SIX Tc-99m LABELLED DIBASIC ACIDS: AN INVESTIGATION OF THEIR POTENTIAL USE AS SCINTISCANNING AGENTS

by

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We accept this thesis as conforming to the required standard

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September, 1975
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ABSTRACT

Six low molecular weight dibasic acids labelled with technetium -99m were investigated for their potential use as scanning agents. Preliminary radioscintiscans of rabbits showed the main areas of localization for all the acids. From this study, the most promising of the compounds, malonic acid, was chosen for further investigation.

Toxicity studies were performed on mice using a complex of stannous chloride and malonic acid given intravenously. The LD₅₀ for the complex was determined to be 800 (734-872) mg/kg.

The excretion rate of the labelled complex was examined in mice and the activity was found to be eliminated in two components. The fast component had a half-life of 46.8 minutes, and consisted of 70.3 percent of the administered dose; the slow component, accounting for the remainder of the administered dose, had a half-life of 196.4 minutes. The excreted urine was found to contain the entire injected dose.

The tissue distribution of technetium -99m - malonate in mice was analyzed with the highest concentration of activity occurring in the kidneys and blood. The liver, spleen, large and small bowels, brain, stomach, lungs, bone, and muscle were also investigated for their content of the labelled complex.

Scintiscans were done on rabbits using technetium -99m - malonate to determine the dynamic flow pattern of the complex and to evaluate the scans at various time intervals after injection.

A renogram was obtained using the Dyna Camera (R) to record
the pattern of activity through the kidneys. This curve demonstrated that a fast component of excretion with a half-life of 5.5 minutes exists in the rabbit kidney.

Absorbed dose calculations were computed for the whole body, kidney, liver, skeleton, and bladder. The bladder component was computed twice, once assuming complete urine retention and again assuming catheterization. The radiation doses were calculated as 26 mrads/10 mCi. for the whole body, 159 mrads/10 mCi. for the kidneys, 103 mrads/10 mCi. for the liver, 2064 mrads/10 mCi. for the skeleton and 11.9 rads/10 mCi. for the bladder assuming complete urinary retention. With the bladder catheterized, the doses fell to: 138 mrads/10 mCi., 43 mrads/10 mCi., 103 mrads/10 mCi., and 1.5 mrads/10 mCi. for the kidneys, liver, skeleton, and bladder respectively.
ACKNOWLEDGEMENTS

I would like to acknowledge both the direction and assistance which I have received from Dr. D.M. Lyster over the last two years. Without his help and friendship it is doubtful this study would have ever taken place.

I wish also to thank the members of my committee; Dr. F. Abbott, Dr. E. Mincey, and Dr. B.E. Riedel for their time and for their comments and suggestions.

Dr. A.R. Fritzberg is also to be thanked for his discussions with me as well as his assistance during portions of the scintiscans. The Nuclear Medicine Technicians at Vancouver General Hospital also deserve a vote of thanks and in particular Ms. J. Sanford for her instruction in radioisotope handling techniques.

To all these people I acknowledge a debt and my sincerest appreciation.

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TO

MS. SUSAN H. J. CLARK
MY BEST FRIEND AND WIFE
A. LITERATURE SURVEY
A. LITERATURE SURVEY

1. Technetium:

Technetium (Tc), element number 43 was the first isotope to be made entirely by artificial means; the name was derived from the Greek work for "artificial". Originally the element was called Masurium and its existence had been predicted from the periodic table long before its discovery. Technetium was first detected in a molybdenum target which had been bombarded by deuterons from a cyclotron. E. Segré and C. Perrier (1) are credited with the discovery of this element.

Technetium can be produced by several types of nuclear bombardment such as neutron or deuteron irradiation of molybdenum (1). It may also be isolated as a product of uranium fission (2,3,4).

The element itself is metallic with a density of 11.49 g/cm³, just slightly denser than the element lead at 11.37 (5, 6). In a chemical analysis technetium would separate out with rhenium as a sulfide, both insoluble in acid solution (5). Of practical importance is the fact that technetium may be separated from molybdenum. This can be accomplished by using 8-hydroxyquinidine to precipitate the molybdenum from an acetic acid solution, with the technetium remaining in the aqueous acid phase (5).

Other methods of effecting a separation involve either a methylethyl ketone extraction or a saline wash over an alumina column. The latter method has proven useful in the development of commercial technetium generators.

2. Chemistry of Technetium:

At present there are sixteen isotopes of technetium ranging
in atomic weight between 92 and 107 (Table 1) (7).

**TABLE 1 (7):**

<table>
<thead>
<tr>
<th>Isotope Mass Number</th>
<th>Half-Life</th>
<th>Mode of Decay</th>
<th>Decay Energy (MeV)</th>
</tr>
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<tbody>
<tr>
<td>92</td>
<td>4.4 min.</td>
<td>B+, E.C.</td>
<td>7.9</td>
</tr>
<tr>
<td>93 meta-stable</td>
<td>43 min.</td>
<td>I.T., E.C.</td>
<td>0.39, 3.6</td>
</tr>
<tr>
<td>93</td>
<td>2.7 hr.</td>
<td>B+, E.C.</td>
<td>3.19</td>
</tr>
<tr>
<td>94 meta-stable</td>
<td>53 min.</td>
<td>B+, E.C.</td>
<td>4.3</td>
</tr>
<tr>
<td>94</td>
<td>293 min.</td>
<td>B+, E.C.</td>
<td>4.26</td>
</tr>
<tr>
<td>95 meta-stable</td>
<td>61 days</td>
<td>I.T., B+, E.C.</td>
<td>0.0389, 1.7</td>
</tr>
<tr>
<td>95</td>
<td>20 hr.</td>
<td>E.C.</td>
<td>1.66</td>
</tr>
<tr>
<td>96 meta-stable</td>
<td>52 min.</td>
<td>I.T.</td>
<td>0.0344</td>
</tr>
<tr>
<td>96</td>
<td>4.3 days</td>
<td>E.C.</td>
<td>2.9</td>
</tr>
<tr>
<td>97 meta-stable</td>
<td>90 days</td>
<td>I.T.</td>
<td>0.0965</td>
</tr>
<tr>
<td>97</td>
<td>2.6x10^6 yr.</td>
<td>E.C.</td>
<td>0.3</td>
</tr>
<tr>
<td>98</td>
<td>1.5x10^6 yr.</td>
<td>B-</td>
<td>1.7</td>
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<tr>
<td>99 meta-stable</td>
<td>6.0 hr.</td>
<td>I.T.</td>
<td>0.1427</td>
</tr>
<tr>
<td>99</td>
<td>2.12x10^5 yr.</td>
<td>B-</td>
<td>0.292</td>
</tr>
<tr>
<td>100</td>
<td>17 sec.</td>
<td>B-</td>
<td>-</td>
</tr>
<tr>
<td>101</td>
<td>14 min.</td>
<td>B-</td>
<td>1.63</td>
</tr>
<tr>
<td>102</td>
<td>4.5 min.</td>
<td>B-</td>
<td>4.4</td>
</tr>
<tr>
<td>102</td>
<td>5 sec.</td>
<td>B-</td>
<td>4.4</td>
</tr>
<tr>
<td>103</td>
<td>50 sec.</td>
<td>B-</td>
<td>2.4</td>
</tr>
<tr>
<td>104</td>
<td>18 min.</td>
<td>B-</td>
<td>5.8</td>
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<tr>
<td>105</td>
<td>8 min.</td>
<td>B-</td>
<td>3.4</td>
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<tr>
<td>106</td>
<td>37 sec.</td>
<td>B-</td>
<td>-</td>
</tr>
<tr>
<td>107</td>
<td>29 sec.</td>
<td>B-</td>
<td>-</td>
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B+ position emission  
E.C. electron capture  
I.T. isomeric transition  
B- beta emission

All the isotopes of technetium are radioactive with half-lives between 5.3 seconds and 2.6 x 10^6 years. The two most common isotopes are Tc-99 and Tc-99m, with half-lives of 2.12 x 10^5 years and 6 hours respectively.
The radiations from all the isotopes of technetium are varied and include beta emissions, electron capture and isomeric transitions. The medically useful technetium 99m emits 140 KeV photons via isomeric transition.

