5) were obtained by the reaction of 2 with, respectively, methyl lithium in diethyl ether, lithium acetylide-ethylenediamine complex in DMSO and lithium chloroacetylide in THF. The 7α-methyl derivatives 6, 7 and 8 were prepared from 7α-methyl-19-nortestosterone acetate using the same procedures as described for the syntheses of 1, 2 and 4.

The affinity of steroids 1-8 for the estrogen receptor was determined by competition studies with [^{3}H]estradiol using the Dextran-coated charcoal method (6) and the results are presented in Table 1. RBA values for estrone, hexestrol and 17α-ethinyl estradiol, determined under the same experimental conditions, are also included. The effect of the addition of 7% DMF on specific binding was measured in order to quantify the effect of binding to non-specific sites (Table 1). Analogs of 4-chloroestradiol with the most promising binding properties include derivatives which are unaltered about the 17β-hydroxyl function. Addition of a 17α-methyl or 17α-ethinyl group diminish binding affinities whereas substitution with a 17α-chloroethinyl group seems to have little effect. In contrast, addition of a 7α-methyl group strongly increases receptor binding properties. Furthermore, it is anticipated that the analogues fluorosteroids will exhibit even higher receptor binding properties with attendant higher target tissue selectivity. Radiosyntheses of the most promising derivatives with ^{18}F for in vivo studies have been projected.

TABLE I. Estrogen Receptor Binding Affinities





1	R_1	: -OH	R_2 : -H	6	R_1	: -OH	R_2 : -H
2	R_1, R_2	: =O		7	R_1, R_2	: =O	
3	R_1	: -OH	R_2 : $-CH_3$	8	R_1	: -OH	R_2 : $-C\equiv CH$
4	R_1	: -OH	R_2 : $-C\equiv CH$				
5	R_1	: -OH	R_2 : $-C\equiv CCl$				

Compd	RBA[1]	RBA (7% DMF)
17α-ethinyl estradiol	100	100
estrone	7.1	1.5
hexestrol	37.6	65.4
1	9.4	8.5
2	0.1	0.1
3	1.3	1.9
4	1.7	2.6
5	11.3	5.3
6	63.1	155.7
7	6.4	6.2
8	12.9	16.0

[1] The binding affinity was determined relative to that of [^{3}H]estradiol, the relative binding affinity (RBA) is 100 times the ratio between the competitor and unlabeled estradiol concentrations required for 50% competition. Incubation times with calf uterine cytosols were 20 h. Incubations were repeated in the presence of 7% DMF in order to assess non-specific binding.

Financial support for this work was provided by the Medical Research Council of Canada.

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ORGANOMERCURY DERIVATIVES OF ANDROSTENE AND PREGENENOLONE. SIMPLE NO-CARRIER-ADDED SYNTHESES OF 6-IODOANDROST-5-EN-3β,17β-DIOL, 6-IODO ANDROST-5-EN-3β-OL,17-ONE, 6-IODOPREGN-5-EN-3β-OL-20-ONE AND 6-IODOPREGN-5-EN-3β,20-DIOL

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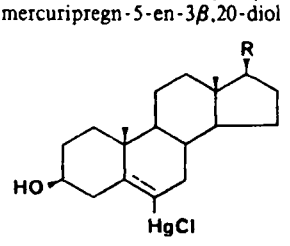
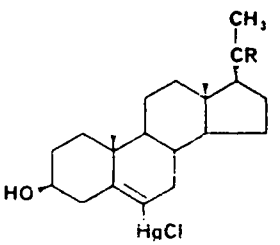
As part of an ongoing program to prepare radiolabelled steroids for steroid receptor mapping we required certain high specific activity no-carrier-added iodinated steroids. The design of these steroids was based on a combination of structure activity relationships and the requirements for the *in vivo* stability of the label. The latter consideration precluded the use of aliphatic iodides and obliged us to use vinylic iodides. Such vinylic iodides are, in general, difficult to prepare especially in high specific activity. We chose to prepare these molecules using precursors in which the labelling position is activated with mercury towards electophilic substitution by radiohalogens. Although mercury is only one of a number of heteroatoms which undergo electrophilic substitution it is particularly suitable for such labelling since simple one step synthetic routes to these precursors exist.

