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RECENT RADIOPHARMACEUTICAL RESEARCH AT THE AAEC RESEARCH ESTABLISHMENT

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J.G. WILSON

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ABSTRACT

During the past few years a large part of the radiochemical research carried out at Lucas Heights has been devoted to the synthesis of ligands capable of forming chelate complexes with technetium-99m, as part of a search for tumour-localising radiopharmaceuticals. An account is given of the synthesis and biological evaluation of a range of these compounds and of the investigation of certain biochemical and biological properties affecting the clinical application of both ligands and radiopharmaceuticals.

In addition to the search for novel ^{99m}Tc-radiopharmaceuticals, major research programs on the development of ^{99m}Tc-generating systems have been in progress at Lucas Heights for several years. Work on the AAEC's Mark III ^{99m}Tc technetium generator has been brought to a successful conclusion. A new type of ^{99m}Tc generator, which uses an insoluble zirconium molybdate gel and provides high yields of pertechnetate by a simple elution technique, has also been developed. Studies are in progress on the osmium-iridium generator.

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PART I
LIGAND SYNTHESIS AND BIOLOGICAL STUDIES

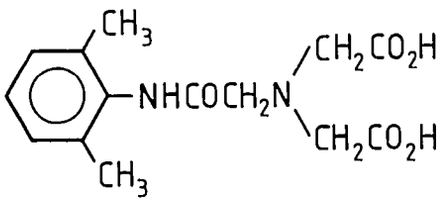
by

J.G. WILSON

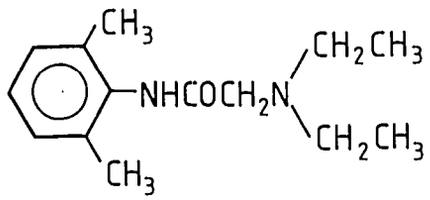
1. INTRODUCTION

One of the major areas of present-day radiopharmaceutical research is the synthesis of new chelating ligands for complex formation with selected radionuclides. Recent advances in this field have led to the development of a number of organ-imaging agents that are widely used in nuclear medicine. In the rational design of these new radiopharmaceuticals, the concept of 'bifunctional' chelating agent has played a significant role. A bifunctional chelating agent is a compound consisting of a strong chelating group capable of forming complexes with a γ-emitting radionuclide and a covalently attached moiety having suitable biological or biochemical properties. This moiety may be expected to confer some degree of *in vivo* organ-specific distribution on the resulting radiopharmaceutical. Using this approach, Sundberg *et al.* [1974] succeeded in labelling fibrinogen by azocoupling the protein with the stable indium-111 complex of azophenyl-EDTA. The method was also the basis of the synthesis of bifunctional analogues of palmitic acid [Eckelman *et al.* 1975] and tolbutamide [Heindel *et al.* 1975] containing the ligands EDTA, DTPA and diethylenetriamine in an attempt to find myocardial and pancreatic localising agents respectively.

More recently the concept of bifunctional ligands in radiopharmaceutical design was applied by Loberg and his group [Loberg *et al.* 1975, 1976] to the synthesis of HIDA (hepatobiliary iminodiacetic acid) as an analogue of lidocaine,



HIDA



LIDOCAINE

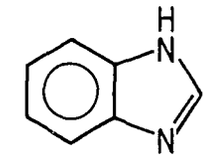
in which the diethylamino group has been replaced by iminodiacetic acid (IDA). Lidocaine is an anti-arhythmic drug that localises in the viable myocardium and the new compound, HIDA, was designed as an ideal ligand for transporting ^{99m}Tc to the heart. The chelating agent, IDA, was chosen as the ligand function because it possesses a number of desirable features. Since it is relatively small, being roughly isosteric with diethylamine, it involved minimal departure in molecular size from lidocaine; it forms stable complexes with most metals, and can be incorporated synthetically into organic molecules with relative ease.

The ^{99m}Tc-complex of HIDA, contrary to expectation, did not localise in the myocardium. After intravenous injection into mice, it was rapidly excreted *via* the hepatobiliary route. HIDA and a number of its derivatives [Loberg *et al.* 1976] were subsequently investigated and evaluated as hepatobiliary agents by several groups [Subramanian and McAfee 1980]; 2,6-dimethyl-HIDA has since been established as the ligand of choice for ^{99m}Tc hepatobiliary scintigraphy - an unexpected 'spin-off' from research directed towards a different goal.

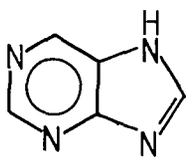
The concept of bifunctional chelating agents has been adopted at Lucas Heights in the search for tumour-imaging agents, and has led to the synthesis of three series of ligands - benzimidazoles, sulphanilamides and acridines — all of which incorporate the IDA chelating group. Studies on their biological evaluation are now described.

2. BENZIMIDAZOLES

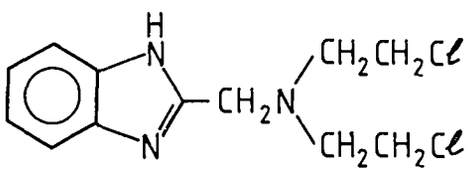
In the field of cancer research, literally hundreds of purine and pyrimidine derivatives and their analogues have been synthesised during the last 30 years in the hope of finding compounds capable of acting as antagonists or false substrates in the normal metabolic processes of DNA synthesis. From this effort, many synthetic compounds were found that possessed anti-neoplastic activity and a small number of these have been used in therapy. The purines, therefore, were an obvious class of compounds with which to begin this kind of radiopharmaceutical research. We simplified our approach, however, by selecting for preliminary study derivatives of benzimidazole, a heterocycle which is isosteric with purine. The benzimidazole nitrogen mustard (I) had already been synthesised in 1957 and found to have pronounced activity against several mouse tumours [Hirschberg *et al.* 1958].



BENZIMIDAZOLE

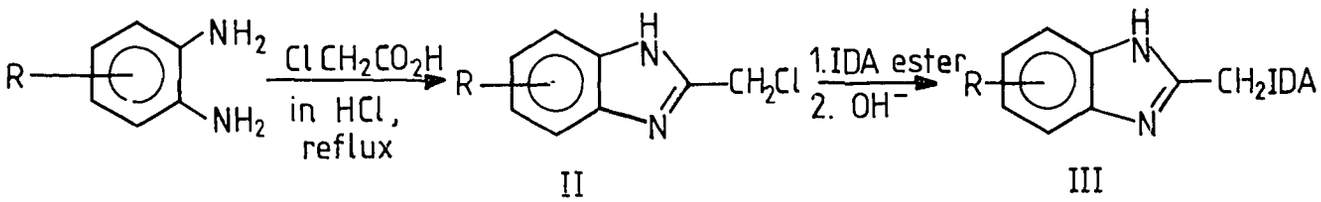


PURINE



I

We therefore synthesised a number of benzimidazoles (III) bearing the IDA ligand in the 2-position [Hunt *et al.* 1979, 1980, 1981]. Alkylation of IDA dimethyl ester with the appropriate 2-choromethyl-benzimidazole (II), followed by mild alkaline hydrolysis, yielded the desired benzimdazolylmethyl-IDA compounds. The chloromethyl-benzimidazoles were prepared from the corresponding *o*-phenylene diamines (scheme I). The benzimidazole iminodiacetic acid (BIMIDA) derivatives are listed in table 1.



II III

SCHEME I

Biodistribution studies on the BIMIDA compounds labelled with ^{99m}Tc by the stannous chloride reduction method were carried out using normal rats. Imaging studies were performed on rabbits. Although the ^{99m}Tc complexes of the ligands showed little affinity for tumours, it was obvious, from their ability to localise preferentially in the gallbladder (GB) and gut, that we had discovered a series of ligands of considerable potential as technetium hepatobiliary radiopharmaceuticals. The results set out in table 2 indicate that the gastrointestinal tract (GIT) activity, which indicates biliary excretion, increases progressively as the substitution in the benzene ring increases. Methoxy-BIMIDA is an exception, with a markedly lower biliary excretion compared to the parent compound and elevated amounts in the kidneys and urine.

When the effects of the alkyl substituents are compared, it is seen that the dimethyl-BIMIDA produces the highest biliary excretion. The *n*-butyl compound was more slowly excreted, 10 per cent of the injected dose being retained in the liver after 1 h. p-Butyl-HIDA also exhibits a slow hepatic clearance rate [Rosenthal 1978].

Of the halogen substituted BIMIDAs, chloro, dichloro and bromo compounds were associated with the highest biliary excretion, all being approximately 92 per cent and hence the best ligands for this purpose. Dihalogen

substituted ligands did not lead to greater hepatobiliary excretion than the mono-halogen compounds. The iodo-compound which, on the basis of lipophilicity, might have been expected to be the best biliary excretor was actually inferior to all the halo-BIMIDAs other than the fluoro compound which produced the lowest hepatobiliary excretion of this group.

Substituents (R^3) on the ring nitrogen dramatically altered the biodistribution pattern. Hepatobiliary excretion was significantly reduced and urinary excretion increased markedly with as much as 65 per cent of the injected dose being observed in the urinary bladder in the case of the N-hydroxyethyl compound. There was also a retarded liver clearance in most cases.

When the IDA moiety was separated from the benzimidazole ring by more than one methylene group, as in the case of dimethyl-BIMPROPIDA, the same kind of biodistribution change occurred. A similar structural effect has been observed with HIDA compounds and was attributed to a change in pKa of the imino nitrogen which reduced the *in vivo* stability of the ^{99m}Tc complex [Chiotellis and Varvarigou 1980].

For comparison, biodistribution studies on several widely used hepatobiliary agents were also determined in these laboratories. Dimethyl-HIDA produced a somewhat lower biliary excretion after 1 h than the halo-BIMIDAs, and a significantly higher urinary output. n-butyl-HIDA is closer to the BIMIDA ligands in its biodistribution. Among the pyridoxylideneaminate Schiff bases, pyridoxylidene p-isopropylphenylalanine approaches closest to the halo-BIMIDAs and, with a urinary excretion of two per cent of the injected dose after 1 h, [Kato-Azuma and Hazue 1981] appears to be a decided improvement on pyridoxylidene glutamate, which has an excessively high urinary output (24 per cent).

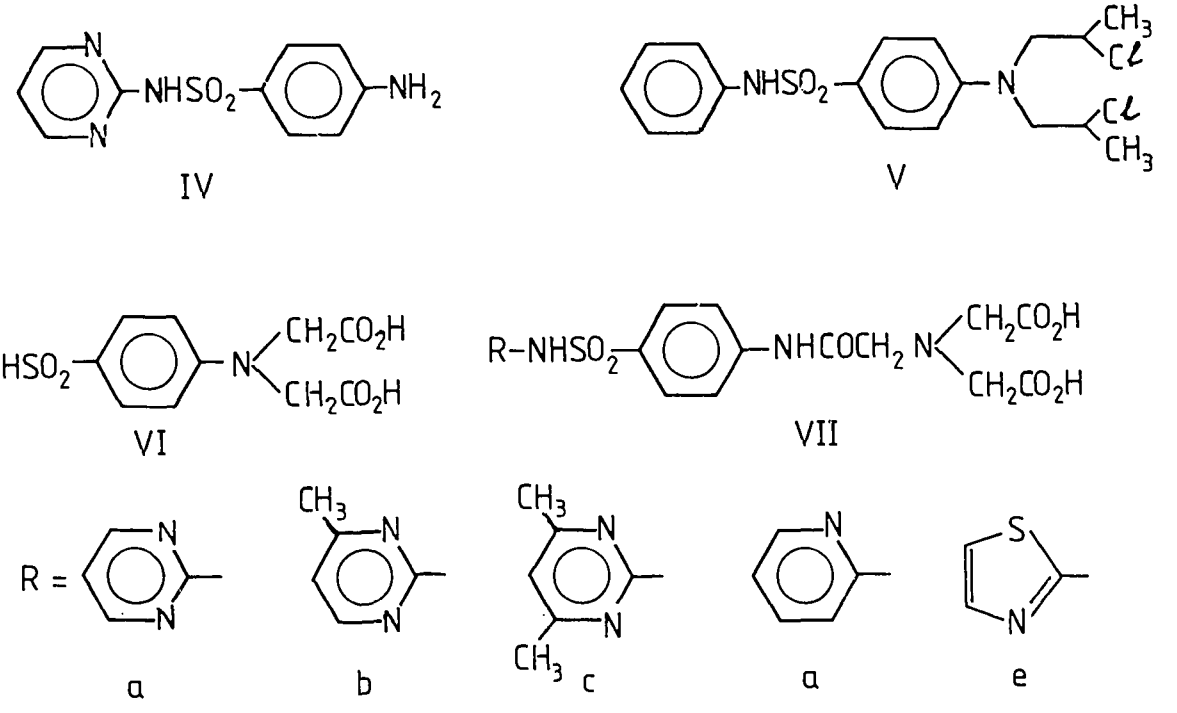
Scintigraphic studies of the ^{99m}Tc-BIMIDA complexes in rabbits are shown in figure 1. After intravenous injection, all the complexes exhibited rapid clearance from the blood by the liver. Imaging of the GB, bile duct and GIT followed as excretion by the biliary system proceeded, the first of these being visualised between five and ten minutes after injection. The elimination route via the kidneys accounted for only very little excretion in the urine. After 60 minutes, the GB was clearly and intensely imaged by the bromo, chloro and dimethyl complexes. In the last case, the liver image was retained longer and, in addition, the image of the urinary bladder was somewhat more intense. The kidneys were only transiently visualised.