Technetium is a group VIIb element occupying the position between rhenium and manganese in that group (8). The chemistry of technetium can be likened more to rhenium than manganese due to the lanthanide contraction of rhenium which gives rhenium an ionic radius and ionization potential similar to technetium (9).

The oxidation states for technetium are between -I and VII, with IV and VII being the preferred states (10). Technetium (like rhenium) does not form simple ions in solution so that its aqueous chemistry consists of soluble compounds and complexes. The most frequently encountered aqueous state is pertechnetate, TcO$_4^-$ (11).

The number of complexes which have been formed with technetium appears to be growing daily. These range from simple acid complexes such as Tc-Ascorbic Acid (12), Tc-D.T.P.A. (13), and Tc-Citrate (14), to macromolecules of albumin (15,16,17) and even antibodies (18). Technetium has also been used to label various cell types including blood cells (19-27).

Due to the high level of radiation given off by Tc-99m, as well as its short half-life, the study of technetium's chemistry has been done with the use of the more stable isotope, technetium 99.

Williams et al (28) evaluated the chromatographic behavior of technetium at various concentrations ranging from trace levels to 10 mg./ml. of technetium. Essentially this study showed that trace amounts of technetium behaved as did the more concentrated solutions of Tc-99. All concentrations of technetium used a small amount of Tc-99m to in-
dicate the location of the more stable isotope.

The various oxidation states which technetium may adopt when binding to chemicals of medical interest have been investigated. The most common chemical ion for technetium, pertechnetate, is capable of a loose binding to serum albumin. This binding takes place with technetium in the (VII) state (29). In this situation, technetium competes for the available binding sites with other ions which possess the following strengths of attraction: $\text{ClO}_4^{-} > \text{I}^{-} = \text{SCN}^{-} > \text{NO}_3^{-} > \text{Br}^{-} > \text{Cl}^{-}$ (29). Pertechnetate appeared to be more strongly bound to albumin than $\text{I}^{-}$ but is displaced by $\text{ClO}_4^{-}$. This type of binding is believed to be purely electrostatic and increases in strength with lowered pH (30). Decreasing the pH appears to affect the binding of pertechnetate in several ways. Initially the albumin expands due to intramolecular electrostatic repulsion, and subsequently positional changes occur with the expulsion of hydrophobic groups e.g. fatty acids (30). With the expulsion of these hydrophobic elements, additional tyrosine groups are exposed, resulting in increased electrostatic attraction, which tends to strengthen the pertechnetate-albumin bond with lowered pH (31).

In order to achieve a more stable bond of technetium to albumin, a reducing agent is necessary. The most common ones used are stannous chloride (32, 33), ferric chloride-ascorbic acid (34), and electrolytic reduction involving zirconium electrodes (35, 36). The actual oxidation state achieved in the preparation of labelled albumin is not certain. Some studies support the theory that unchanged pertechnetate adheres to albumin in a complex formation with zirconium (IV) (36), while others suggest the presence of a technetium (V) albumin complex (34,37). The best evidence appears to have been put forth in
favor of the Tc (V)-albumin complex. It has been shown (38), using microgram quantities of Tc-99, that Fe (3+)-ascorbic acid will reduce TcO$_4^-$ to the (V) state, as demonstrated by the formation of a red thio-cyanate complex. Furthermore, in this study, the Tc (V) entity was shown to be stable at low pH. At high pH, Tc (V) will disproportionate into Tc (IV) and Tc (VII); however, this is a pH reversible reaction (39-41) and the Tc (V) would also be subject to the stabilizing effects of ligands. In general, for a successful Tc label of albumin, the pH must be low, usually less than pH 2 (32,33,34,42).

Steigman et al. (42) did extensive work to prove that reduced Tc was necessary for a stable tag to albumin. Steigman showed that any oxidizing agent present such as H$_2$O$_2$, Cl$_2$, HClO during the labelling process will destroy the labelling efficiency. In his study Steigman showed what he believed to be evidence of the Tc (V) state taking place in the binding to albumin, although the basis for his conclusions were not sufficient to rule out some other reduced state of technetium.

Some of the smaller molecules such as diethylene-triamine pentaacetic acid (D.T.P.A.) (41) and citric acid (43,44) have had their technetium complexes investigated. By the use of carrier Tc-99, it has been demonstrated that technetium exists in the (IV) state with D.T.P.A.. The stannous ion in this preparation, as in the albumin preparation, is believed to reduce the technetium to the (IV) state (41). The technetium in the citric acid complex is also probably in the (IV) state. Using carrier Tc-99 and an equal amount of stannous chloride, a Tc (V) citric acid complex was formed. However, when an excess of stannous chloride was used, as would be the case if using Tc-99m, the complex became a Tc (IV) citrate (43).
A technetium-citric acid complex has also been made using a thiocyanate salt at low pH. This product is said to react via an intermediate Tc (V)-SCN complex which subsequently reacts to form Tc (V)-citrate (44). However, arguments for the (V) state are based upon inconclusive chromatography evidence, especially since it has been shown that the Tc (V)-SCN complex is easily reduced (45). Thus, it is quite possible that the complex is actually Tc (IV)-citrate.

Sulfur has also been labelled with technetium in the form of Tc-sulfur colloid, which is the only medically useful complex other than pertechnetate where technetium is in the (VII) state. The Tc-sulfur colloid has been made using Tc -99 and stoichiometrically, the complex was found to be TC₂S₇ (46). It is also known that rhenium, the closest analogue of technetium, also forms Re₂S₇ (47, 48).

In the past, it was thought necessary to add rhenium as a carrier when making the Tc-99m sulfur colloid. However, this has recently been shown not to be required (49). With or without the addition of carrier rhenium, the colloid requires the same amount of time to form. This would imply that despite the low concentration of Tc -99m, the inclusion of the technetium into the compound is not the rate limiting step.

There are many other technetium labelled complexes which have been used in the field of nuclear medicine, but for only a few has the chemistry of technetium been seriously studied.