The efficient reaction of 6-chloromercuricholest-5-en-3β-ol with iodine to give 6-iodocholest-5-en-3β-ol is well known (**1**). . This reaction has been used by us in the preparation of high specific activity [^{131}I]-6-iodocholest-5-en-3β-ol (**2**). The difficult part of this chemistry, if any, is the preparation of the organomecury precursor. We have expanded this synthetic method to other steroids and have prepared a number of chloromecury analogues of androstene and pregenenolone eg. 6-chloromercuriandrost-5-en-3β-ol,17-one (**1**), 6-chloro mercuriandrost-5-en-3β,17β-diol (**2**), 6-chloromercuripregn-5-en-3β-ol-20-one (**3**) and 6-chloro mercuripregn-5-en-3β,20-diol (**4**).



1 R = =O

2 R = 



3 R = =O

4 R = 

These compounds were prepared by direct mercuration of the corresponding steroids with mercuric acetate in acetic acid. Typical yields after isolation were 20%. The competing Triebs reaction is the main cause of these low yields since it reduces the mercuric acetate to mercurous acetate (**3**). In practice the low yields do not constitute a problem since a couple of milligrams of chloromercury precursor will suffice for many labelling experiments. These chloromercury compounds undergo smooth reaction with [^{131}I]-iodine monochloride in ethanol to give the corresponding iodinated product. Because of the wide range of solvents in which this reaction will proceed we generally carry out labelling experiments in the same solvent as used for HPLC so that the product mixture can be directly injected and chromatographed. The rf values of chloromercury steroids are very different from the corresponding iodo-steroids so that the entire product can be chromatographed in a number of injections on an analytical column.

The corresponding reaction with bromine was also very successful. It is important to note that in the case of those chloromercury compounds containing ketone functions, the rate of reaction of bromine with the carbon mercury bond was sufficiently fast to preclude bromination of the ketone function.

These chloromercury derivatives demonstrated excellent stability with respect to water and oxygen and so could be stored for long periods at room temperature. They were too reactive in most cases to allow much subsequent chemistry and attempts to oxidize the pregnenolone derivatives to progesterone derivatives always met with demercuration and introduction of an oxygen function at C-6.

The reactivity and the long term stability of these compounds can be altered by changing the substituent on the mercury. Thus a chlorine group which is electron withdrawing makes

the carbon bond less liable to undergo electrophilic substitution while an electron group such as a thioethyl or a thioproplycarboxyl increases to make it more reactive. We have found the latter two groups to be particularly useful.

Preliminary *in vivo* studies in rats indicate that the radiolabel is not rapidly cleaved. These compounds show promise as adrenal function agents and tumour imaging agents.

Financial support for this work was provided in part by an NSERC/Merck Frosst Fellowship (RJF), an Alberta Heritage Foundation for Medical Research, Research Allowance (RJF) and an Alberta Heritage Foundation for Medical Research - Applied Research Cancer Grant (VJJ).

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FUNCTIONALIZATION OF THE ESTRADIOL B-RING VIA ORGANOMERCURY INTERMEDIATES.

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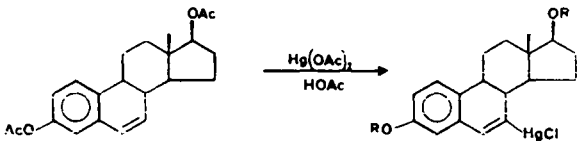
The major effort in the use of organometallic compounds as substrates for electrophilic labelling of radiopharmacuticals lies in the synthesis of the organometallic precurosr. The synthetic routes to these compounds play an important role in the choice of heteroatom to be used. Mercury offers a large number of synthetic approaches, especially when compared to boron, tin and group 4 metals such as germanium and silicon **(1)**. Of particular interest to us was the ability of mercury to functionalize molecules which contain double bonds, to produce vinyl mercury compounds **(2)**. If such a reaction could be carried out in the presence of a phenolic group elsewhere in a molecule then mercury would offer a distinct advantage over other heteroatoms.