As a measure of the rate of uptake of the ligand complexes in the liver after intravenous injection, the time of injection to the time of maximal liver radioisotope concentration, T max., and the time from maximal liver concentration to time of 50 per cent of this concentration $T^{1/2}$ max., of several complexes were recorded. Time activity curves were prepared with the aid of a gamma camera and computer. Results given in table 3 show the effect of various substituents in the ligands on these biological parameters [Fawdry and Hunt 1982]. The three halogen-substituted BIMIDA compounds exhibit virtually the same pharmaco-kinetic profile, whereas of the five ligands studied the dimethyl derivative shows the highest urinary activity after 20 min. and the lowest excretion rate ($T^{1/2}$). The nitro-BIMIDA complex is markedly different from the others showing the most rapid uptake in the liver, the lowest $T^{1/2}$ and consequently the highest slope at $T^{1/2}$. The powerful electron withdrawing effect of the nitro-substituent on the basicity of the benzimidazole nitrogens and also, although to a lesser extent, on the nitrogen of the IDA moiety is doubtless a contributing factor. It is the strongest acid of the BIMIDA ligands studied — $pKa_1 = 3.06$. The pKa_1 values of the bromo and dimethyl compounds are 3.30 and 4.09 respectively.

These studies show that the benzimidazole heterocycle may replace the acetanilido group of HIDA to give technetium-99m radiopharmaceuticals having properties very similar to those of HIDA in laboratory animals. Halogen substituents in the benzene ring of benzimidazole provided the optimal ligands in terms of rapid and maximal excretion *via* the liver into the GIT and minimal urinary excretion.

3. SULPHANILAMIDES

The need for new tumour-labelling radiopharmaceuticals arises largely from the lack of specificity of gallium-67 citrate which is currently the clinical agent of choice for tumour localisation [Edwards 1979]. Although ^{67}Ga exhibits some degree of differential concentration in many human and animal tumours, the uptake is not tumour-specific and high activities are observed in inflammatory lesions, whereas smaller amounts are found in most normal tissues. Abel and colleagues at the Chester Beatty Institute, London, and at the CEA's Nuclear Research Centre at Saclay, France [Abel *et al.* 1973, 1975], found that certain sulphanilamides, such as sulphadiazine (IV), concentrated selectively in the Walker rat carcinoma and the Yoshida sarcoma; this seemed to offer an attractive lead to the type of ligand that might confidently be expected to transport a radionuclide to a tumour. Their studies indicated that the mechanism of selective deposition was quite specifically related to structure. The primary amino group did not appear to be essential and the pyrimidine moiety could be replaced by other heterocycles without loss of activity. Abel *et al.* synthesised several nitrogen-mustard derivatives of various sulphanilamides, of which (V) proved to have outstanding activity against the rat tumours [Calvert *et al.* 1975].



SCHEME II

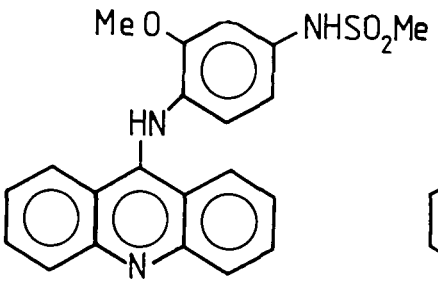
Two series of IDA derivatives were therefore prepared from five well-known sulpha-drugs, sulphadiazine, sulphamerazine, sulphamethazine, sulphapyridine and sulphathiazole (VI and VII, a-e, Scheme II). The first series consisted of N^4, N^4-*bis*carboxymethyl derivatives (VI) which represent the simplest modification of the sulphanilamide structure using IDA; the second consisted of N^4-carbonylmethyliminodiacetic acids (VII) which recent studies have suggested are better suited for complexing with ^{99m}Tc than simple IDA derivatives [Fields *et al.* 1978]. The former group was made by carboxymethylation of the sulphanilamides; the latter by the Burns reaction [Burns *et al.* 1978], a relatively recent method that allows the addition of the group $-COCH_2N(CH_2CO_2H)_2$ to an aromatic amine in one step.

None of the sulphanilamide-IDA compounds were successful in transporting technetium-99m to the tumour under test. Although tumour/muscle ratios were greater than unity at both time intervals (table 4), the tumour/blood ratios are less than unity, indicating a lack of specific concentration in the tumour. This was confirmed by a lack of tumour delineation in the gamma camera studies. The biodistribution of the ^{99m}Tc complexes of the compounds (table 5) revealed that the sulphadiazine and sulphamethazine ligands were excreted primarily *via* the bladder (59.2 and 49.3 per cent respectively in the urine after 2 h), whereas the sulphathiazole derivative was found in the gut (59.3 per cent after 2 h), indicating predominant hepatobiliary excretion [Hunt *et a* 1982a].

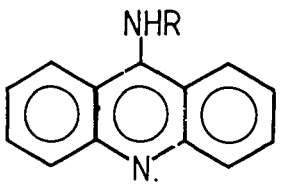
4. ACRIDINES

Certain acridines have been known for some time to localise selectively in tumours and this has been the basis for exploiting this class of compounds for tumour therapy and diagnosis [Ackerman 1972; Davis and Soloway 1967]. A major contribution to research in cancer chemotherapy based on acridines has been made by the New Zealand Cancer Society's Experimental Chemotherapy Laboratory in Auckland, where Dr Bruce Cain (Director of the Laboratory until his untimely death in 1981) and his co-workers synthesised hundreds of acridine compounds in their search for an anti-cancer drug over the past fifteen years. One of their most successful compounds was *m*-AMSA which was judged to have provided "substantial promise" against a number of tumours.

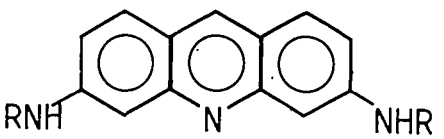
The compound *m*-AMSA provided such an obvious structural clue to the design of ligands for tumour imaging that a number of acridines were modified by attaching an IDA chelating group to an appropriate site in the molecule. Five compounds representative of the acridine types studied by Cain and his group [Atwell *et al.* 1977], each bearing an IDA group, were synthesised (scheme III).



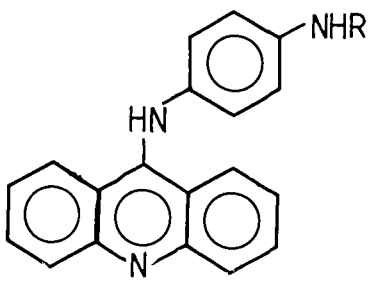
m-AMSA



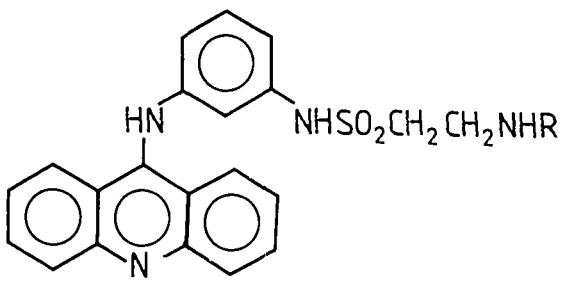
VIII



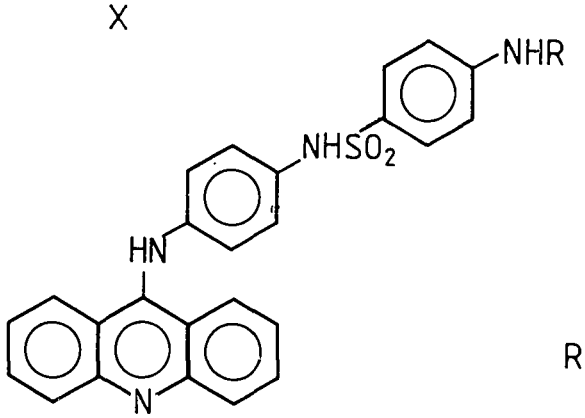
IX



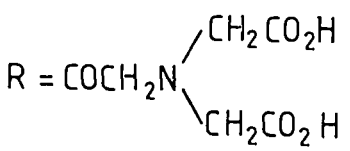
X



XI



XII



SCHEME III

The IDA ligand group was introduced into the compounds (VIII-XII) by a Burns reactions [Burns *et al.* 1978] on the respective aminoacridines (VIII-XII, R=H). For the synthesis of VIII and IX, the starting materials 9-amino- and 3,6-diaminoacridine were obtained commercially. The aminoacridines required for the synthesis of X and XI and XII were made as follows:

X, R=H - condensation of 4-aminoacetanilide with 9-chloroacridine [Atwell *et al.* 1977; Cain *et al.* 1977] and removal of the acetyl group;

XI, R=H - reaction of 2-phthalimidosulphonyl chloride with 3-nitroaniline, hydrazine removal of the phthalimido group, hydrogenation of the nitro group and condensation with 9-chloroacridine under acid conditions [Winterbottom *et al.* 1947];

XII, R=H - reaction of N-acetylsulphanilychloride with X, R=H, and removal of the acetyl group.

The results of biodistribution experiments are shown in table 6. The amounts in the various organs

demonstrate both urinary and hepatobiliary excretion pathways indicating, not surprisingly, the partially lipophilic nature of the ligands.

With the exception of XI, the tumour/blood ratios (table 7) do not exceed unity, indicating a lack of specific concentration in the tumour model used. This is also revealed by the low tumour concentration (%/g) which decreased in the interval between two and twenty-four hours after injection. It is concluded that these compounds are not suitable for scintigraphic tumour localisation, at least in this tumour model [Hunt *et al.* 1982b].

Modification of the sulphanilamides and acridines by attachment of ligand groups and subsequent labelling with ^{99m}Tc has obviously altered their biological distributions and tumour localising properties, probably in a similar way to that observed [Callery *et al.* 1976] in the lidocaine-based IDA compounds (HIDAs). The behaviour of the HIDA compounds was attributed to the formation of ^{99m}Tc complexes with two ligands per atom of technetium, thus forming a molecule with greatly increased molecular weight compared with that of the original lidocaine molecule [Loberg and Fields 1978]. It is probable that the sulphanilamide and acridine IDA compounds also occur as similar *bis*-ligand complexes. The excellent hepatobiliary properties of the BIMIDA compounds also suggest a close resemblance to the HIDA ligands in the type of complex they form with ^{99m}Tc.

Work at Lucas Heights on the development of new radiopharmaceuticals is continuing with the exploratory synthesis of new ligands derived from the attachment of chelating groups other than IDA to molecules of structural and biological interest. By using tetra- and higher-dentate ligands with ^{99m}Tc it might be reasonable to expect the formation of 1:1 metal-ligand complexes with lower molecular weights and hence different lipophilic properties of biodistributions.