3. Dicarboxylic Acids:

The compounds used in this study were the low molecular weight dicarboxylic acids, oxalic acid to adipic acid plus maleic acid. The structures of these acids are depicted in Figure 1, and their dissociation constants are listed in Table 2.
FIGURE 1:

Six Dicarboxylic Acids

Oxalic Acid
HOOC - COOH

Malonic Acid
HOOC - CH₂ - COOH

Succinic Acid
HOOC - CH₂ - CH₂ - COOH

Glutaric Acid
HOOC - CH₂ - CH₂ - CH₂ - COOH

Adipic Acid
HOOC - CH₂ - CH₂ - CH₂ - CH₂ - COOH

Maleic Acid
HOOC - CH = CH - COOH

TABLE 2 (50):

Dissociation Constants for Oxalic, Malonic, Succinic, Glutaric, Adipic and Maleic Acids

<table>
<thead>
<tr>
<th>Acid</th>
<th>Dissociation Constants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K1</td>
</tr>
<tr>
<td>Oxalic Acid</td>
<td>$5.4 \times 10^{-2}$</td>
</tr>
<tr>
<td>Malonic Acid</td>
<td>$1.4 \times 10^{-3}$</td>
</tr>
<tr>
<td>Succinic Acid</td>
<td>$6.4 \times 10^{-5}$</td>
</tr>
<tr>
<td>Glutaric Acid</td>
<td>$4.5 \times 10^{-5}$</td>
</tr>
<tr>
<td>Adipic Acid</td>
<td>$3.7 \times 10^{-5}$</td>
</tr>
<tr>
<td>Maleic Acid</td>
<td>$1.0 \times 10^{-2}$</td>
</tr>
</tbody>
</table>

Although the acids are members of an homologous series, they have a wide range of toxicities (Table 3).
**TABLE 3:**

**Toxicity of Oxalic, Malonic, Succinic, Glutaric, Adipic and Maleic Acids**

<table>
<thead>
<tr>
<th>ACID</th>
<th>TOXICITY</th>
<th>ANIMAL</th>
<th>ROUTE OF ADMINISTRATION</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxalic Acid</td>
<td>MLD 100mg/kg</td>
<td>Rabbit</td>
<td>Subcutaneous</td>
<td>(52)</td>
</tr>
<tr>
<td>Malonic Acid</td>
<td>LD$_{50}$ 1.54g/kg</td>
<td>Rat</td>
<td>Intraperitoneal</td>
<td>(51)</td>
</tr>
<tr>
<td>Succinic Acid</td>
<td>MLD 2.0g/kg</td>
<td>Frog</td>
<td>Subcutaneous</td>
<td>(53)</td>
</tr>
<tr>
<td>Glutaric Acid</td>
<td>Low order of toxicity</td>
<td>---</td>
<td>---</td>
<td>(54)</td>
</tr>
<tr>
<td>Adipic Acid</td>
<td>LD$_{50}$ 680mg/kg</td>
<td>Mouse</td>
<td>Intravenous</td>
<td>(55)</td>
</tr>
<tr>
<td>Maleic Acid</td>
<td>LD$_{50}$ 708mg/kg</td>
<td>Rat</td>
<td>Oral</td>
<td>(56)</td>
</tr>
</tbody>
</table>

Oxalic acid, the most toxic member of the group, is widely distributed in nature and can be isolated from mammalian urine. The toxicity of oxalic acid is due to its physically corrosive action and its ability to form poorly soluble calcium salts.

35-44% of endogenous oxalic acid is a metabolic product of dietary ascorbic acid (57, 58) and 40% is formed from glycine (59). The remaining 20% come from glycollate and other dietary sources (60). The proportions are variable, generally depending upon the amount of calcium within the diet (61). Normal excretion of oxalic acid ranges from 5 to 38 mg. per 24 hours. The amount of acid excreted at any one time varies during the day, with the highest output occurring after a meal (62).

Oxalic acid is excreted by glomerular filtration and proximal tubular secretion, but approximately 96% of the cleared oxalate is reabsorbed by passive back diffusion in the tubules (61, 63). The majority of endogenous oxalic acid is cleared via the kidneys, 88-89%, with the remaining 11 to 12% excreted via the feces (64).

The fate of exogenous oxalic acid varies with the amount taken into the body. In a study by Just (65), most of a large dose of
oxalic acid (60 mg/kg i.v.) was found to be retained in the kidneys, while for a smaller dose (2.5 mg/kg i.v.) a larger percentage was excreted. Large amounts of retained oxalic acid in the kidney may lead to the formation of oxalate calculi (66).

Malonic acid was first synthesized in 1858 by the oxidation of malic acid with potassium chromate (67). Like oxalic acid, malonic acid is frequently found as an end product of oxidative degradation. Malonic acid is less toxic than oxalic acid and in the form of derivatives such as malonyl CoA, it is endogenous to the body. The kidney, liver and diaphragm are capable of rapid metabolism of malonic acid, while in the spleen, brain, testes and lung metabolism is slow (68). The major metabolizing organs are able to convert the malonate to acetoacetate by decarboxylation and recombination, followed by a second decarboxylation (68). The conversion of malonate to acetyl CoA in the human placenta has also been demonstrated (69). Malonic acid therefore plays an important part in the Kreb's cycle as a precursor to acetyl CoA and as a potential inhibitor of the oxidation of succinic acid to fumaric acid (70).

 Succinic acid, described in 1595 as "Flos Succini", has been demonstrated to be a constituent of the Kreb's cycle (72,73). Synthetically the chemical is an important precursor to the formation of succinyl chloride, pyrrole, and pyrrollidine (71). The mammalian body is capable of synthesizing succinic acid from a ketoglutaric acid via succinyl CoA with the succinic acid being used to synthesize porphyrins. Alternatively, the succinic acid can be metabolized to fumaric, malic and/or oxaloacetic acid and pyruvic acid (74).

 Glutaric acid is of little commercial value but it can be
chemically synthesized in a variety of ways (53). In the body, glutarate is converted to acetate by way of glutaconate, beta-hydroxyglutarate and acetonedicarboxylate (75). Large amounts of glutaric acid have been fed to humans with no apparent ill effects; it is the largest chain dicarboxylic acid which can be utilized by the body (76).

Adipic acid is readily formed from the oxidation products of fatty acids and is used mainly as an intermediate in the synthesis of nylon, polyurethans and polyester resins (77). Labelled adipic acid has been fed to rats, where it was shown that the majority of the acid is excreted unchanged (78). Using C-14 labelled adipic acid, 70% of the activity was shown to be eliminated via the lungs, the activity still in the body being located in the kidneys and liver. The oxidation of succinic acid appears to be via decarboxylation (55).

Maleic acid, the only unsaturated acid in the group, is useful as an antioxidant in the protection of the fats and oils from rancidity (79). Maleic acid exerts its toxic effect upon the kidney (80). The effect upon the kidney includes a sharp increase in urine pH which lasts up to 4 hours. Creatinine clearance and para-aminohippuric acid secretion is reduced but the effect is temporary (80).

Bergeron (81) likens maleic acid toxicity to the Fanconi syndrome, including aminoaciduria, glycosuria, and phosphaturia. Maleic acid blocks the energy utilization of the renal cells thus increasing the efflux of the above mentioned species.

4. Chemistry of the Dicarboxylic Acids:

Chemically the dicarboxylic acids should behave in a similar manner, modified only by the increasing carbon chain length. All the acids may act as ligands, either mono-or-bidentate, and each acid has a
number of transition metal complexes. Investigation by Li et al (82) showed that the stability constants of the manganese complexes decreases with the chain length of the dicarboxylic acids for one-to-one complexes with Mn IV (Table 4).