As part of a current program to synthesize radiolabelled steroids we required an estrogen which was labelled in the B-ring with radioiodine. Structure activity relationships **(3)** mandated that such a label be at the 7-position and preferably vinylic for reasons of *in vivo* stability. Thus we sought to produce an organometallic precursor with a Δ-6 double bond and a C-7 organometallic substituent. Our experience with organomercury chemistry encouraged us to consider the use of a precursor such as 7-chloromercuri-estradiol-6-ene **(1)**. It is known that double bonds which allow good stabilization of a carbonium ion intermediate are much more likely to undergo substitution with mercury rather than addition. The styrene nature of the double bond in **2** was likely to direct mercuration to the 7-position and promote substitution. This molecule also allowed a measurement of the relative rate of reaction of a phenol versus an vinylic system with mercuric acetate.



2 **1** R = OH
3 R = OAc

Treatment of estradiol-6-ene-3,17-diacetate **(2)** with mercuric acetate in acetic acid gave the required 7-chloromercuri-estradiol-6-ene-3,17-diacetate **(3)** in 80% yield after isolation. The reaction was complete in 15 mins. No addition products of the Δ-6 double bond were observed. The reaction was performed on the diacetate to reduce the possibility of electrophilic mercuration on the phenol ring. Indeed, it was found, that in the case of the unprotected phenol the A-ring competed effectively with the Δ-6 double bond for mercuration. Removal of the acetic acid groups with KOH/methanol gave the desired product **(1)**. The chloromercury group was quite stable under such conditions.

Both the diacetate **2** and the free phenol **1** reacted smoothly with bromine and iodine to give the corresponding 7-halo-estradiol-6-ene. It is important to note that no ring halogenation was observed under these conditions implying that the chloromercury function is more reactive towards electrophilic substitution than the phenol ring.

This sequence of reactions demonstrates one of the particular advantages of using organomercury compounds as precurors in radiolabelling experiments. We believe that the **ability of mercuric** acetate to react with double bonds is a powerful tool in the production **of such precursors.**

Financial support for this work was provided in part by an NSERC/Merck Frosst Fellowship (RJF) and an Alberta Heritage Foundation for Medical Research - Applied Research Cancer Grant (VJJ).

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THE PREPARATION OF [21-^{11}C]PROGESTERONE. A POTENTIAL RECEPTOR BINDING RADIOTRACER.

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The relevance of the presence of progesterone receptors in tumour tissue for the response to chemotherapy has been discussed widely. Clinically, the in vivo determination of progesterone receptor density by PET is of interest to estimate the response to chemotherapy. Since PET, in combination with a suitable radiopharmaceutical provides a useful tool for the in vivo localisation and quantification of receptors in tissues, we decided to investigate the potential of [21-^{11}C]progesterone as a radiopharmaceutical. The synthesis of this compound will be discussed.

Methylation of tosmic derivatives with $^{11}CH_3I$, followed by acid hydrolysis provides a general route to ^{11}C-acetylated compounds [1]. By this method we prepared [21-^{11}C]progesterone. The reaction scheme is given in figure 1.