5. DRUG INTERACTIONS

Report of *in vivo* interactions between therapeutic drugs and *in vivo* diagnostic radiopharmaceuticals are only slowly appearing in the literature. The potential for such interactions is considerable as many patients referred to nuclear medicine clinics have often been prescribed various drugs by a general practioner before treatment with a radiopharmaceutical. Patients with high blood pressure are often prescribed anti-hypertensive drugs. Two drugs of this type, propranolol and frusemide, have recently been found to have marked affects on the distributions of ^{201}Th in heart and liver tissues of mice [Bossuyt and Jonckheer 1978] and on the distribution of the ^{201}Th and ^{99m}Tc-glucoheptonate complex in the heart tissue of dogs [Hamilton *et al.* 1978; Cahill *et al.* 1978].

Several well-known skeletal imaging agents have also been found to be sensitive to interactions with certain therapeutic drugs. Perturbations of their biodistributions are known to occur in disorders associated with chronic iron overload [Parker *et al.* 1976; Virgilio *et al.* 1980; Choy *et al.* 1985a], although the interaction between these radiopharmaceuticals and iron, when the latter is administered as parenteral therapy, is not so widely recognised. The soft tissue concentrations of ^{99m}Tc-diphosphonates and ^{99m}Tc-polyphosphate associated with intramuscular injections of iron-dextran were reported by Van Antwerp *et al.* [1975] and Buyun *et al.* [1976], who put forward an explanation of the formation of an iron-Tc-PYP complex or a dextran-Tc-PYP complex. More recently, an excessive blood pool activity accompanied by gross reduction of bone uptake was observed in a patient who, at the Prince of Wales Hospital, Sydney, had undergone skeletal scintigraphy with ^{99m}Tc-pyrophosphate (Tc-PYP) 24 hours after an intravenous infusion of iron-dextran [Choy *et al.*, 1985a]. A joint investigation with a clinician of the hospital succeeded in elucidating the phenomenon. The abnormal scan was found to be the result of the formation of circulating complex of iron-dextran and Tc-PYP.

In vivo experiments with rats confirmed the clinical observations. When injected with Tc-PYP rats primed with iron in the form of iron-dextran had significantly higher whole blood activity than controls which could be accounted for solely by the increase of plasma activity. The results also support earlier findings that dextran alone does not combine with Tc-PYP and that pertechnetate does not combine with iron-dextran. They also strengthen the concept of complex formation between iron-dextran and Tc-PYP. *In vitro* experiments added further confirmation to these findings. When a mixture of iron-dextran and Tc-PYP was applied to a Sephadex-G25 column, one of the eluted fractions was identified as a complex of the two compounds. When injected into rats, this fraction concentrated in the plasma and kidneys with little uptake by the skeleton. Scintigraphy of the rats revealed excessive blood pool labelling and an increased renal uptake similar to that observed in humans who have received iron-dextran before skeletal scintigraphy with Tc-PYP. These changes were produced only by a combination of iron-dextran and Tc-PYP, suggesting complex formation between these compounds [Choy *et al.*, 1985b].

Scintigraphic appearances of a rat (A) injected with Tc-PYP, another (B) given iron-dextran before an injection of Tc-PYP and a third (C) injected with eluted iron-dextran-Tc-PYP complex are shown in figure 2. Rat A shows the

normal skeletal scan; the bone scan of rat B is spoiled by increased blood pool activity and renal uptake; and rat C shows mainly renal and blood pool activity with little bone uptake.

Our investigations of the interactions of therapeutic drugs with *in vivo* diagnostic radiopharmaceuticals are continuing. It is an area of vital importance to nuclear medicine and a deeper understanding of these phenomena will benefit both the clinician, by eliminating sources of false diagnosis, and the manufacturers of radiopharmaceuticals by preventing the label 'poor quality' from damaging the integrity of their products.

6. TOXICOLOGICAL EVALUATION OF A PROSPECTIVE HEPATOBILIARY IMAGING AGENT

The study of the biological reactions elicited by radiopharmaceutical ligands on injection into laboratory animals is an important part of their total evaluation before clinical use.

The first ligand selected for toxicological evaluation was dimethyl-BIMIDA (DMB). Although concerned primarily with the unlabelled compound, the investigation included comparative studies of the ^{99m}Tc complex where relevant. The amount of technetium required for one human equivalent dose (HED) is 0.4 ng or 4×10^{-10} g, an amount that could not be expected to have any toxic effects.

The LD_{50} values determined in rodents of each sex following intraperitoneal injection of DMB were 150 mg kg^{-1} for rats and 90 mg kg^{-1} for mice. This route of administration was chosen to reduce the possibility of haemoconcentration of a chelating agent leading to variable dose effect and mortality at a spuriously low dosage. A major reason for estimating the LD_{50} of a pharmaceutical is the need to assess the safety margin associated with its use. Dimethyl-BIMIDA, when labelled with ^{99m}Tc, belongs to the class of diagnostic radiopharmaceuticals designed for once only use, *i.e.* a single intravenous injection of the complex at a human dose of 5 mg/70 kg. Thus, one HED provides a margin between the dose required for diagnosis and lethal toxicity of about 2000 fold for rats and more than 1000 fold for mice.

The route of administration of high concentrations of DMB appeared to be significant with respect to the occurrence of clinical signs antemortem and gross postmortem lesions. There was no immediate physiological change in animals treated by intraperitoneal injection, although significant vascular lesions were evident postmortem. Rats treated intravenously displayed a range of susceptibility to DMB antemortem with acute responses highly suggestive of clinical signs associated with hypocalcaemia, *i.e.* muscle tremors and intermittent toxic spasms characteristic of increased neuromuscular irritability, followed by recovery, death or, in some animals, immediate mortality with no intervening symptoms.

To confirm the role of the iminodiacetic acid moiety in the death of animals treated with DMB, blood samples taken from rats were found to have no measurable quantity of calcium 30 seconds after intravenous injection. Another group of rats was pretreated with calcium gluconate and, together with a control group, intravenously administered known lethal concentrations of DMB. Animals with artificially raised blood calcium levels survived whereas the control group without calcium gluconate died.

Other experiments measured a broad spectrum of biochemical parameters in blood from rats treated intraperitoneally with high doses of DMB. The most significant finding was the reduction of serum alkaline phosphatase (ALP) to 20-25 per cent of control values thoughout the time intervals tested. Alkaline phosphatase is a ubiquitous enzyme, its function being dependent on the tissue in which it is present; recent work has suggested that intestinal ALP is involved in calcium absorption. A characteristic of the enzyme, independent of its source of origin, is its requirement for calcium as an activating ion. *In vitro* experiments confirmed that when an excess of free calcium ions was supplied before administration of DMB, there was no reduction in serum alkaline phosphatase activity. *In vivo* experiments, in which liver and intestinal microsomal preparations from rats treated with labelled and unlabelled DMB were assayed for ALP activity, also indicated a significant change with up to an 80 per cent reduction in liver activity following DMB administration. Concentration of free calcium ions was measured in tissue homogenates of liver and intestine from rats similarly treated with DMB and, although no significant change was apparent in duodenal or ileal samples, the calcium concentration of liver homogenates was still only one third of the control values 15 minutes after injection of DMB.

In the liver, degenerative changes seen under haematoxylin and eosin (H&E) in hepatic parenchymal cells were confirmed using oil red O to demonstrate the presence of lipid. The extent of these tissue changes appeared to increase with higher concentrations of DMB. A lack of significant lesions at the highest dose administered (200 mg kg^{-1}) may have been associated with the rapid mortality following treatment.

The presence of calcium in the renal medulla has been reported as an incidental finding in the rat, dog and cat, deposits being small though often involving several tubules near the corticomedullary junction [Benirschke *et al.* 1978]. When rats were given 100 HED of DMB, the effect on the mineral deposits in the renal medulla of the

animals was minimal. Figure 3 shows deposits seen in the lumen of a collecting duct after staining by H&E. Staining by von Kossa's method confirmed that the deposits were calcium. Rats administered doses of 1400 HED of DMB or more and sacrificed four days after treatment displayed more significant mineralisation. Again, using von Kossa's staining technique, numerous concretion-like deposits were observed scattered throughout the cortex and, to a much lesser extent, the medulla (figure 4). The cortical deposits appeared to be located within the lumina of the proximal convoluted tubules and extended also into the interstitial tissue. The associated tissue damage, however, makes interpretation difficult.

When administered lethal doses of DMB intraperitoneally, rats developed acute lesions with marked vacuolations of cytoplasm, fragmentation and loss of nuclei. Cytoplasmic degeneration was also evident in renal tissue from animals receiving 700 HED of DMB. Whether the degenerative changes and areas of necrosis precipitated the deposition of calcium, as is commonly found, or whether the mineral deposits caused the associated tissue damage, is still a matter of speculation.

With respect to the biochemical aspects of DMB toxicity, studies in rats are concerned at present with measurement of the common drug metabolising enzyme, benzpyrene monooxygenase, by radioactive assay. Following DMB administration (100 HED) there is a significant reduction in the capacity of the enzyme to metabolise benzpyrene, suggesting that DMB could be preventing hydroxylation of the substrate or competing with the substrate benzpyrene for its own metabolism by the enzyme.

To determine whether DMB alters the metabolism and hence toxicity of other compounds, phenobarbitone and benzpyrene are being used as representatives of the two groups of compounds able to stimulate the hepatic microsomal metabolism of other drugs — in the case of phenobarbitone this is a large number, whereas for benzypyrene they are relatively few.

Rats pretreated with phenobarbitone *via* their drinking water displayed an increased ability to metabolise benzpyrene. This, however, was again significantly reduced following DMB administration (100 HED). Further groups of rats pretreated with benzpyrene and subsequently with DMB also showed a reduction in the amount of benzpyrene substrate metabolised.

The possible repercussions of this type of drug interaction in a clinical situation are important. Many drugs and exogenous compounds have the ability to induce the microsomal enzyme responsible for their conversion to less toxic metabolites; it appears, however, that DMB does not induce the mixed function monooxygenase but rather, by interfering with the hepatic microsomal metabolism of other drugs and exogenous compounds that might be present in a patient, it could lead to a toxic accumulation of these substances [Keayes 1982].

Further studies are required in this area to evaluate more completely the significance of DMB and associated hepatobiliary agents in relation to other drugs, and to assess whether the prediction of a clinical response may confidently be based on an animal model.

7. ANIMAL STUDIES

Radiopharmaceutical research and development requires a constant supply of high quality laboratory animals for *in vivo* evaluation of new compounds. The specialised laboratory animal resources facility at Lucas Heights has recently introduced two internationally recognised specific pathogen-free (SPF) in-bred laboratory rat strains. Each of these rodent strains carries a transplantable tumour line, one being a 'T-cell' leukaemia which may be transported as a lymphoma, and the other a mammary carcinoma. These animal models are being developed for labelled imaging agent tumour studies [McNeill 1980; ILAR 1977].

An arrhythmic, immunoincompetent, pathogen-free mouse strain is also available for tumour xenograft studies with possible collaborative radiopharmaceutical research applications using human tumours [ILAR 1976].

8. ASPECTS OF CAESIUM METABOLISM IN THE MAMMALIAN THYROID

Although poorly understood, caesium metabolism is being more widely studied through the use of caesium radioisotopes. Studies comparing caesium and potassium metabolism in rat, bovine and porcine thyroid tissues were carried out using the radioisotopes ^{134m}Cs and ^{42}K. In the rat tissue, both radionuclides showed the same distribution, although, caesium was more concentrated in the thyroid lobes than in other tissue. With tissue culture techniques, caesium and potassium concentrated in the thyroid tissue of the three species at levels of several times the media concentration. Tissue uptake of both ions was inhibited by the absence of sodium or the presence of ouabain or 2,4-dinitrophenol. Both ions also supported the uptake of ^{99m}Tc-pertechnetate at similar rates, the uptake also being inhibited by the presence of ouabain or 2,4-dinitrophenol. It is therefore proposed that caesium is actively transported in thyroid tissue by a mechanism almost identical to that by which potassium is transported.

Potassium transport is known to be associated with an enzyme, Na^+/K^+-ATPase. Using the partially purified enzyme complex in beef thyroid lobe homogenate fractions prepared by differential centrifugation, the Michaelis constants for caesium and potassium activation of the enzyme complex were estimated, and caesium was found to have a lower affinity for the enzyme than potassium.