**TABLE 4 (82):**

<table>
<thead>
<tr>
<th>Acid</th>
<th>Log Kf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxalic Acid</td>
<td>2.93</td>
</tr>
<tr>
<td>Malonic Acid</td>
<td>2.30</td>
</tr>
<tr>
<td>Succinic Acid</td>
<td>1.26</td>
</tr>
<tr>
<td>Glutaric Acid</td>
<td>1.13</td>
</tr>
<tr>
<td>Adipic Acid</td>
<td>1.13 - 108&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Maleic Acid</td>
<td>1.68</td>
</tr>
</tbody>
</table>

<sup>a</sup> Adipic acid constant estimated from data (82).

Li (82) points out that a chelate ring size of 5 atoms (e.g.: oxalic acid) is the configuration for maximal stability. The longer chain dicarboxylic acids tend to behave like two mono-carboxylic acids because each acidic group acts like independent ligand. The log Kf of a long chain dicarboxylic acid becomes approximately twice that for the equivalent single acid groups.

The most frequently occurring complex between oxalic acid and manganese (II) or (IV) is a 3 to 1 complex as depicted: (83-85)

\[ \text{Mn (C}_2\text{O}_4\text{)}_3^{3-} \]

Similarly, the formula for the complex between malonic acid and manganese is given as an equilibrium (83 - 85):

\[ \text{Mn (C}_3\text{H}_2\text{O}_4\text{)}_3^{3-} = \text{Mn (C}_3\text{H}_2\text{O}_4\text{)}_2^{2-} \]

The oxalate complex and the malonate complex depicted on the left of the equilibrium suggest an octahedral arrangement similar to Figure 2.
Rhenium IV shows a different type of complex with oxalic acid: (86)

\[
\{\text{OH}_3 \ \text{Re} \ (C_2\text{O}_4) - \text{H}_2\text{O}\} = \{\text{OH}_4 \ \text{Re} \ (C_2\text{O}_4)\}^{2-}
\]

This is a 6 coordinate complex, but instead of oxalate making up all the ligands, hydroxyl groups maintain the majority of the coordinate positions. With a larger amount of oxalic acid present, it is possible that some of the hydroxyl ligands might be replaced by additional oxalate ligands.

Manganese (VII) is a strong oxidizing agent, unlike Tc (VII) and Re (VII). It can be reduced in the presence of either oxalic or malonic acid to give Mn III (87). Although these acids are not able to reduce Tc and Re, they may be able to stabilize these ions once they have attained the (III) oxidation state.

Succinic and maleic acid have been shown to form the following compounds with Mn II: (88, 89)

\[
\text{Mn} \ (C_2\text{H}_2\text{O}_2)_2 \quad \text{Succinate} \\
\text{Mn} \ (C_2\text{H}_2\text{O}_2) \cdot \text{H}_2\text{O} = \text{Mn} \ (C_2\text{O}_4\text{H}_3)_2 \quad \text{Maleate}
\]

Maleic acid, which on the basis of the ligand ring size formed, (82) should make stable bi-dentate ligands, has a stronger tendency to form
mono-dentate ligands. This is believed due to the tendency for maleic acid to form intramolecular hydrogen bands (89).

Both oxalic and malonic acids are capable of forming complexes with the stannous ion: (90)

\[
\begin{align*}
\{\text{Sn } (\text{C}_2\text{O}_4)_2 \text{CH}_2\}^{2-} & \quad \text{Malonate} \\
\{\text{Sn } (\text{C}_2\text{O}_4)_3\}^{2-} & \quad \text{Oxalate}
\end{align*}
\]

The presence of these complexes may explain the long-term stability of solutions of malonic acid with stannous chloride.

Of the six acids only oxalic is acutely toxic. Oxalic acid causes the physical accumulation of insoluble calcium salts (66). Maleic acid is also nephrotoxic by exerting an inhibiting effect upon energy production within the renal cortex (80, 81).

Malonic, succinic and glutaric acids are important constituents of normal mammalian cells, so that the body has adequate means for handling the presence of these acids. Adipic acid, although not normally present in the body, can be handled by the normal cellular enzymes, which transforms the adipate ion to succinate and acetate derivatives (55).

5. Radiolabelled Compounds for Renal Studies:

These agents are useful in determining both renal morphology and renal perfusion. Generally, these compounds tend to localize in the kidney as compared to surrounding tissues. The ideal agent would be one which localized in all parts of the kidney including the medulla, but most agents reported to date localize in the cortex only. The information these agents give is useful in the surgical intervention of some pathological conditions. The transplanted kidney has been studied with radiolabelled drugs which enable identification of a rejected
kidney as opposed to a kidney with acute tubular necrosis (91). Renal tumors, cysts, and hydronephrosis are all conditions which may be diagnosed by radionuclide study (92).

The ideal radionuclide for renal scanning agents is generally technetium -99m, although indium -113m and iodine -123 are also useful. Technetium -99m possesses the advantages of a short half-life, good energy for accurate collimation, and a ready availability. The only drawback to technetium is that it often will not label some compounds as well as iodine.

a) Labelled Mercurial Diuretics:

\[
\begin{align*}
\text{Cl} & \quad \text{Hg} \quad \text{CH}_2 \quad \text{CH} \quad \text{CH}_2 \quad \text{N} \quad \text{C} \quad \text{NH}_2 \\
\text{O-CH}_3 & \quad \text{H} \quad \text{O}
\end{align*}
\]

Chlormerodrin

Mercury -203 chlormerodrin was first used in 1960 for the detection of space-occupying lesions in the kidney (93). The agent was found to be tightly bound to the serum proteins and was excreted via the proximal tubules in the renal cortex (94). In the normal individual, 86% of the injected dose is eliminated via the kidneys, while the remainder is excreted via the feces (95).

Although this compound shows good accumulation in the kidneys, approximately 45% within 2 hours (95), it has the disadvantage of delivering a high radiation dose. This is caused partly by the long biological half-life of the chlormerodrin (28 days) (96) and partly by the long radioactive half-life of the mercury -203 (46.9 days) (Table 5).