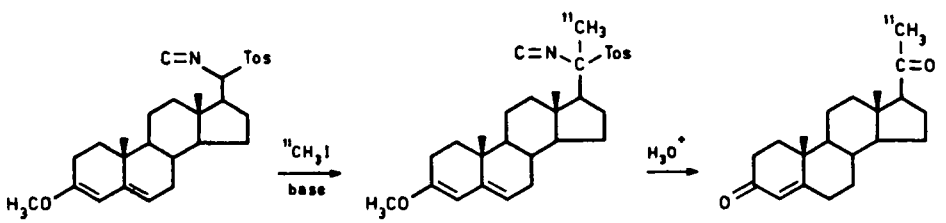

fig. 1

Amounts of 2 mg of starting isocyanide and 4 mg of triethylbenzylammoniumchloride were transferred to a 50 ml round bottom flask, followed by 2 ml of 50% aqueous NaOH solution. To this mixture about 100 mCi of $^{11}CH_3I$ in 1 ml of toluene was added, followed by 5 ml of toluene. The reaction mixture was heated at 100 °C under reflux and stirred vigourously for 15 minutes. After cooling to room temperature the NaOH-layer was removed and the organic layer extracted once with 3 ml of water. Finally 3 ml of concentrated HCl was added. Hydrolysis was achieved by vigourously stirring for 5 min at 70 °C. After cooling the HCl-layer was removed. The toluene solution was extracted with water and with a saturated $NaHCO_3$ solution. After drying of the organic layer

with $MgSO_4$, filtering and evaporation to dryness, the residue was dissolved in 1.5 ml of chloroform/hexane (4/6 , v/v). Purification of the final product was achieved on HPLC using a Partisil 10 column (50x0.9 cm, chloroform/hexane, 4/6, v/v). The ^{11}C-progesterone was collected, the eluents evaporated to dryness and the residue redissolved in 0.5 ml of ethanol. To this solution 2 ml of physiological saline was added. The solution was sterilised by aseptic membrane filtration for application in rats.

The [21-^{11}C]progesterone was obtained 60 min after the introduction of the $^{11}CH_3I$ into the reaction mixture. The yield was about 13% (corrected for decay) and the specific activity 20 Ci/mMol. The identity and radiochemical purity of the final product was determined by comparison to authentic material.

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NO CARRIER-ADDED FLUORINE-18 LABELLING OF NEUROLEPTICS WITH DIFFERENT 18F SOURCES

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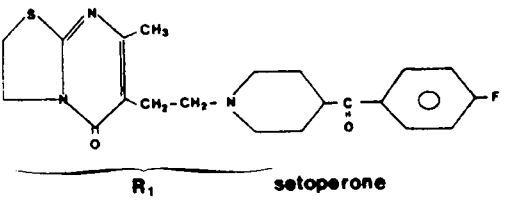
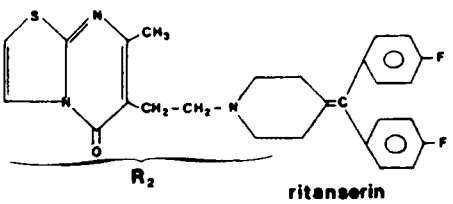
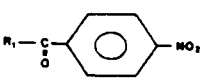
Labelling conditions have been studied for two fluorinated neuroleptics : setoperone and ritanserin (1), with different sources of fluorine-18.

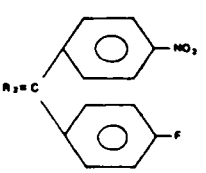




The synthesis method used involves a nucleophilic type substitution reaction, displacement of a phenyl-carried nitro group by the fluoride anion, this substitution being strongly influenced by the nature of the group in the para position of the NO_2 group. The reaction thus takes place only with the nitro derivative of setoperone :

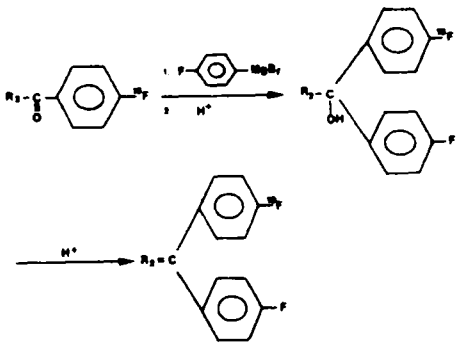


The reaction does not occur with the nitro precursor of ritanserin.