The relative permeabilities of caesium and potassium in rat lobes at different media cation concentrations and temperatures were investigated by radioisotope efflux methods. In this way, caesium proved to be approximately one half as permeable as potassium under most conditions. Finally, ultracentrifugation, equilibrium dialysis and ultrafiltration methods were used to examine the binding of caesium and potassium to components of beef thyroid lobe homogenate fractions, and although potassium was found to bind to the soluble protein fraction, no evidence of the binding of caesium could be detected.

The studies so far indicate that caesium is metabolised in the same way as potassium by the thyroid gland of the three species and that it too is transported by the same transport mechanism but less efficiently than potassium; the high tissue concentration of caesium observed both *in vivo* and *in vitro* is most probably due to its lower membrane permeability [Maddalena 1979].

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10. REFERENCES

Abel, G., Connors, T.A., Ross, W.C.J., Nguyen-Hoang-Ham, Hoellinger, H., Pichat, L. [1973] - *Eur. J. Cancer*, 9:49.

Abel, G., Connors, T.A., Goddard, P., Hoellinger, H., Nguyen-Hoang-Nam, Pichat, L., Ross, W.C.J., Wilman, D.E.V. [1975] - *Eur. J. Cancer*, 11:787.

Ackerman, N.B. [1972] - *J. Surg. Oncol.*, 49:447,495.

Atwell, G.J., Cain, B.F., Denny, W.A. [1977] - *J. Med. Chem.*, 20:1128

Benirschke, K., Garner, F.M., Jones, T.C. [1978] - Pathology of Laboratory Animals. Springer-Verlag, New York, p.154.

Bossuyt, A., Jonckheer, M.H. [1978] - *J. Nucl. Med.*, 19:973.

Burns, H.D., Sowa, D.J., Marzilli, L.G. [1978] - *J. Pharm. Sci.*, 67:1434.

Buyun, H.H., Rodman, S.G., Chung, K.E. [1976] - *J. Nucl. Med.*, 17:374.

Cahill, P., Jacobstein, J., Alonso, D., Kine, S., Post, M. [1978] - *J. Nucl. Med.*, 19:748.

Cain, B.F., Atwell, G.J., Denny, W.A. [1977] - *J. Med. Chem.*, 20:987.

Callery, P.S., Faith, W.C., Loberg, M.D., Fields, A.T., Harvey, E.B., Cooper, M.D. [1976] - *J. Med Chem.*, 19:962.

Calvert, N., Connors, T.A., Ross, W.C.J. [1968] - *Eur. J. Cancer*, 4:627.

Chiotellis, E., Varvarigou, A. [1980] - *Int. J. Nucl. Med. Biol.*, 7:1.

Choy, D., Murray, I.P.C., Hoschl, R., [1985a] - *Radiology* (in press).

Choy, D., Maddalena, D.J., Murray, I.P.C. [1985b] - *Int. J. Nucl. Med. Biol.* (in press).

Davis, M.A., Soloway, A.H. [1967] - *J. Med. Chem.*, 10:730.

Eckelman, W.C., Karesh, S.M., Reba, R.C. [1975] - *J. Pharm. Sci.*, 64:764.

Edwards, C.L. [1979] - *Semin. Nucl. Med.*, 9:186.

Fawdry, R.M., Hunt, F.C. [1982] - Abstracts, 13th Annual Scientific Meeting of the Australian and New Zealand Society of Nuclear Medicine, Melbourne, 5-7 May, p.16.

Fields, A.T., Porter, W.H., Callery, P.S., Harvey, E.B., Loberg, M.D. [1978] - *J. Labelled Compd. Radiopharm.*, 15:387.

Hamilton, G.W., Narahara, K.A., Yee, H., Ritchie, J.L., Williams, W.L., Gould, K.L. [1978] - *J. Nucl. Med.,* 19:10.

Heindel, N.D., Risch, Y.R., Burns, H.D., Honda, T., Brady, L.W., Micalizzi, M. [1975] - *J. Pharm. Sci.,* 64:687.

Hirschberg, E., Gellhorn, A., Gump, W.S. [1958] - *Ann. N.Y. Acad. Sci.,* 68:888.

Hunt, F.C., Maddalena, D.J., Wilson, J.G. [1979] - Proc. Second Int. Symp. on Radiopharmaceutical Chemistry, Seattle, Washington, 18 February to 2 March. In *Radiopharmaceuticals II.* Society of Nuclear Medicine Int., New York. p.587.

Hunt, F.C., Maddalena, D.J., Wilson, J.G. [1980] - Proc. Third Int. Symp. on Radiopharmaceutical Chemistry, St. Louis, Missouri, 16-20 June, p.191.

Hunt, F.C., Maddalena, D.J., McLaren, A.B., Wilson, J.G. [1981] - Abstracts, Royal Australian Chemical Inst. Organic Synthesis Conf., Sydney University, 30 November, p.4.

Hunt, F.C., Maddalena, D.J., McLaren, A.B., Wilson, J.G. [1982a] - *J. Labelled Compd. Radiopharm.,* 19:203.

Hunt, F.C., Maddalena, D.J., McLaren, A.B. [1982b] - Proc. Third World Congress on Nuclear Medicine and Biology, Paris, 29 August to 2 September.

ILAR [1976] - Guide for the Care and Use of the Nude Mouse in Biomedical Research. Institute of Laboratory Animal Resources; US National Academy of Sciences, Washington, D.C.

ILAR [1977] - Laboratory Animal Management: Rodents. Institute of Laboratory Animal Resources; US National Academy of Sciences, Washington, D.C.

Kato-Azuma, M., Hazue, M. [1981] - *J. Labelled Compd. Radiopharm.,* 18:133.

Keayes, M.C. [1982] - 14th Annual Meeting of the Australian Society for Experimental Pathology, 18-20 August, Dunedin, New Zealand.

Loberg, M.D., Callery, P.S., Harvey, E.B., Faith, W.C., Cooper, M.D. [1975] - *J. Nucl. Med.,* 16:546.

Loberg, M.D., Cooper, M., Harvey, E., Callery, P., Faith, W. [1976] - *J. Nucl. Med.,* 17:633.

Loberg, M.D., Fields, A.T. [1978] - *Int. J. Appl. Radiat. Isot.,* 29:167.

Maddalena, D.J. [1979] - M.Sc Thesis, New South Wales Institute of Technology.

McNeill, J.R. [1980] - Laboratory Rat and Mouse Colonies: Some Research Considerations.AAEC/E492.

Parker, J.A., Jones, A.G., Davis, M.A. [1976] - *Clin. Nucl. Med.,* 1:267.

Rosenthal, L. [1978] - *Can. J. Surg.,* 21:279.

Subramanian, G., McAfee. D.G. [1980] - Proc. Int. Symp. on Medical Radionuclide Imaging, Vol.I, p.1 (and references therein).

Sundberg, M.W., Meares, C.F., Goodwin, D.A., Diamanti, C.J. [1974] - *J. Med. Chem.,* 17:1304.

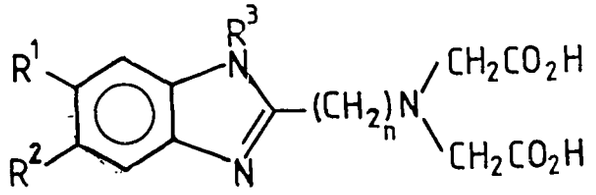
Van Antwerp, J.D., Hall, J.N., O'Mara, R.E. [1975] - *J. Nucl. Med.,* 16:577.

Virgilio, A., Jacobstein, V., Jacobstein, J.G. [1980] - *J. Nucl. Med.,* 21:47.

Winterbottom, R., Clapp, J.W., Miller, W.H., English, J.P., Roblin, R.A. [1947] - *J. Amer. Chem. Soc.,* 69:1393.

TABLE 1

BENZIMIDAZOLE IMINODIACETIC ACID (BIMIDA) LIGANDS



Substituents	R^1	R^2	R^3	n
H	H	H	H	1
Methyl	CH_3	H	H	1
Dimethyl	CH_3	CH_3	H	1
n-Butyl	C_4H_9	H	H	1
Methoxy	CH_3O	H	H	1
Fluoro	F	H	H	1
Chloro	Cl	H	H	1
Dichloro	Cl	Cl	H	1
Bromo	Br	H	H	1
Iodo	I	H	H	1
Nitro	NO_2	H	H	1
N-Methyl	H	H	Me	1
N-Benzyl	H	H	$CH_2C_6H_5$	1
N-Phenethyl	H	H	$CH_2CH_2C_6H_5$	1
N-Hydroxyethyl	H	H	CH_2CH_2OH	1
Dimethyl-BIMPROPIDA	CH_3	CH_3	H	3

TABLE 2

DISTRIBUTION OF ^{99m}Tc-LABELLED BIMIDA AND OTHER LIGANDS IN RATS

% dose in organ at 1 h (means ± s.d. of 3 to 6 animals)

BIMIDA Substituents	Blood	Liver	Kidneys	GIT + GB	Urine
H	3.6 ± 0.2	3.6 ± 0.4	3.9 ± 0.4	60.0 ± 6.0	16.7 ± 2.7
Methyl	1.9 ± 0.1	2.6 ± 0.5	1.8 ± 0.1	75.2 ± 7.5	10.3 ± 1.2
Dimethyl	0.9 ± 0.1	1.7 ± 0.3	0.8 ± 0.08	84.0 ± 2.9	6.0 ± 0.8
n-Butyl	5.1 ± 0.4	10.3 ± 0.5	2.2 ± 0.1	74.6 ± 2.2	5.1 ± 0.7
Methoxy	6.0 ± 1.0	4.3 ± 0.2	7.4 ± 2.0	45.7 ± 8.0	25.4 ± 3.3
Fluoro	1.0 ± 0.1	1.6 ± 0.3	0.8 ± 0.0	76.0 ± 2.1	6.4 ± 0.1
Chloro	0.7 ± 0.1	1.3 ± 0.2	0.3 ± 0.0	92.3 ± 2.2	1.7 ± 0.4
Dichloro	1.5 ± 0.2	2.2 ± 0.2	0.8 ± 0.2	91.0 ± 1.5	1.2 ± 0.2
Bromo	1.2 ± 0.3	2.5 ± 1.5	1.6 ± 0.2	92.1 ± 2.5	6.1 ± 2.6
Iodo	5.1 ± 1.8	3.6 ± 0.5	1.3 ± 0.1	83.6 ± 0.7	2.4 ± 0.1
Nitro	3.1 ± 0.4	7.4 ± 0.1	1.5 ± 0.1	76.1 ± 1.8	4.6 ± 0.5
N-Methyl	5.0 ± 0.5	32.2 ± 0.8	12.6 ± 1.2	7.8 ± 0.4	26.6 ± 3.0
N-Benzyl	5.0 ± 0.4	47.1 ± 0.3	6.8 ± 0.6	16.8 ± 0.7	14.0 ± 2.2
N-Phenethyl	7.2 ± 0.3	3.7 ± 0.7	18.8 ± 1.6	8.0 ± 0.4	39.0 ± 5.9
N-Hydroxyethyl	2.8 ± 0.5	1.3 ± 0.2	2.5 ± 0.1	9.5 ± 1.6	65.0 ± 2.8
Dimethyl-BIMPROPIDA	8.7 ± 0.3	16.4 ± 0.8	10.4 ± 0.4	14.3 ± 1.0	26.6 ± 1.5
	Other Widely Used Ligands				
Dimethyl HIDA	0.3 ± 0.0	0.6 ± 0.1	0.4 ± 0.0	81.2 ± 2.8	125 ± 1.0
n-Butyl HIDA	2.1 ± 0.8	2.6 ± 0.4	1.5 ± 0.7	86.7 ± 4.5	4.6 ± 1.7
PG*	2.0 ± 0.1	1.8 ± 0.3	0.9 ± 0.1	53.8 ± 1.2	24.0 ± 1.0
PiproPhe**	0.08	7.48	0.41	87.57	2.13

GIT = Gastrointestinal tract; GB = Gallbladder.