### TABLE 5:

**Radiation Doses from Selected Radiopharmaceuticals**

<table>
<thead>
<tr>
<th>Radiopharmaceutical</th>
<th>Radiation Dose</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Kidney</strong></td>
<td><strong>Whole Body</strong></td>
<td></td>
</tr>
<tr>
<td>Hg-197 Chlormerodrin</td>
<td>500 mrad/mCi.</td>
<td>97</td>
</tr>
<tr>
<td>Hg-197 Chlormerodrin</td>
<td>5000 mrad/mCi.</td>
<td>98</td>
</tr>
<tr>
<td>Hg-203 Chlormerodrin</td>
<td>51-153 rad/mCi.</td>
<td>98</td>
</tr>
<tr>
<td>Tc-99m (fe) Ascorbate</td>
<td>270 mrad/mCi.</td>
<td>99</td>
</tr>
<tr>
<td>Tc-99m Gluconate</td>
<td>200 mrem/mCi.</td>
<td>100</td>
</tr>
<tr>
<td>Tc-99m D.M.S.A.</td>
<td>1400 mrad/mCi.</td>
<td>101</td>
</tr>
<tr>
<td>Tc-99m D.T.P.A.</td>
<td>---</td>
<td>92</td>
</tr>
<tr>
<td>Tc-99m D.T.P.A.</td>
<td>420 mrad/mCi.</td>
<td>102</td>
</tr>
<tr>
<td>Tc-99m D.T.P.A.</td>
<td>42 mrad/mCi.</td>
<td>99</td>
</tr>
<tr>
<td>Tc-99m H.E.D.P.</td>
<td>11 mrad/mCi.</td>
<td>103</td>
</tr>
<tr>
<td>Tl Citrate or Chloride</td>
<td>40 rad/uCi.(medulla)</td>
<td>---</td>
</tr>
<tr>
<td>I-131 Hippuran</td>
<td>---</td>
<td>70 mrad/mCi.</td>
</tr>
<tr>
<td>In-113m D.T.P.A.</td>
<td>---</td>
<td>9 mrad/mCi.</td>
</tr>
<tr>
<td>In-113m D.T.P.A.</td>
<td>72 mrad/mCi.</td>
<td>---</td>
</tr>
<tr>
<td>In-111 D.T.P.A.</td>
<td>---</td>
<td>20 mrad/mCi.</td>
</tr>
<tr>
<td>Cr-51 D.T.P.A.</td>
<td>---</td>
<td>1 mrad/mCi.</td>
</tr>
<tr>
<td>Cr-51 D.T.P.A.</td>
<td>1.7 mrad/mCi.</td>
<td>---</td>
</tr>
<tr>
<td>Yb-167 D.T.P.A.</td>
<td>---</td>
<td>20 mrad/mCi.</td>
</tr>
<tr>
<td>Yb-169 D.T.P.A.</td>
<td>22 mrad/mCi.</td>
<td>---</td>
</tr>
<tr>
<td>Yb-169 D.T.P.A.</td>
<td>10 mrad/mCi.</td>
<td>---</td>
</tr>
<tr>
<td>Ga-68 D.T.P.A.</td>
<td>55.7 mrad/mCi.</td>
<td>---</td>
</tr>
<tr>
<td>Hg-197 E.D.T.A.</td>
<td>63.8 mrad/mCi.(cortex)</td>
<td>---</td>
</tr>
<tr>
<td>Cr-51 Inulin</td>
<td>1.8 mrad/mCi.</td>
<td>---</td>
</tr>
<tr>
<td>Co-57 Vit. B&lt;sub&gt;12&lt;/sub&gt;</td>
<td>4.0 mrad/mCi.</td>
<td>---</td>
</tr>
</tbody>
</table>

Mercury -197 has been used in chlormerodrin because it has a shorter half-life of 65 hours. The radiation dose of Hg -197 chlormerodrin is approximately one-fifth of the whole body dose for Hg -203.
chlormerodrin (Table 5). Mercury -197 chlormerodrin is less than ideal as the lower energy of Hg -197 (67-80 KeV) causes limited resolution due to tissue attenuation (108).

Mersalyl, a similar compound, has been used by Winkel et al (109).

\[
\begin{align*}
&\text{H} - \text{O} - \text{Hg} - \text{CH}_2 - \text{CH} - \text{N} - \text{C} - \text{N} \\
&\text{NaO} - \text{C} - \text{CH}_2 - 0'
\end{align*}
\]

Mersalyl

This compound was found to be cleared more quickly from the kidneys, thus reducing the radiation dose. However, mersalyl has a higher percentage elimination via the gut, which tends to obscure the renal images. As a result, it is not used to any extent for renal scanning.

b) Iodine -131, 1-4-Iodophenyl)-3-(3-chlormercu)-2-methoxypropyl urea (I.P.C.M.)

\[
\begin{align*}
&\text{Cl} - \text{Hg} - \text{CH}_2 - \text{CH} - \text{CH}_2 - \text{N} - \text{C} - \text{N} - \text{I}
\end{align*}
\]

I.P.C.M.

In attempts to use the chlormerodrin structure and specificity for the kidney, I.P.C.M. was synthesized using iodine -131 as the radio-nuclide (110). Tanaka et al (110) felt that the Cl - Hg - R structure would give the compound good specificity for the renal cortex and, that like chlormerodrin, cleavage would occur at the Hg - R bond in vivo. If this were the case, the high radiation dose associated with Hg tagged chlormerodrin would be avoided, as the I-131 in the cleared R - group
would be rapidly eliminated from the kidney. However, experiments showed that the expected cleavage did not take place, as evidenced by the large amount of I.P.C.M. recovered in the urine. The compound was also found to have a large uptake by the liver. The distribution patterns showed that the kidney/liver ratio increased with time but not sufficiently to give good kidney scans (110).

Tanaka et al (110) attributed the liver activity to the lipid character of the iodophenyl groups, a feature which chlormerodrin does not possess. However, it is possible that the liver uptake may have been due to the solvent used for injection, namely 50% dimethyl sulfoxide (D.M.S.O.). This excellent solvent may have been chosen because of the limited water solubility of I.P.C.M.. Upon dilution of the D.M.S.O. in the blood stream, the I.P.C.M. may have precipitated out in the form of a colloid. Colloids have been shown to localize in the liver, spleen, and bone marrow (111).

The agent I.P.C.M. is an interesting approach to a problem but like some derivatives it fails to behave like the parent compound. The other disadvantage to I.P.C.M. is the nuclide I-131, which in itself can give a high radiation dose to surrounding tissues, particularly as 10% of radioactivity is still within the body up to 6 days post-injection (110).

c) Technetium - 99m - (Fe) - Ascorbic Acid

This complex was first developed in 1966 by Harper et al (112) who demonstrated that Tc-Fe-ascorbic acid behaved differently from either pertechnetate or ferric ions. By using dual labels (Tc-99m and Fe-59) the authors showed that the technetium and the iron either remain associated together or behave in an identical manner
when complexed with ascorbic acid (112). The complex was shown to maintain a fixed concentration in the kidney over a 24 hour period (113) and to leave the body in a biphasic excretion curve (114).

More recently, Winston et al (115) did extensive work on this complex with human subjects. His experiments showed adequate renal scans for those patients with a creatinine level of less than 3mg%. Good scans could be obtained in normal patients after a one hour delay, while patients with varying degrees of renal impairment might require delays of up to 3 hours for scans of the same quality. Some liver image was noted in all patients but was more pronounced in patients with diminished renal function. The clearance ratio was determined at 36% of inulin due to 56% of the complex being associated with plasma proteins (115).

The complex broke down in the body, as free pertechnetate and was detected in the urine (115). Stern et al (116) believed the breakdown was due to competition between sulfhydryl groups in the kidney and the ascorbate anions for the technetium. Autoradiograms indicate that the activity is located within the cortex but whether this is due to the intact complex or just to the pertechnetate ion was not decided (115).

This agent is not an ideal scanning agent because of image deterioration when the patient has impaired renal function. It is also not satisfactory as a measure of glomerular filtration rate as the complex is not stable in the kidney and the possibility of tubular reabsorption has not been ruled out (117). The dosimetry is given in Table 5.