Since the ethylene group is not very reactive, the preparation of ^{18}F-ritanserin needs to be carried out in several stages. The first is common to both molecules :

The synthesis of ritanserin requires two further stages :



These substitution reactions were tried with fluorine-18 from 3 sources :

Target	Nuclear reaction	Particle energy	Activity produced EOB
Neon	Ne (^{3}He, αn) ^{18}N → ^{18}F	30 MeV	100 mCi
Natural water	^{16}O (^{3}He, p) ^{18}F	30 MeV	200 mCi
Enriched water 50 %	^{18}O (p, n) ^{18}F	16 MeV	500 mCi

The substitution was carried out in DMSO at 170°C, in the presence of K_2CO_3 with or without aminopolyether potassium complex (kryptofix 2.2.2.) (2,3), for 30 minutes. The setoperone results are given in the following table :

^{18}F source	Yield without K.2.2.2.	Yield with K.2.2.2.	Specific radioactivity EOS
Neon	8 - 10 %	35 - 55 %	50 mCi/µMol
Natural water	5 %	8 - 25 %	150 mCi/µMol
Enriched water	-	8 - 25 %	400 mCi/µMol

The addition of kryptofix 222 increases the yield by a factor 4 to 5. Fluorine-18 production by irradiation of neon with helium-3 seems to be easily the most reactive of the three methods. Unfortunately however, whereas the mass of stable fluorine is about the same as in the other sources (within a factor 2 nevertheless), the low radioactivity produced is the factor limiting the specific radioactivity obtainable.

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RADIOCHEMISTRY OF SALICYLIDENE-AMINATES (SCHIFF BASES) LABELED WITH Tc-99m

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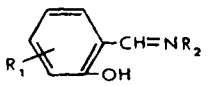
Current work on the development of ^{99m}Tc-radiopharmaceuticals focuses on improving their specificity for individual organs by devising new chelating agents for this radionuclide. ^{99m}Tc-pyridoxylidene-aminates, reported as potential hepatobiliary agents, were prepared either by heating pyridoxal, the aminoacid and pertechnetate in autoclave (1) or by mixing the reactants with stannous chloride in alkaline pH (2). However, various by-products derived from the transamination reaction occurring simultaneously, may bind technetium as well. Therefore the ligand of technetium in these preparations cannot be well defined. On the other hand stable complexes of pyridoxal or salicylaldehyde-amino acid derivatives with divalent or trivalent metal ions are already known (3-5). In order to examine the chelation ability of isolated Schiff bases with Tc-99m a series of salicylidene aminates was prepared and labelled with this radioisotope using the stannous ion reduction method. Radiochemical study of the complexes formed, accompanied with preliminary biological data are presented. The structure of azomethine derivatives studied, as confirmed by elemental analysis and NMR spectroscopy, are listed in Table 1.

Salicylidene-aminates were labelled with Tc-99m in pH 7.4 . Optimization study of the labeling procedure was carried out using various concentrations of salicylidene-phenylalanine (S-Phe) and $SnCl_2$ at the appropriate pH, in 4 ml final volume. The optimal ligand to reducing agent molar ratio for efficient Tc-binding was found to be 46:1 in concentrations 18.5×10^{-2} mmol and 0.4×10^{-2} mmol, respectively. Concentrations of S-Phe beyond 9.2×10^{-2} mmol yielded low percentages of ^{99m}Tc-chelate. Radiochemical purity of the labeled compounds normally exceeded 90% as determined by ITLC and electrophoresis proving the good chelation ability of the Schiff bases studied. The amount of free pertechnetate in the preparations was always low indicating the almost complete reduction of heptavalent technetium under the experimental conditions tested. Labeling yield was not significantly affected neither by various substitutions in salicylidene-phenylalanine molecule (compounds 2-10) nor by different aminoacid moieties (compounds 10-16).