* Pyridoxylidene glutamate;

** Pyridoxylidene *p*-isopropylphenylalanine (values taken from Kato-Azuma and Hazue [1981])

TABLE 3
RABBIT LIVER KINETICS OF ^{99m}Tc-BIMIDA COMPLEXES

BIMIDA	% ID Urine at 20 min	T Max. (min)	$T^{1/2}$ Max. (min)	Slope at T½ Max. (counts s^{-1})
Nitro	4.0 ± 1.1	2.8 ± 0.5	5.9 ± 0.3	-34 ± 9
Iodo	3.6 ± 1.3	4.1 ± 0.9	10.8 ± 0.5	-19 ± 1
Chloro	2.5 ± 0.6	5.1 ± 0.7	11.3 ± 2.5	-20 ± 7
Bromo	1.7 ± 0.5	5.3 ± 0.3	10.3 ± 1.2	-16 ± 1
Dimethyl	7.0 ± 3.3	4.5 ± 0.5	12.8 ± 1.1	-9

TABLE 4
TUMOUR/TISSUE RATIO
[% per g tumour/% per g tissue]

Compound	Tumour/Muscle	Tumour/Blood
^{99m}Tc-VIIa	3.2[a]	0.70
	2.9[b]	0.79
^{99m}Tc-VIIc	6.9[a]	0.58
	10.1[b]	0.43
^{99m}Tc-VIIe	4.0[a]	0.58
	4.0[b]	0.61

[a] Two hours and [b] 24 hours after injection.

TABLE 5
BIODISTRIBUTION OF ^{99m}Tc CHELATES OF
N^4-SULPHANILAMIDE CARBONYLMETHYLIMINODIACETIC ACIDS[a]
[Means of three animals]

Compound	t[b]	Liver	Kidneys	Muscle	Blood	Urine	GIT + Faeces	Tumour
Sulphadiazine -	2	1.8	4.32	5.14	3.13	59.2	14.9	0.41
(VIIa)	24	0.61	2.89	1.26	0.78	76.1	12.1	0.15
Sulphamethazine -	2	2.77	3.01	2.90	4.93	49.3	25.7	1.30
(VIIc)	24	0.73	2.28	0.48	1.55	59.7	30.1	0.23
Sulphathiazole -	2	1.59	2.50	2.48	2.48	19.8	59.3	0.36
(VIIe)	24	0.58	2.31	1.22	0.94	35.6	49.1	0.21

[a]Percentage of injected dose per organ. [b]Hours after injection.

TABLE 6
BIODISTRIBUTION OF 99m-TECHNETIUM ACRIDINYL IMINODIACETIC ACID CHELATES

Compound	t	Liver	Kidneys	Muscle	Blood	Urine	GIT + Faeces	Tumour
VIII	2	9.5 ± 0.1*	5.5 ± 0.1	4.4 ± 0.1	3.5 ± 0.3	29.0 ± 2.3	37.5 ± 1.0	0.17 ± 0.07
	24	5.0 ± 0.6	3.8 ± 0.2	1.2 ± 0.1	0.8 ± 0.0	43.6 ± 1.3	38.4 ± 7.6	0.11 ± 0.00
IX	2	6.6 ± 0.2	3.7 ± 0.4	2.7 ± 0.1	2.5 ± 0.1	70.0 ± 11.1	6.1 ± 0.0	0.10 ± 0.00
	24	8.7 ± 0.9	6.0 ± 0.4	1.2 ± 0.1	1.2 ± 0.0	70.4 ± 4.8	14.0 ± 0.7	0.19 ± 0.06
X	2	13.5 ± 0.5	12.2 ± 1.3	4.1 ± 0.4	5.9 ± 0.4	25.8 ± 2.2	47.2 ± 0.7	0.20 ± 0.02
	24	3.8 ± 0.3	7.1 ± 0.3	1.0 ± 0.1	0.8 ± 0.1	51.1 ± 1.4	40.6 ± 5.2	0.17 ± 0.04
XI	2	17.8 ± 0.8	10.6 ± 0.3	3.3 ± 0.2	6.7 ± 0.1	18.9 ± 0.5	29.5 ± 1.4	0.11 ± 0.00
	24	10.0 ± 0.3	6.7 ± 0.3	1.4 ± 0.1	0.9 ± 0.1	32.8 ± 3.0	40.4 ± 3.4	0.09 ± 0.09
XII	2	11.3 ± 0.4	14.4 ± 0.9	2.7 ± 0.2	4.7 ± 0.2	34.2 ± 1.1	23.1 ± 0.4	0.13 ± 0.02
	24	4.8 ± 0.2	6.4 ± 0.5	1.0 ± 0.2	1.1 ± 0.1	31.2 ± 2.9	51.5 ± 2.5	0.18 ± 0.11

*Means ± standard deviations of the % ID in three rats with implanted leukaemia tumours.

GIT = gastrointestinal tract t = time post injection (h).

TABLE 7
TUMOUR CONCENTRATION AND TUMOUR/TISSUE RATIOS
[% per g tumour/% per g tissue]

Compound	Tumour %/g	Tumour/Blood	Tumour/Muscle
^{99m}Tc-VIII	[a] 0.08 ± 0.03	0.30 ± 0.08	2.0 ± 0.9
	[b] 0.04 ± 0.01	0.70 ± 0.17	3.4 ± 0.7
^{99m}Tc-IX	[a] 0.07 ± 0.30	0.30 ± 0.20	2.6 ± 0.5
	[b] 0.05 ± 0.01	0.8 ± 0.20	5.5 ± 1.6
^{99m}Tc-X	[a] 0.10 ± 0.05	0.4 ± 0.20	3.5 ± 1.1
	[b] 0.06 ± 0.003	1.1 ± 0.30	4.4 ± 2.5
^{99m}Tc-XI	[a] 0.20 ± 0.03	0.40 ± 0.10	6.0 ± 1.4
	[b] 0.10 ± 0.002	1.60 ± 0.08	7.4 ± 0.1
^{99m}Tc-XII	[a] 0.10 ± 0.01	0.30 ± 0.02	4.0 ± 1.02
	[b] 0.06 ± 0.02	0.90 ± 0.20	7.2 ± 3.30

[a] Two hours and [b] 24 hours after injection.

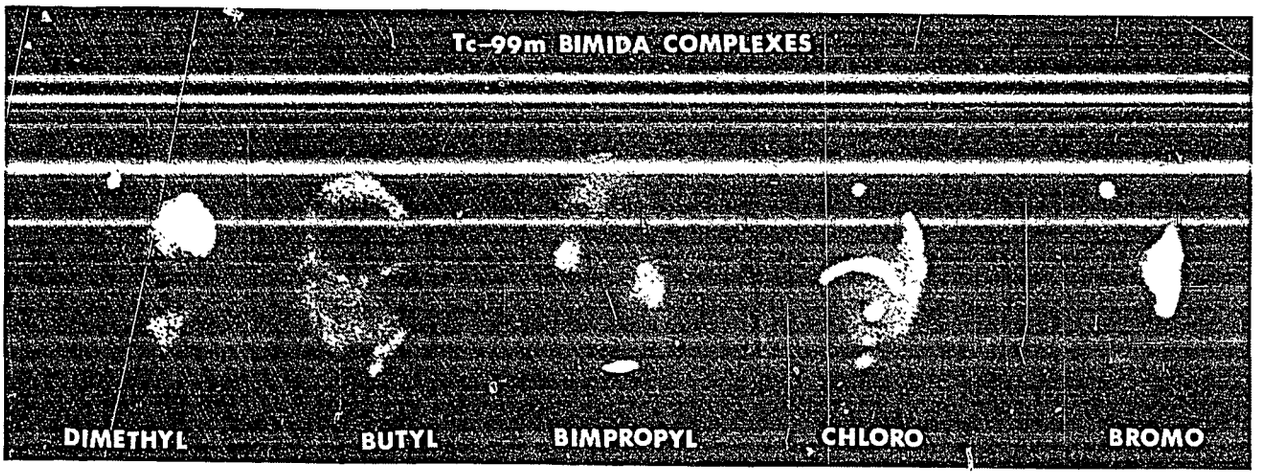
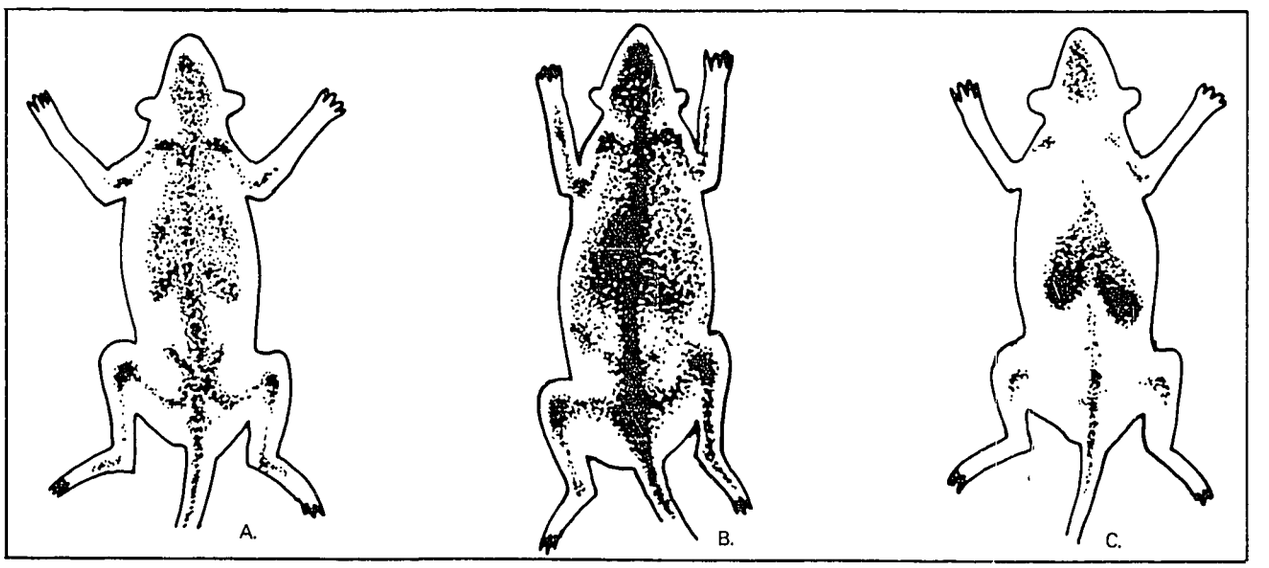


Figure 1 Scintigraphic studies of ^{99m}Tc-BIMIDA complexes in rabbits 1 h after injection



Uni of NSW Dept Medical Illustration photos

Figure 2 Drug interactions

A. Bone scan from the radiopharmaceutical technetium-pyro-phosphate (Tc-PYP)

B. Bone scan is spoiled by the image of the kidney and bloodstream. Prior to injection with Tc-PYP, the 'patient' had been given the drug iron dextran (ID), which is used for treating iron deficiency

C. Injection of the radiopharmaceutical, obtained when Tc-PYP and ID are allowed to interact in the laboratory, produces a clear image of the kidneys and the bloodstream. Similar images can also be obtained in medical practice, when patients treated with ID are also given Tc-PYP

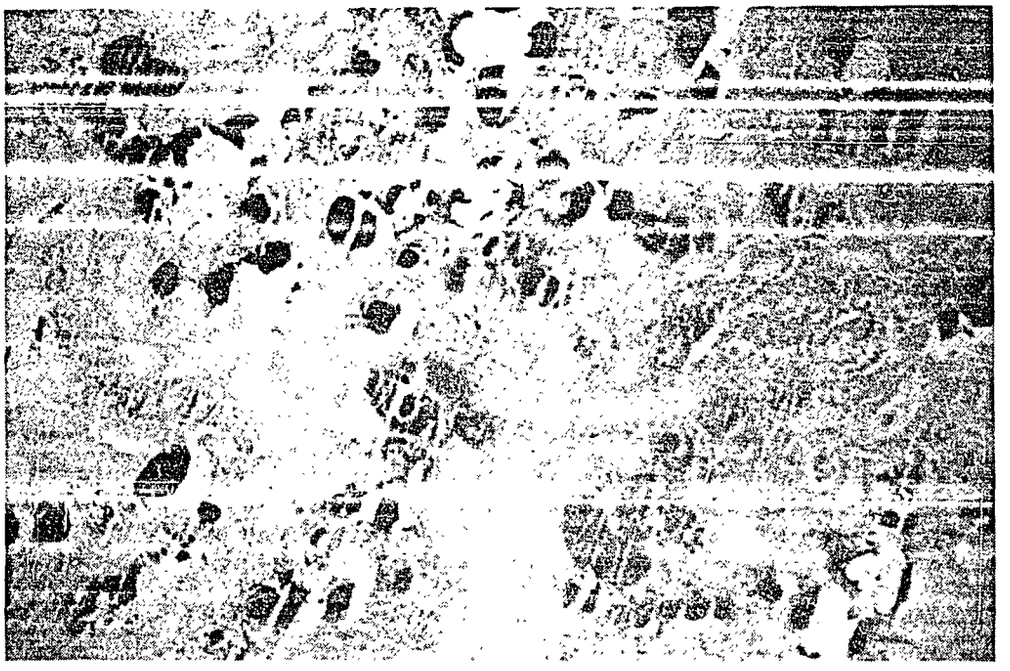


Figure 3 Von Kossa's stain, rat kidney four days after treatment with 100 mg/kg BIMIDA. Renal cortex shows numerous calcium deposits within tubule lumina extending also into interstitial tissue