The Tc-99m-ascorbate complex has been observed to be unstable in vitro as well as in vivo (115). It was found that the addition of di-
ethylene triamine pentaacetic acid (D.T.P.A.) improved the shelf life of the complex (118). This complex, commercially available as Renotec (R) clears via glomerular filtration, although some tubular reabsorption does occur (117). Its whole-body clearance is similar to the Tc-Fe-ascorbate complex (114). The radiation doses are also similar (Table 5), as are the quality of the scans.

d) Tc-99m - Penicillamine - Acetazolamide (T.P.A.C.)

This complex was investigated by Holpern et al (119) as a renal scanning agent useful in patients suffering from renal impairment. It was thought that an agent which was excreted by tubular processes would show an acceptable kidney scan in individuals with moderate to severe renal impairment. Unlike the majority of radiopharmaceuticals which are labelled with technetium, this complex uses an organic reducing agent penicillamine.

T.P.A.C. is strongly plasma-protein-bound with some breakdown taking place \textit{in vivo} as shown by chromatography of the urine, although little technetium appears in the \textit{(VII) state}. Autoradiograms showed that the activity in the kidney was localized within the cortex.

Compared to inulin, T.P.A.C. has a much slower clearance; probably due to 97% being bound to plasma proteins. The radioactivity which did localize in the kidneys was retained rather than being immediately excreted, producing good kidney-to-background ratios (119). The authors (119) claimed better results for the impaired kidney with this agent than with I-131 hippuran, Hg-197 chlormerodrin or Tc-99m D.T.P.A., and equal results with the normal kidney.

e) Tc-99m Tetracyclines

Four tetracyclines have been labelled with technetium-99m
by Dewanjee et al (120) and the distribution studies suggest that they might be useful as renal scanning agents. The tetracyclines used were: tetracycline, oxytetracycline, chlortetacycline and dimethylchlortetacycline. Tetracycline showed the highest renal concentration with 30% of the injected activity being located in the kidneys. The other three agents showed a 15% accumulation within the kidneys. The liver activity for the agents increased with the highest being from chlortetacycline and dimethylchlortetacycline at 7% and 10% respectively.

Within the kidney, the complexes all showed a high cortex to medulla ratio (10.8:1 for tetracycline) and a high cortex-to-blood ratio (13.4:1 for tetracycline).

Double isotope labelling of tetracycline with Sn-119m and Tc-99m showed that the different complexes localized in different organs. Tc-99m - tetracycline showed a high specificity for the kidney while the Sn-119m - tetracycline showed a higher activity level in the liver with only one-quarter of the kidney activity of the Tc-99m complex. The Sn-119m labelled tetracycline also showed additional activity in the skeleton not present with the Tc-99m tetracycline.

Although human trials were not done by the authors (120) there are some problems which might prevent these agents from being used as renal scanning agents. There is a considerable amount of intestinal activity with all the complexes: tetracycline 13.8%, oxytetracycline 16.1%, chlortetacycline 24.7%, and dimethylchlortetacycline 21.8%. The best agent, Tc-99m tetracycline showed an optimum kidney-to-intestine ratio of only 2.0 to 1.0, which is excessively high background as evidenced by the scans. The complexes were also shown to be unstable, with the amount of free pertechnetate in-
creasing with time if the preparation is left at room temperature. Some improvement in stability is observed by storing the complex in a refrigerator out of contact with oxygen and light.

f) Tc -99m - Caseidin

Caseidin is a small polypeptide with a molecular weight of about 2500, produced by controlled hydrolysis of casein. The labelled complex largely locates in the renal cortex, with peak activity in the kidney occurring 15 minutes after injection: 32% of the injected dose is located in the kidney at this time (121). One third of the administered activity is found in the kidney 24 hours post-injection. The optimum kidney-to-liver ratio of 8.3:1 was reached at 2 hours although by this time the total percentage activity in the kidney had dropped from 32% to 25.8% (121). The compound's clearance curve was found to be triphasic with half-lives of 3 minutes, 10 minutes, and 3.5 hours. The time for 50% of the activity to disappear from the blood was found to be 14 minutes (122).

Clinically, the agent appeared to localize well within 8 minutes and scans were of good quality after more than 2 hours delay, thus making the time interval between injection and scanning less critical than with some agents (122). Tc -99m - caseidin was found to localize in patients with mild to moderate renal impairment, although in cases of serious impairment no visualization was possible (122).

g) Tc -99m - Dimercaptosuccinate

This agent was developed specifically by Medi-Physics (R) as a technetium-labelled replacement for the mercury-labelled diuretics e.g.: mellaride (109) and chlormerodrin (93-96, 108). Tc -99m dimercaptosuccinate is a relatively nontoxic agent showing high spec-
ificity for the renal cortex and with considerable retention in this area allowing successful images to be obtained after a 24 hour delay (101). It has been demonstrated that the half-life for the plasma clearance is 45 minutes (123), yet no appreciable excretion from the kidney occurs over the first 2 hour period (101).

Studies in rats demonstrated that 54.2% of the injected dose was located in the kidneys 1 hour post-injection, while the liver and spleen contained only 5.3% of the dose at the same time interval (123).

In a clinical review of dimercaptosuccinate, it was felt that the agent showed good renal images with little renal excretion (101). In the normal patient, a 2 hour delay affords excellent scans. Kidneys of patients suffering from severe renal impairment could be visualized, but only after delays of up to 24 hours. In the clinical study of 65 patients only one patient's kidneys could not be imaged, and this patient had a serum creatinine of 9 mg.% and a B.U.N. of 210 mg.%.

Because there is little excretion of Tc-99m-dimercaptosuccinate even in the normal patient, the radiation doses listed in Table 5 would be expected to remain similar for a patient with renal impairment.

h) Tc-99m Sulfur Colloid

Although this compound is normally used as a liver-spleen scanning agent, there have been two recent reports of Tc-99m sulfur colloid accumulating in the kidneys (124, 125). These reports, concerned with 3 patients, could not be attributed to the preparation as the sulfur colloid was used on other patients the same day with apparently normal results. One of the authors (124) used indium-113m colloid on his patient and obtained renal uptake identical to that obtained with
sulfur colloid.

In animal studies it has been demonstrated that less than 2% of the injected Tc-sulfur colloid accumulates in the kidneys (111). No known migration of the reticulo-endothelial cells from the liver to the kidney has been reported, as it has from the liver to the lungs (126), so it is difficult to imagine sulfur colloid kidney images resulting from the same cause as the lung images.

The patients in both reports did have in common congestive heart failure, but it remains to be shown how this disease could cause the accumulation of Tc-99m-sulfur colloid in the kidneys.

i) Thallium chloride and Citrate (Mixture of $^{198}\text{TI}$, $^{199}\text{TI}$, $^{200}\text{TI}$, $^{201}\text{TI}$, $^{202}\text{TI}$).

These salts of thallium are possibly the only agents which have been proposed as specific renal medulla scanning agents (104). Unlike most other heavy metal ions, thallium does not localize in the cortex but rather in the medulla by mechanisms yet unknown. At approximately 2 hours post-injection a medulla to cortex ratio of 40-to-1 is obtained with 7.5% of the total injected activity present in the kidney (104). The agent is not ideal as there is intestinal uptake and fecal excretion, which diminishes the quality of the scans (104).