Dual label studies of salicylidene-phenylalanine were performed using $^{113}SnCl_2$ for reduction of Tc-99m in order to investigate tin retention in the final ^{99m}Tc-complex. Salicylidene-phenylalanine was also labeled with ^{113}Sn for comparison purposes. Radioanalytical study of ^{113}Sn- ^{99m}Tc-S-Phe preparation (a) and ^{113}Sn-S-Phe (b) revealed different Rf of complexes (a) and (b) the day of labeling while identical seven days later, when Tc-99m had decayed away. Therefore, it was estimated that tin does not participate in the final complex because the Rf values of (a) should remain unchanged. Tin acts only as electron-donor for the reduction of pertechnetate in a lower valence state. Under the labeling conditions tin probably forms a complex with S-Phe exhibiting different radiochemical behavior. The above results were confirmed by biological studies of preparations (a) and (b) in mice. Complex (a) appeared hepatotropic as expected,whereas complex (b) was found to excrete mainly via the urinary system.

Table 1 summarizes the comparative 1 hour biodistribution data of ^{99m}Tc-salicylidene-aminates in mice. The values present the percent dose of administered radioactivity in various organs or tissues. The results indicated a high liver affinity for the most of the complexes and negligible urinary excretion. Considerable amounts of radioactivity were recovered into the urine only for compounds ^{99m}Tc-salicylidene-isoleucine, ^{99m}Tc-salicylidene-cycloleucine and ^{99m}Tc-salicylidene-alanine. (Table 1, compounds 12, 14, 15). Low water-octanol partition coefficient values correlated with the biological data of these three ^{99m}Tc-salicylidene-aminate complexes.

TABLE 1. Schiff Bases Studied and Comparative 1 h Biodistribution Data in Mice of their ^{99m}Tc-Complexes



Compounds	R_1	R_2	Percent dose per organ			
			Blood	Liver	Intestines	Urine
1	H	Phe	2.673	24.421	75.017	1.171
2	H	O-F-Phe	1.587	19.526	63.610	0.910
3	H	m-F-Phe	1.610	19.292	64.313	1.775
4	H	p-F-Phe	1.270	21.795	71.473	2.024
5	H	p-I-Phe	2.040	51.826	34.754	5.802
6	H	p-OCH_3-Phe	4.581	28.977	50.661	6.152
7	4-OC_4	Phe	3.231	46.254	39.143	3.482
8	5-OH_3	Phe	5.020	19.741	45.350	8.784
9	5-NO_2	Phe	4.717	34.469	67.445	4.630
10	4-OH	Phe	3.728	15.300	82.237	1.813
11	H	Tr	4.184	13.083	77.231	0.662
12	H	iso-Leu	7.571	22.025	37.619	35.603
13	H	nor-Leu	5.191	12.524	63.573	6.432
14	H	cyclo-Leu	10.761	16.047	15.326	39.750
15	H	Al	7.826	10.472	17.905	46.663
16	H	Me	5.919	8.489	58.495	5.208

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SYNTHESIS AND COMPARATIVE BIOLOGICAL STUDIES OF ^{99m}Tc COMPLEXES WITH FUNCTIONAL DERIVATIVES OF ARYLCARBAMOYLMETHYLIMINODIACETIC ACID

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Complexes of ^{99m}Tc with alkylphenylcarbamoylmethyliminodiacetic acids discovered by Loberg more than ten years ago (I) are now widely used for investigation of hepatobiliary diseases, but the literature data on the ^{99m}Tc complexes with functional derivatives of iminodiacetic acid are rather limited (2,3). We have synthesized complexones – derivatives of iminodiacetic acid of general formula p-$XC_6H_4NHCOCH_2N(CH_2COOH)_2$, where X = H, OH, COOH, NO_2, SO_2NH_2 and $CONHCH_2COOH$, prepared their complexes with ^{99m}Tc and studied distribution of the complexes in vivo. The complexones have been obtained by two stage synthesis from corresponding aromatic amino derivatives by acylation with chloroacetylchloride and then alkylation of iminodiacetic acid with the isolated arylcarbamoylmethylchlorides in aqueous ethanol. Complexation with ^{99m}Tc has been carried out by the addition of eluate from Tc-generator to aqueous or freeze-dried solutions of the sodium salts of the complexones containing $SnCl_2$ with subsequent analysis by electrophoresis on paper or celluloseacetate strips in phosphate or borate buffers with pH 6-7. Radiochemical purity of the complexes was 90-95% with the major admixture being reduced hydrolyzed technetium and with pertechnetate content no more than I.5%.