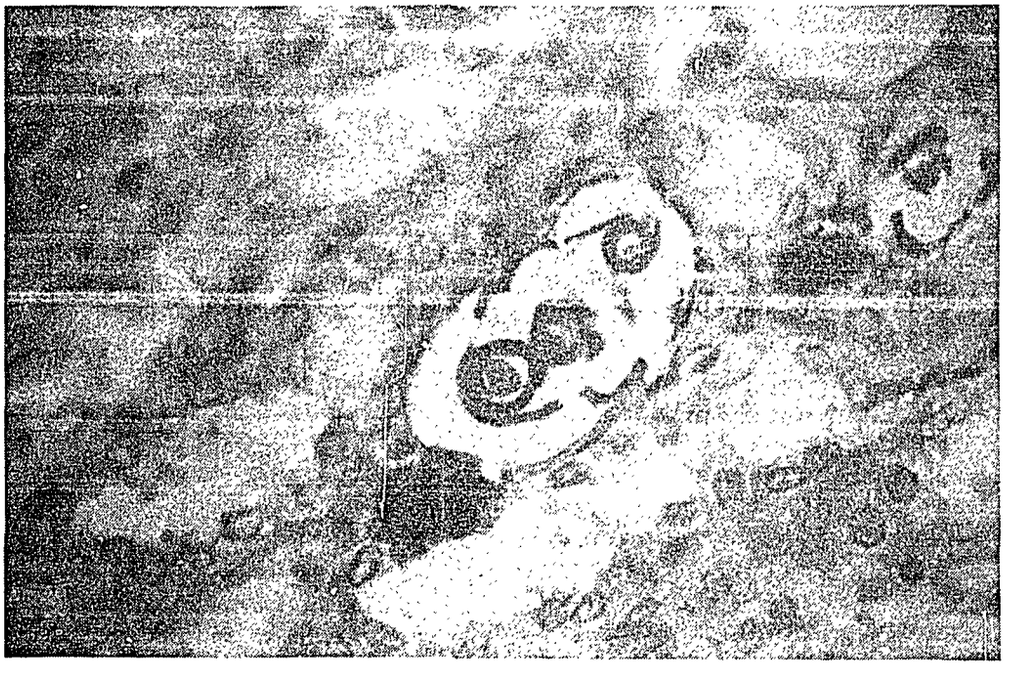


Figure 4 H & E, rat kidney 24 hours after 100 human equivalent doses BIMIDA (7.1 mg/kg). Concretion-like mineral deposit within collecting duct lumen, renal medulla

PART II
GENERATOR SYSTEMS

by

R.E. BOYD

1. INTRODUCTION

Radionuclide generators are a practical solution to the logistic problems caused by the demand for short-lived radiopharmaceuticals. Whether based on the secular or the transient equilibrium decay process, these generators provide a long-lived source for a short-lived daughter radionuclide. Consequently, they have removed the constraints of time and distance which, otherwise, would have limited the spread of nuclear medicine.

Few, if any, would dispute the claim [Richards *et al.* 1982] that nuclear medicine owes its emergence and continued existence to the important role played by technetium-99m (^{99m}Tc). An exponential increase in the use of ^{99m}Tc has placed this radionuclide in a pre-eminent position in contemporary nuclear medicine. In the 1960s, workers at the Brookhaven National Laboratory, Long Island, USA, overcame the problem associated with a short half-life by developing a ^{99m}Tc generator based upon fission product molybdenum-99 (^{99}Mo) adsorbed on chromatographic alumina. Since that time there have been many other innovations.

The inevitability that, despite the growing availability of cyclotron radioisotopes and generators based upon other parent-daughter systems, the practice of nuclear medicine in Australia will continue to rely on ^{99m}Tc for the base load of patient scans, provides a substantial justification for continued scientific investigation into a better understanding of the ^{99m}Tc generator and a search for improved versions. This section of the report summarises work done at Lucas Heights on the development of new ^{99m}Tc generators in the period 1978-82.

Notwithstanding the validity of the above statements, ^{99m}Tc lacks certain properties which impose constraints on the scope of its applications. For example, its radioactive half-life ($T_{½}$)is too long for applications where repeat studies are required over a short time. The use of ultra short-lived photon-emitting radionuclides has several advantages. The radiation dose to the patient is minimised and the higher photon fluxes improve accuracy and image quality. A number of generator systems have been proposed (table 1). The initial studies performed on one of these newer generator types are discussed.

2. THE MARK III TECHNETIUM GENERATOR

The Mark III generator (figure 1) has been designed to meet the present standards of Australian Federal and State health regulatory authorities and the practical demands of the nuclear medicine community.

A new production facility has been constructed to produce the generators in a controlled, clean environment and, as an additional precaution against microbial contamination, the generators are being autoclaved and then assembled under aseptic conditions. The development program has taken about four years to complete, during which time a number of difficult, yet scientifically interesting, problems have had to be solved. Several of these are described here.

The first major problem encountered was the detrimental effect of autoclaving on the elution efficiency of the generator. Several potential solutions were investigated; these have been described in a general review of ^{99m}Tc generators by Boyd [1982].

The 'dry' generator, in which the saline remaining in it after elution is displaced with air, is one method by which high elution efficiency can be maintained throughout the life of the generator. High elution efficiency can also be maintained when either pure nitrogen or pure water is used to displace the saline, although the latter gives rise to chemically active species by radiolysis. It was observed that reduction in elution efficiency seemed to be

caused by the chloride ions in the eluant; this was subsequently confirmed in experiments using sodium sulphate and sodium perchlorate eluant solutions. It was further observed that traces of organic substances dissolved in the saline had a profound effect on the performance of an autoclaved generator, and that the presence of these residues potentiated the effect of the chloride ions. This aspect of our work has contributed significantly to the understanding of mechanisms underlying the successful elution of ^{99m}Tc from the dry generator, and dispelled previous misconceptions.

On the practical side, however, the dry generator had a number of defects, such as exposed upward pointing needles, which could be a source of possible microbial contamination and potentially dangerous to the operator; associated with this problem was the difficulty of making less than full volume elutions.

These defects prompted a search for a design which allowed the generator to be connected permanently to a reservoir of saline by a system sealed from the external environment. In the interests of safety, the saline reservoir had to be held within a non-fragile (not glass) container. Saline contained in vinyl sachets is known to be contaminated with the plasticiser ethyl hexyl phthalate (at a concentration of about 0.5 ppm). Not only has this material been proscribed in the United States as a potential carcinogen but also its presence in the saline has, as already stated, a detrimental effect on ^{99m}Tc elution. This effect was satisfactorily overcome by placing a small charcoal cartridge [Boyd and Sorby 1984] between the reservoir and the generator bed; high elution efficiencies were thus achieved with the removal of a potential source of toxicity. This technique for improving generator performance has been patented by the AAEC.

As a result of continued work on the refinement of generator design, a number of features were incorporated into the Mark III generator to improve performance characteristics. When the alumina is coated with an insoluble scavenger of radiation-induced hydrated electrons (*e.g.* chromate- and manganese-coated alumina) the elution efficiency of a ^{99m}Tc generator is enhanced. Similar results were obtained at Lucas Heights with ceric- and silver-coated alumina and were sufficiently novel for the AAEC to seek and be granted international patents covering Ce(IV)- and Ag-modifications to alumina [Boyd and Matthews 1978]. Subsequently however, Ce(IV) was found to be superior, particularly with respect to autoclaved generators. Cerium, an element whose insoluble compounds possess only slight toxic properties resembling those of aluminium, should be considered as a pharmaceutically acceptable additive to the generator system, particularly as it is present in the form of its refractory oxide. Furthermore, the incorporation of 0.1 wt % of CeO_2 into the alumina provides the added advantage of significantly reducing the level of radio contaminants in the eluted ^{99m}Tc. Advantages which accrue from the use of cerium-coated alumina are essential if the goal of optimum generator performance is to be achieved.

The overall program for the development of the Mark III generator has become somewhat protracted because of the number of very difficult problems (not only scientific) which had to be overcome. A completely new sterile production facility had to be built to a design acceptable to the Australian National Biological Standards Laboratory, the construction of which was delayed for several months because of an industrial dispute. Staff had to be retrained in aseptic operations, and it was necessary to validate the proposed manufacturing procedures in order to satisfy the requirements of the Code of Good Manufacturing Practice. In addition the generator package had to be tested under rigorous conditions in order to comply with new transport regulations required by the International Atomic Energy Agency and the International Air Transport Association (IAEA/IATA).

The final pre-clinical trial runs have been completed and the data generated will define the generator in terms of its various properties and qualities. The total work program associated with the development of this generator has called upon the ingenuity and expertise of a large team of research workers at Lucas Heights.

3. THE TECHNETIUM GEL GENERATOR

A new ^{99m}Tc generator has been developed at Lucas Heights to the stage where a number of 50 Ci generators have been prepared and tested [Evans and Matthews 1978]. The generator uses an insoluble zirconium molybdate gel prepared with an open matrix structure to allow the free diffusion of the pertechnetate ion (TcO_4^-), and hence the ability to 'milk' in high yield, by a simple elution technique. The gel is prepared from molybdenum produced by the neutron activation ($(n,\gamma)^{99}$Mo) in a process that avoids the problems of cost, safety, processing and waste disposal associated with molybdenum obtained from the fission of uranium ($(n,f)^{99}$Mo). At the same time, the problem of low activity, which restricts the use of chromatographic generators prepared from $(n,\gamma)^{99}$Mo, has been overcome by the use of a gel that is approximately 25 per cent molybdenum. Elution profiles depend on a number of factors including particle size, elution time, solid/liquid ratio, and bed dimensions.

The results in table 2 show that elution efficiencies are typically in the range 75 to 90 per cent. Furthermore, no decrease in efficiency occurs over the life of the generator.

3.1 Radionuclidic Impurities

Molybdenum-99 is the nuclide most likely to be present in the eluates in significant quantities *via* a release mechanism from the gel. Analysis of all eluates showed that the ^{99}Mo content of the ^{99m}Tc solutions was between 0.001 to 0.1 per cent of total activity; this reduced to less than 0.0001 per cent on passage of the eluate through a suitably designed zirconia bed. The British Pharmacopoeia [BP 1980] quotes a limit of 0.1 per cent for ^{99}Mo. The ^{99}Mo values did not significantly increase over the life of the generator, thus confirming the chemical stability of the gel under elution conditions. The zirconia beds retained their adsorptive capacity when used daily for two weeks.

Most radionuclidic impurities arise from neutron activation of impurities in the target MoO_3. By careful analysis of fresh and partly decayed samples, more than 20 radionuclidic impurities were identified. Soluble nuclides such as ^{24}Na, ^{42}K, ^{134}Cs, ^{186}Re and ^{188}Re were largely eliminated in the filtrate and washings during gel preparation. Others were fixed in the gel and produced no soluble impurities. A few, such as ^{95}Zr and ^{113}Sn produced traces of daughter nuclides such as ^{95}Nb and ^{113m}In in some eluates. Apart from ^{99}Mo, the commonest impurity in the eluates was ^{92m}Nb which was produced by the reaction ^{92}Mo (n,p) ^{92m}Nb. However, the amounts present never exceeded 5×10^{-5} per cent, and were frequently less than 1×10^{-5} per cent, the statistical limit of detection.