Scans done on human patients were judged acceptable although not of the quality obtained with Hg-197 (or 203) chlormerodrin. The radiation dose is listed on Table 5.

j) Tc-99m-Gluconate

Tc-99m-gluconate was initially prepared by Charamza et al (127) in 1969 and was reported to be a stable complex suitable for renal scintigraphy. An electrolytic method of preparation for the complex
using ferrous ion as the reducing agent was later reported (128). The main work on Tc-99m gluconate was done by Boyd et al in an extensive animal and clinical study (129).

Boyd (129) felt that this agent could provide information regarding renal function, patency of excretory pathways and renal morphology with a single study. His work involved the Tc-99m - gluconate complex made using either stannous ion as a reducing agent or the ferrous electrolytic reduction method. He concluded that the complex was identical when prepared by either method. Both methods yield a complex which contains less than 5% free pertechnetate and which is stable for up to 24 hours. Lyophilized kits could be made and stored indefinitely prior to reconstitution with pertechnetate (129).

The animal distribution studies showed good localization in the kidneys with an optimum uptake of 18.1% at 2 hours post-injection. Autoradiography demonstrated that the majority of the activity was located in the renal cortex. It was postulated (129) that upon injection this agent spreads rapidly into the extracellular fluid pool and then is slowly released back into the circulation to maintain equilibrium between vascular and extracellular spaces (129).

In the human trials (129), 17% of the complex was located in the kidneys at 10 minutes; this amount slowly declined over the next 24 hours. Excretion of the agent was shown to be dependent upon renal function. In the normal patient the scans are good within 60 minutes while in the patient with impaired function, the scans are poor and must be delayed up to six hours for optimum definition.

The disadvantage to this agent is the slight liver image usually present, which can be more pronounced in some patients despite
renal function. Occasionally, good gallbladder scans have been obtained (129). Dosimetry is given in Table 5. Commercially this agent is available as Tc-99m glucoheptonate (130).

6. Radioisotopic Renograms:

The renogram was first developed in 1956 by Taplin et al (131) using I-131 labelled idopyracet (Diodrast(R)). The author demonstrates the classic triphasic or "renogram curve" which results from monitoring the activity over a patient's kidneys. A number of patients were done using the renogram technique and results were tabulated for various types of pathology including hydronephrosis, ureteral obstruction and absent kidneys (131).

Since the first published work there have been disputes and changes in the test, such as choice of agent, narrow-angle collimation vs wide angle collimation (132), third probe placement (133), and patient hydration (134). A current interpretation of the test may be found in the work of Formelant et al (135) as it applies to the use of I-131 hippuran. The authors (135) split the curve into two portions, the rapidly rising initial curve showing the accumulation of activity within the view of the collimator, and the falling curve showing the drop off in activity as the nuclide flows down the ureter and out of the field of view. Other comprehensive reviews are available (136-138) on the renogram. The test as done at present enables the detection of pelvic obstruction, low-urine flow, renal artery stenosis and renal tubular disorders (135).

7. Radioisotopic Compounds used for the Renogram Test:

To be useful for renogram studies, a radiopharmaceutical must be cleared rapidly by glomerular filtration or tubular secretion.
Lower body background will be the result if a tubular agent is used. Any material which is not cleared must be considered when interpreting the results. Approximately 20% of the cardiac blood supply enters the kidney and of this, approximately 20%, or 5% of the total available activity, will enter the glomerular filtrate. An agent which is cleared by tubular secretion shows an 18% decrease in total activity during the same time period, thus yielding a lower body background.

a) I-131 Hippuran

\[
\begin{align*}
\text{Hippuran} & \quad \text{Sodium o-iodohippurate} \\
\text{Chemically, I-131 ortho-iodohippurate behaves much like para-amino hippurate (P.A.H.), the agent which has been used classically for the estimation of renal plasma flow. In a single passage through the kidney, approximately 90% of the P.A.H. present was eliminated into the urine (142). O.I.H. clearance was found to be very close to that of }
\end{align*}
\]
P.A.H. with the ratio of O.I.H. to P.A.H. clearance measured at 0.85 (143, 144). The presence of free I-131 in the O.I.H. preparation was postulated as the reason for the lower clearance value (142). Free I-131 can be formed from O.I.H. by light, temperature, and high specific activity (142). The difference in clearance values could also be due to greater plasma protein affinity of O.I.H..

The radiation doses are listed in Table 5 with a normal dose of 10-40 uCi. depending upon the patient's weight and kidney function (145).

b) I-131 Iodopyracet

\[
\text{Iodopyracet}
\]

This was the first agent to be used as a radiopharmaceutical for performing the renogram test (131). As a tracer dose it was found that the clearance was 20-25% of P.A.H., while if non-radioactive carrier iodopyracet was injected first, the clearance ratio of iodopyracet to P.A.H. became close to unity (146).

Because of the liver accumulation, interpretation of the right kidney was difficult (131) and the agent is seldom used anymore.

c) I-131 Sodium Diatrizoate and I-131 Sodium Diprotizzoate

\[
\text{Sodium Diatrizoate} \quad \text{Sodium Diprotizzoate}
\]
These contrast media were labelled with I-131 and studied by Winter et al (147). Both agents are secreted mainly by tubular processes but are not as rapidly cleared as is iodopyracet. Unlike iodopyracet, the presence of carrier compound has no effect upon the measured renal clearance, and both agents show less liver uptake than does iodopyracet. The authors (147) concluded that sodium diatrizoate and sodium diprotrizoate would be useful renogram agents, but that the renogram curves would take longer owing to the slower clearance.

d) Cr -51 Ethylenediamine Tetraacetic Acid (Cr -51 E.D.T.A.)

This was probably the first agent to be used for a renogram which was not labelled with I-131 (148). Chromium -51 has several advantages over I-131 in that it is a monoenergetic gamma emitter with no beta emission (149).

Myers et al (148) found that Cr -51 E.D.T.A. had a rapid renal clearance in animals plus a long shelf life, factors which would make it an ideal renogram agent. Unfortunately, it was later (150) found that humans had a slow clearance of the compound, giving a renogram curve of low amplitude and questionable value.

e) I-131 2,6 - Diodosulfanilate (I-131 D.I.S.A.)

This agent was investigated by Winter et al (151) and found to have good characteristics in mice, but these were not duplicated in humans (152). The compound had a clearance half-time of about one hour (152), so that compared to I-131 hippuran it showed much lower amplitude and slower changes in the shape of the renogram curve.

f) Diethylene Triamine Pentaacetic Acid (D.T.P.A.)

The compound D.T.P.A. has been used to make several complexes with radioactive nuclides which are of interest for the renogram test.
In this group of complexes are In \(-113m\), In \(-111\), and Yb \(-169\) D.T.P.A. (114, 116-118, 153). The D.T.P.A. complexes are best considered together as they are all handled in the kidney by glomerular filtration (154). Because they are glomerular agents, the time taken for 50% of the dose to be excreted is approximately 80-90 minutes (92) which is somewhat slower than I-131 hippuran (143, 144). The result of the slower clearance has been mentioned previously as giving a flatter uptake and excretion segment for the renogram curve. The doses for In \(-113m\) D.T.P.A. and Yb \(-169\) D.T.P.A. are given in Table 5.