Biological studies of the prepared ^{99m}Tc complexes have been carried out on rats by investigation of their distribution at certain time intervals after intravenuous injection. Our studies have clearly demonstrated the effect of functional group in the phenyl ring of the complexone on the excretion mode of the corresponding ^{99m}Tc complex. Thus, nonpolar (lipophilic) groups enhance preferential hepatobiliary excretion, while polar groups – renal one. Complexes with OH, COOH and $CONHCH_2COOH$ groups are characterized by specific nephrotropic distribution and high excretion rate by kidneys that suggest their hippurate-like mechanism of tubular filtration. For the case of X = $CONHCH_2COOH$ (Tc-PAHIDA) this mechanism has been proved by Chervu (4). The obtained results

suggest the possibility of application of such complexes for the differential investigation of excretory organs diseases.

TABLE I. Distribution of ^{99m}Tc Complexes of $p\text{-}XC_6H_4NHCOCH_2N(CH_2COOH)_2$ in Rats at I Hour after Intravenuous Injection, % Dose/Organ

Organ	X					
	H	NO_2	SO_2NH_2	OH*	COOH	$CONHCH_2COOH$**
Blood	0.58	0.78	2.I	8.4	3.6	4.9
Stomach	0.02	0.03	0.2	0.7	0.2	0.3
Liver	0.9	0.9	I.3	5.2	0.6	4.7
Intestine	60.0	77.6	I2.I	5.5	2.0	
Bone	0.4	0.5	I.4			3.3
Kidneys	0.85	I.3	II.5	4.9	2.8	3.5
Bladder	34.3	II.8	39.6	42.0	72.5	6I.5

*At I5 min. **At 20 min.

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PHARMACOKINETIC STUDIES OF ^{99m}Tc- PYROPHOSPHATE WITH ^{99m}Tc, ^{113}Sn AND ^{32}P TRACERS.

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During last years more and more attention is paid to studying the radiochemical composition of the radiopharmaceuticals based on ^{99m}Tc complex compounds. For example, in the case of some osteotropic agents (^{99m}Tc-MDP, ^{99m}Tc-EHDP, ^{99m}Tc-pyrophosphate) the presence of several complexes of the radionuclide was shown which differ by their biological distribution (1-4). The studies of this kind provide data for the better knowledge ^{99m}Tc complex formation and enable the correct choice of parameters and methods of analysis of ^{99m}Tc radiopharmaceuticals that have great importance both for radiopharmaceutical chemistry and practical nuclear medicine.

In order to study distribution of the components of ^{99m}Tc-pyrophosphate in animals after intrevenous injection we have performed its labeling with ^{113}Sn and ^{32}P. ^{32}P-pyrophosphate has been prepared by drying the solution of $Na_2H^{32}PO_4$ at 100°C and then 200°C with the subsequent heating at 500°C for 5 hours. Metal ^{113}Sn has been dissolved in conc.HCL and added to required amount of the prepared dry ^{32}P-pyrophosphate. This freshly prepared mixture has been used for the complexation with ^{99m}Tc, being carried out by adding 4 ml of ^{99m}Tc-pertechnetate and adjusting the solution to pH 6-7. The final ^{99m}Tc- ^{113}Sn- ^{32}P-pyrophosphate solution has had the following characteristics: Sn^{2+} - 0,25 mg/ml, sodium pyrophosphate -5 mg/ml, ^{113}Sn- 37 MBq/ml, ^{32}P- 33 MBq/ml, ^{99m}Tc-70 MBq/ml, pH 5-7.