3.2 Chemical Impurities

Analysis of eluates gave the following metals:

- Mo $<$ 1 ppm (limit of detection); and
- Zr $<$ 0.5 ppm (limit of detection).

These did not increase over the life of the generator. The occluded salt in the gel was easily removed by washing and, in early elutions, the nitrate concentrations were between $<$ 1 to 10 ppm (dependent upon degree of washing), which further reduced in later elutions. There was no evidence of colloidal matter in the eluates.

The gel generator has several advantages over the solvent extraction method for the production of pertechnetate solution in a central dispensing laboratory. The fact that the daily supply of ^{99m}Tc is obtained by a simple elution reduces demands on the skill and training of the production staff. The product is obtained quickly in high yield by a process that is essentially free from organic impurities. These features will make it easier for a producer to maintain a reliable supply of high quality pertechnetate. The high efficiency also ensures that levels of the ^{99}Tc isomer in the pertechnetate are kept at a minimum.

Present indications are that after full development the gel generator will be an economic and convenient source of ^{99m}Tc. It should appeal especially to countries that have reasonably high flux reactors and either lack the facilities for, or wish to avoid the problems of, processing uranium fission products [Evans *et al.* 1982].

4. THE OSMIUM-IRIDIUM GENERATOR

Iridium-191m ($T_{½}$ 4.9 s), which decays with the emission of a 120 keV gamma, has been proposed as th agent of choice for a number of medical procedures, particularly the diagnosis of cardiovascular diseases i newborn infants, where serial studies involve considerable radiation exposure.

Iridium-191m is formed in the decay of ^{191}Os ($T_{½}$ = 15.3 days) and an Os-Ir generator system can be used for a continuing supply of the short-lived nuclide [Treves *et al.* 1979; Cheng *et al.* 1980]. Although the AAEC has commenced studies on the production of ^{191}Os, by irradiation of ^{190}Os in the materials testing reactor HIFAR, and the development of a practical generator system, the experimental generators so far prepared have shown excessive ^{191}Os breakthrough and the presence of other significant radionuclidic impurities [Hetherington and Sorby 1984].

Progress in this work has been hampered by the unavailability of highly enriched ^{190}Os target material which is required to increase the attainable ^{191}Os specific activity and to eliminate other osmium and iridium nuclides produced during the reactor irradiation.

5. RESULTS

The results of experiments performed in the study of factors influencing ^{99m}Tc generator performance are given in graphical form in Appendix A. Factors investigated were

- the effect of radiolysis on generator performance;
- the sensitivity of the generator to organic materials;
- the effect of autoclaving the generator and the importance of saline containers;
- identification of the deleterious effects of chloride ions;
- Neutralisation of the effects of radiolysis and organic impurities;
- the effect of nitrate concentration in the saline on elution efficiency; and
- a comparison of nitrate doped versus saline purified generators.

6. CONCLUSIONS

The radionuclide generator was conceived to permit short-lived radiopharmaceuticals to be used many thousands of miles away from the point of manufacture. Recent history proves that the spread of nuclear medicine techniques was due to the technological refinements which made ^{99m}Tc widely available.

The use of generators will continue, but the development of imaging modalities, such as computer-aided tomography and nuclear magnetic resonance, with their vastly superior powers of spatial resolution, has changed the emphasis in nuclear medicine away from visualising anatomic structures to the study of function.

Positron-emitting cyclotron radioisotopes will play a larger role in nuclear medicine, for example in the technique of positron emission tomography (PET). Many of these have ultra-short lives and may be restricted in use to the immediate vicinity of the cyclotron. A number of short-lived positron emitters are produced by the decay of longer-lived parent radionuclides; these provide the opportunity for further generator development.

When access to a radioisotope-producing cyclotron becomes a reality, the future demands of the demographically dispersed Australian nuclear medicine community will be satisfied *via* these newer types of generator.

7. ACKNOWLEDGEMENTS

The author gratefully acknowledges the cooperation of Eric Hetherington, Peter Sorby, Phillip Moore and other colleagues in the Isotope Division whose combined efforts are presented in this report.

8. REFERENCES

B.P. [1980] - British Pharmacopoeia

Benjamins, H.M., Kortenoeven, K., Panek-Finda, H. [1974] - Method of manufacturing a generator which produces radioisotopes and has an improved elution efficiency, and generator obtained by this method. US Patent 3,785,990; 15 January.

Boyd, R.E. [1982] - *Radiochim. Acta*, 30:123-145.

Boyd, R.E., Matthews, R.W. [1978] - Technetium-99m generators - improvements to performance. US Patent 4,206,358, 16 October.

Boyd, R.E., Sorby, P.J. [198] - *Int. J. Appl. Radiat. Isot.*, 35:993.

Charlton, J.C., Lyons, D. [1975] - Technetium-99m generators. Australian Patent 4,640,43B; 29 July.

Cheng, C., Treves, S., Samuel, A. and Davies, M.A. [1980] - *J. Nucl. Med.*, 21:1169.

Evans, J.V. and Matthews, R.W. [1978] - Australian Patent 515808, together with six other world patent applications.

Evans, J.V., Moore, P.W., Shying, M.E. and Sodeau, J.M. [1982] - Abstracts, 13th Annual Scientific Meeting of the Australia and New Zealand Society of Nuclear Medicine, Melbourne, 5-7 May.

TABLE 1

GENERATORS OF SHORT HALF-LIFE RADIONUCLIDES FOR NUCLEAR MEDICINE

Parent	Half-life	Daughter	Half-life	Decay Mode (%)	Gamma MeV (%) other than 0.51 MeV
^{52}Fe	8.3 h	^{52m}Mn	21.1 m	β^+(98) EC(2)	1.43(100)
^{62}Zn	9.1 h	^{62}Cu	9.7 m	β^+(100	0.59(22)
^{68}Ge	287 d	^{68}Ga	68.3 m	β^+(88) EC(12)	1.8(3.5)
^{77}Br	57 h	^{77m}Se	17.5 s	IT	0.162(52)
^{81}Rb	4.58 h	^{81m}Kr	13.3 s	IT	0.191(67)
^{82}Sr	25 d	^{82}Rb	76 s	β^+(96) EC(4)	0.78(9)
^{113}Sn	115.1 d	^{113m}In	1.66 h	IT	0.392(64.1)
^{118}Te	6.0 d	^{118}Sb	3.5 m	β^+(75) EC(22)	1.23(3)
^{122}Xe	20.1 h	^{122}I	3.5 m	β^+(100)	0.56(14)
^{128}Ba	2.43d	^{128}Cs	3.8 m	β^+(51) EC(49)	0.44(27)
^{191}Os	15.4 d	^{191m}Ir	4.9 s	IT	
^{195m}Hg	40 h	^{195m}Au	30.6 s	IT	0.262(68.2)

Boyd, R.E., Hetherington, E.L.R., Moore, P.W. [1984] - From *Radionuclide generator technology, status and prospects*, Proc. Int. Conference on Radiopharmaceuticals and Labelled Compounds, IAEA, Tokyo, October pp.79-94.

TABLE 2

ELUTION EFFICIENCIES

Generator Activity (Ci)	Number of Daily Elutions	Mean Elution Efficiencies (%)
29.2	9	83.2
17.3	12	86.9
54.7	12	90.4
46.9	10	98.4
41.9	7	82.0
17.8	7	95.8
0.91	11	78.7
1.86	8	81.1
1.86	6	88.4
1.61	8	76.5
1.41	8	70.3
0.41	8	72.7