Biodistribution studies have been performed on white rats (weight 150-180 g). Animals were injected in the tail vein with 0,1-0,2 ml of preparation. At certain time intervals the animals were killed by decapitation. The content of ^{99m}Tc, ^{113}Sn and ^{32}P in the studied organs and tissues has been measured by direct radiometry by commonly used methods and calculated as a percent from the injected amount per organ.

The content of ^{99m}Tc and ^{113}Sn has been found to be identical in all studied organs (see table I). However ^{32}P has greater accumulation in liver and skeletal muscles and to much lesser extent is excreted by kidneys.

TABLE I. Distribution of Radionuclides in Rats after i/v Injection of ^{99m}Tc- ^{113}Sn- ^{32}P-pyrophosphate (% injected dose per organ, M± m)

	I hr after injection			24 hrs after injection		
	^{99m}Tc	^{113}Sn	^{32}P	^{99m}Tc	^{113}Sn	^{32}P
Blood [1]	3,0±0,5	3,0±0,4	3,0±0,I	I,5±0,I	0,5±0,05	3,0±0,2
Liver	5,0±0,2	4,2±0,7	I2,5±0,I	4,0±0,2	3,2±0,5	5,0±0,8
Bone [2]	38,5±I,0	38,0±2,0	40,0±I,3	40,2±2,5	4I,5±I,0	42,2±I,5
Skeletal muscles [3]	6,0±0,5	5,2±0,3	20,0±I,5	I,5±0,5	2,0±0,3	30,0±2,0
Kidneys	4,0±0,7	5,5±0,2	I,5±0,3	4,6±I,0	0,5±0,04	I,2±0,3
Exeretion with urine	35,0±0,2	33,0±2,0	3,5±0,I			

1-7% of body weight ; 2 - femur x 20; 3 - 45 % of body weight

The presented results enable us to assume the following: I. tin is included into the complex molecule ^{99m}Tc-Sn-pyrophosphate, which is intensely concentrated in bones; 2. in the radiopharmaceutical of ^{99m}Tc -pyrophosphate there is another complex of ^{99m}Tc with tin, which is rapidly excreted with urine;

3. a certain part of the pyrophosphate in the radiopharmaceutical exists in the non-complexed form, being not involved neither in ^{99m}Tc-Sn-pyrophosphate, nor in Sn-pyrophosphate.

The authors consider these conclusions as preliminary. It is necessary further confirmation by radioanalytical methods.

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THE STUDY OF RADIONUCLIDE PHYSICO-CHEMICAL STATE IN RADIOPHARMACEUTICAL PREPARATIONS SOLUTIONS

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As a rule the preparation of radiopharmaceutical medicines results in a complex compound or a colloidal form of radionuclide in a solution.

This work describes obtaining of radionuclides complexes of indium, technetium and rare-earth elements with complexons containing acetate and phosphone subgroups. By using the techniques of extraction and gel-chromatography in the field of pH-solutions from 1,5 to 8 the composition of complexes being formed is determined and their stability constants are calculated, the possibility of formation and simultaneous presence of complexes in solutions is shown, which corresponds to the mole ratio metal:ligand 1:1 and 1:2 and which are characterized by different extent of ligand protonation.

By methods of electrophoresis, thin-layer chromatography, gel-filtration, dialysis and ultracentrifugation the investigation of the processes of complex formation and colloidal forms of reduced technetium in phytate solutions is carried out. Different parameters influence on the velocity and the extent of binding the tracer with an organic carrier is studied. It is established that under certain conditions co-existence of calcium (magnesium) and stunnum dichloride in the solution of phytate salts is possible, that in turn makes it possible to obtain further a stable practically monodispersed colloidal solution of technetium-99m in one step.