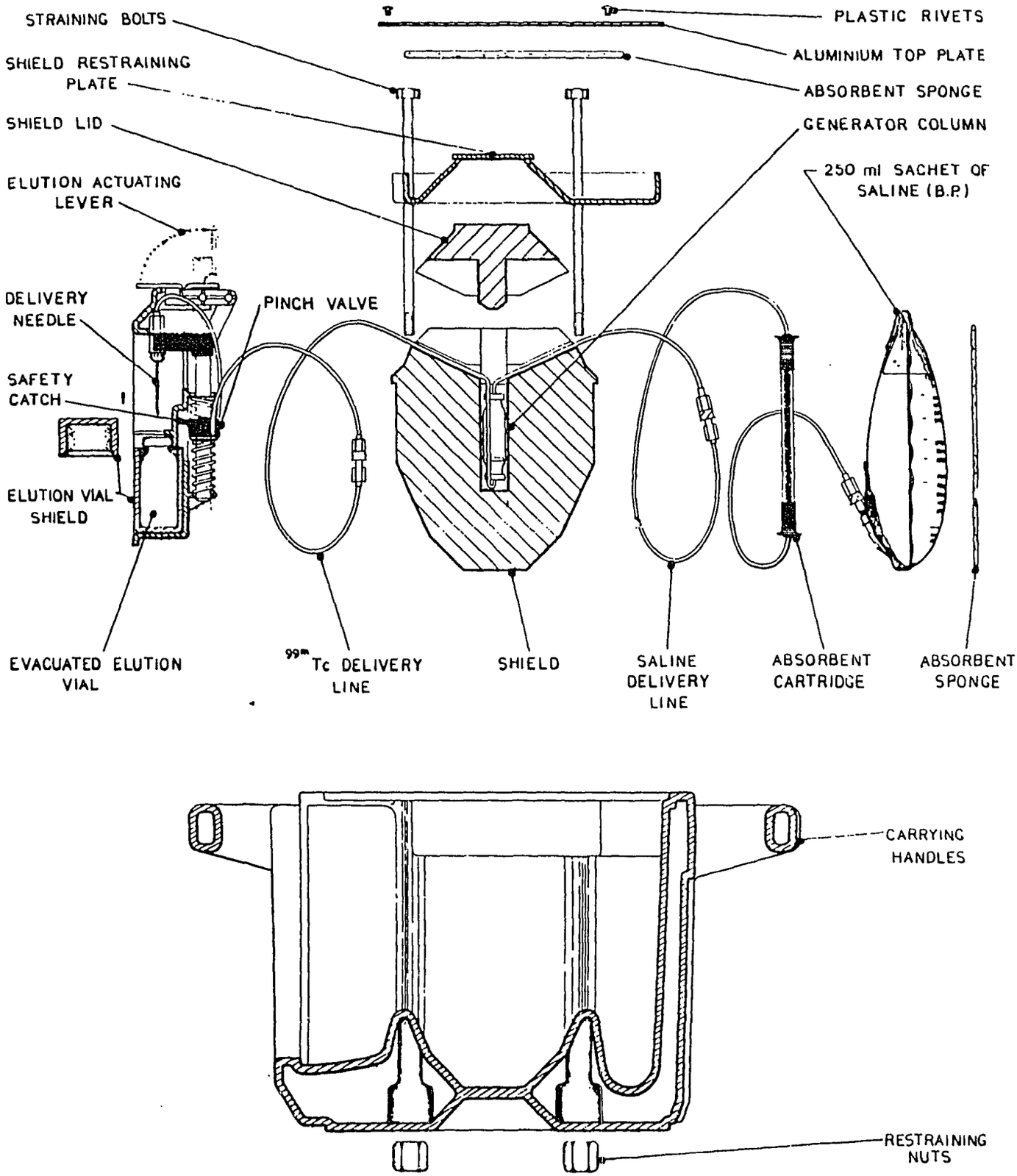


Figure 1 ^{99m}Tc generator

APPENDIX A
EXPERIMENTAL RESULTS

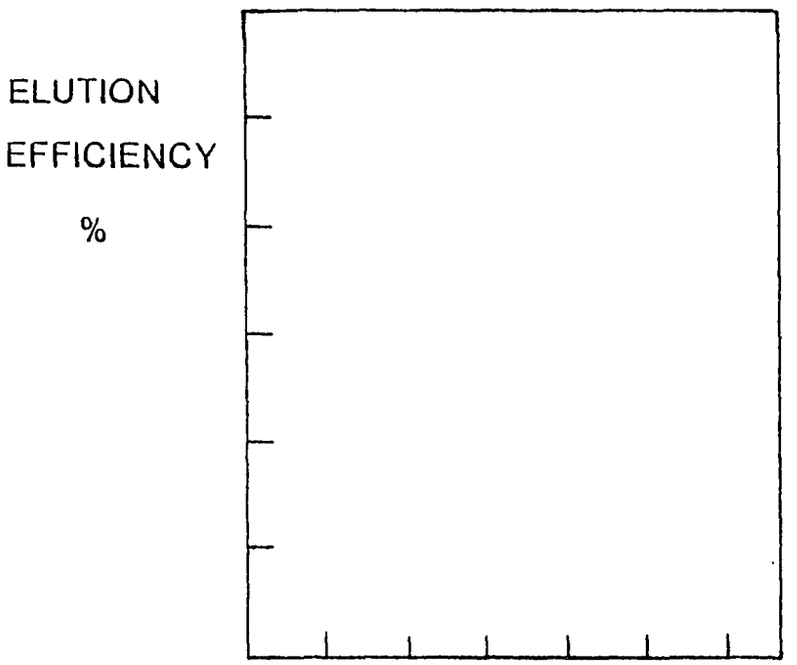
KEY TO DISPLAYING RESULTS GRAPHICALLY

Standard format for reporting experimental results

NOT AUTOCLAVED / AUTOCLAVED

DETAIL OF GENERATOR

SUBSTRATE



DETAIL OF ELUENT

COMPOSITION

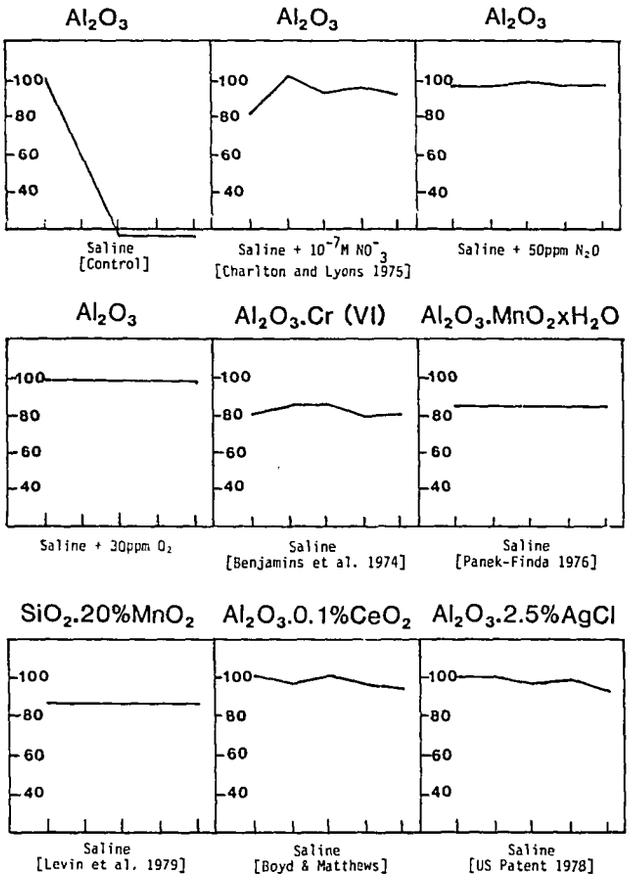


Figure A1 The effect of radiolysis on generator performance; efficiency is enhanced by the scavenging of hydrated electrons

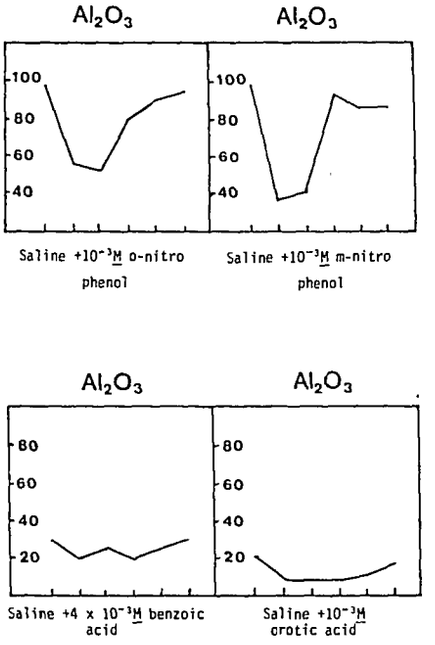


Figure A2 Sensitivity of the generator to organic materials. Not all electron scavengers enhance generator performance

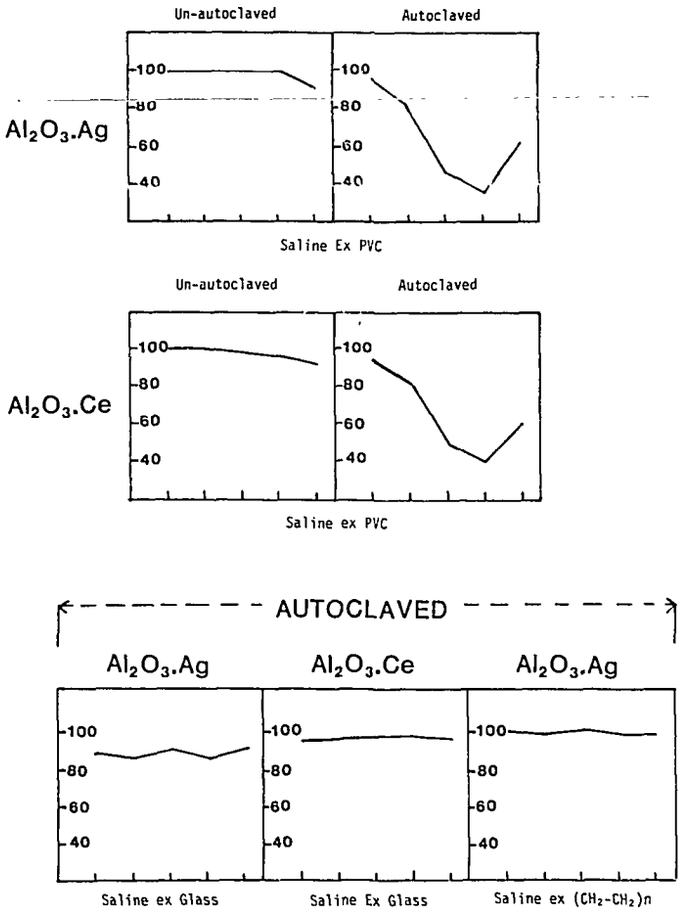


Figure A3 The effect of autoclaving the generator and the importance of saline containers

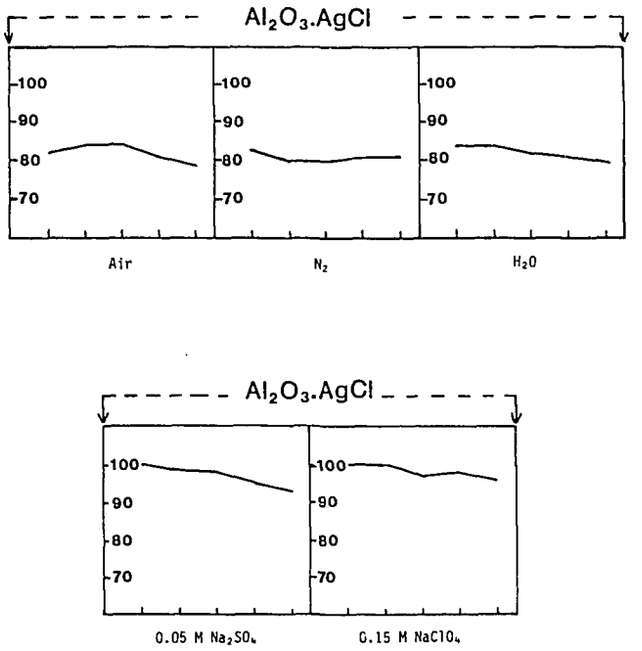


Figure A4 Identification of the deleterious effects of chloride ions

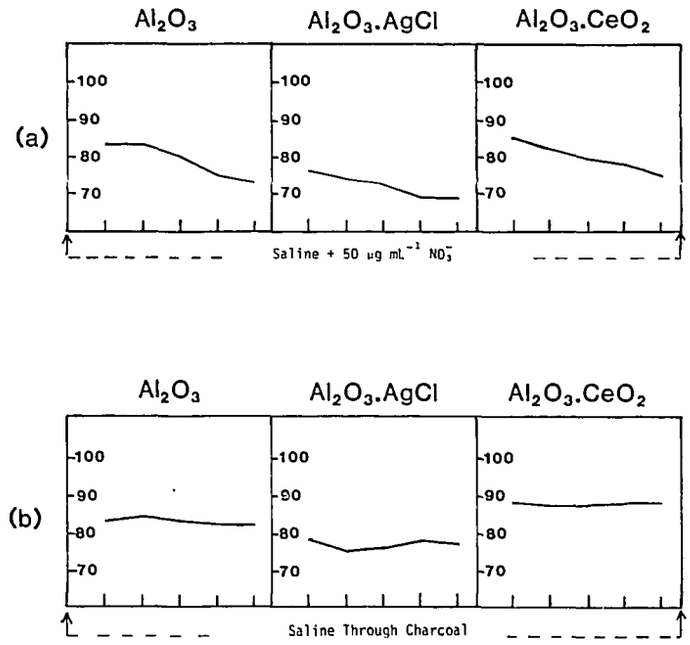


Figure A5 Neutralisation of the effects of radiolysis and organic impurities

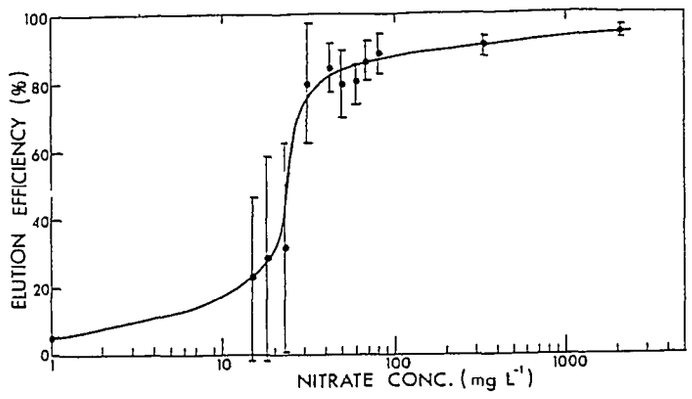


Figure A6 Effect of nitrate concentration on elution efficiency

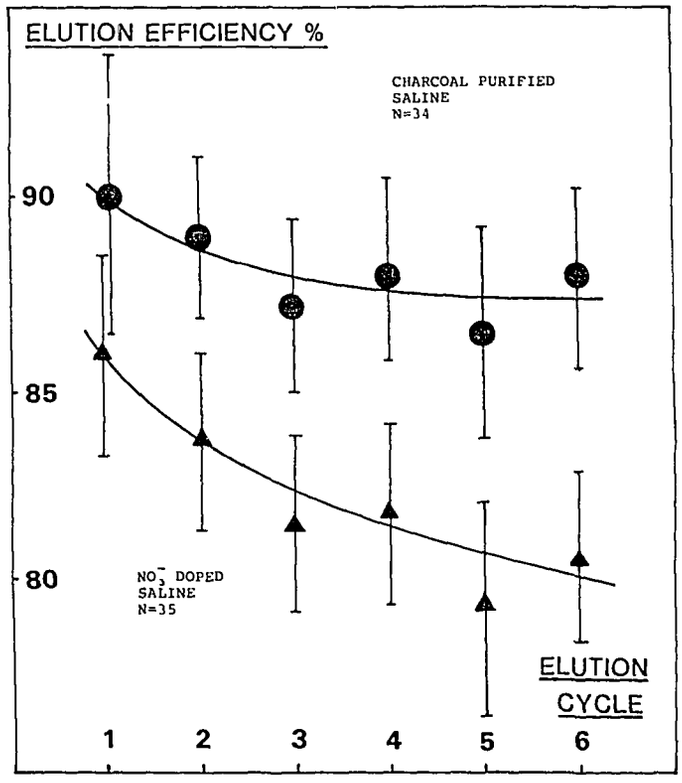


Figure A7 Comparison of nitrate-doped *versus* saline-purified generator