8. Agents for Specific Renal Function Tests:

Although renograms and renal scans are the most frequently used nuclear medicine procedures for the study of the kidney, there are other renal function tests which can be performed.

a) Glomerular Filtration Rate Agents

Glomerular filtration rate has traditionally been measured using inulin (155, 156) a polysaccharide which is eliminated entirely by glomerular filtration but for which assays are difficult to perform. Inulin has been labelled with C \(-14\) quite successfully and without changing the chemical behavior of the molecule (157). Carbon \(-14\) has the drawback of requiring liquid scintillation for detection so that other nuclides have been used to label inulin. Iodine \(-125\) has been attached to an allyl derivative of inulin (158) and this molecule also behaves like non-radioactive inulin if the I-125 allyl inulin is purified before use (159). Chromium \(-51\) labelled inulin has also been prepared and shown to be cleared by glomerular filtration (160).

Cyanocobalamin (Vitamin $B_{12}$) labelled with either Co \(-57\)
or Co-58 has been used to measure glomerular filtration rates quite successfully, provided the body was saturated by non-radioactive carrier prior to the test to ensure that all the Vitamin B$_{12}$ binding sites were saturated (161, 162). Some exchanged between the radioactive cyanocobalamin and the bound non-active cyanocobalamin has been demonstrated (163). Hydroxycobalamin, a more strongly bound form of the vitamin, has been used instead of cyanocobalamin to minimize this exchange (163).

Many of the metal chelates of E.D.T.A. and D.T.P.A. have been used for the determination of glomerular filtration. These agents include: Cr-51 E.D.T.A., Tc-99m D.T.P.A., Yb-164 D.T.P.A., and In-113m D.T.P.A. (114, 117, 118, 148, 154), all of which have been discussed previously. Other nuclides that have been chelated to D.T.P.A. and E.D.T.A. are Co-57, Co-58, Ga-68, In-114m, In-115m, Hg-197 (97, 164, 165).

b) Renal Plasma Flow Agents

Renal plasma flow can be measured by any agent which is cleared completely from the blood in a single pass through the kidney. Parahydroxyhippuric acid (P.A.H.) has been used as a measure of this function, as it is known to be 90% cleared in a single pass through the kidney (142). Ortho-iodohippuric acid (O.I.H.) labelled with I-131 has found widespread use because it compares closely with P.A.H. in its clearance values if the O.I.H. is purified of free I-131.

c) Renal Blood Flow Agents

The renal blood flow may be measured directly by the use of radioactive inert gas washout methods (166-168). The isotopes used include xenon-133, and krypton-85. Oxygen-15 incorporated as water has recently been used as well (169).
B. EXPERIMENTAL METHODS AND MATERIALS
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1. Animals:

The mice used in this study were of the Swiss strain and included both males and females weighing between 25 and 35 gms. The mice were maintained in plastic cages containing not more than 12 mice at a time. They had free access to Purina Laboratory Chow \(^{(R)}\) and tap water. The mice were used for the distribution, excretion and excretion products study.

For the dynamic and static scans, New Zealand White rabbits of both sexes were used. The rabbits were housed individually in mesh cages with tap water and Purina Rabbit Chow \(^{(R)}\) allowed ad libitum.

2. Chemicals:
   a) Stannous Chloride

   Solutions of stannous chloride were made up by exact weighing of small quantities of stannous chloride dihydrate (Mallinckrodt), usually less than 10 mg., on a Mettler \(^{(R)}\) analytical balance. After weighing, the crystals were quickly placed in a 7 ml. vacutainer (Beckton-Dickinson Ltd.) and dissolved in a drop of concentrated hydrochloric acid (Fischer Reagent Grade). The stannous chloride solution was then made up to a volume of 0.5 ml. with distilled water and subsequently purged with nitrogen gas for 1-2 minutes by means of hypodermic needles placed through the vacutainer seal. The amount of stannous chloride needed for the preparation to be made was determined, and the volume of solution containing that amount was withdrawn via a 25 gauge needle into a 1.0 cc. syringe. The measured tin solution was then placed in a 6 ml. evacuated vial (New England Nuclear) for reaction with the desired dibasic acid. Tin (II) solutions are readily oxidized by air, especially in the presence
of light, to form tin(IV) (170). Therefore, at all times it was attempted to ensure that the stannous chloride solutions were not exposed to air and that they were refrigerated when not being used.

b) Dibasic Acids

The acids used for this study were: oxalic acid (Merck Reagent Grade), malonic acid (Matheson Coleman and Bell Manufacturing Chemists), succinic acid (Merck Reagent Grade), glutaric acid (B.D.H. Laboratory Reagent) adipic acid (Fischer Reagent Grade), and maleic acid (Matheson Coleman and Bell Manufacturing Chemists). The desired amount of each acid was weighed on a Mettler® analytical balance and placed in a 7 ml. vacutainer (Beckton and Dickinson Ltd.). The weighed acid was dissolved in 2 ml. of distilled water and then adjusted to the desired pH with the use of 1.0N sodium hydroxide (Certified Fischer Scientific) and made up to 5 ml. final volume with distilled water. The pH was determined by the use of a Fischer Accumet Model 230 pH/ion meter. An aliquot of the acid solution at the correct pH was then transferred to a 6 ml. evacuated vial (New England Nuclear). The acid solution once placed in the vial, was purged with nitrogen gas for 5 minutes and the previously determined amount of stannous chloride solution was then added to it.

3. Technetium -99m:

Technetium -99m, in the form of sodium pertechnetate, was obtained daily by elution of a New England Nuclear Tc -99m Generator. The eluate was measured for its specific activity at a given reference time.

The concentration of molybdenum -99, a possible contaminant of the sodium pertechnetate solution, was determined by the use of selective lead shielding which attenuates the lower energy Tc -99m gamma
rays. The allowable limit of molybdenum contamination is 1 uCi. per mCi. of Tc -99m for the eluate or 5 uCi. per dose of radiopharmaceutical.

The aluminum contamination was also measured in the eluate by the use of a New England Nuclear Aluminum ion Indicator Kit. This test is performed by comparing the colour of a drop of eluate with that of a standard solution when placed on a treated indicator paper. The Atomic Energy Commission's limit on the presence of aluminum ion is set at 20 µg per ml.

The amount of activity used when labelling the dibasic acids varied according to the type of study to be done. The rabbit scans were done with 1.0 mCi. of activity calculated to be present at scanning time. The mouse distribution and excretion study used 100 uCi. of activity, while the excretion products study involved only a 20 uCi. dose of activity.

4. Technetium -99m Labelled Dibasic Acids:

The dibasic acids were labelled by first adding stannous chloride solution to the dibasic acid solution in the 6 ml. N.E.N. elution vial. The acid and the tin solutions were mixed together for 20 to 30 seconds and then the vial was placed inside lead shielding. A measured amount of technetium -99m activity was then added to the vial and inverted several times by hand. The solution in the vial was made up to 6.0 ml. total volume with sterile water for injection U.S.P. and stored at 4° C until used.

5. Technetium -99m Labelled Malonic Acid Kit:

As a part of this study, a kit was developed which required only the addition of pertechnetate solution to make the labelled malonate complex. Malonic acid in the amount of 15.6 g. was weighed out on
an analytical balance and dissolved in approximately 10 ml. of distilled water. The pH was adjusted to pH 7.4 and then distilled water was added to make a final volume of 25.0 ml. Using a 10 ml. syringe, one ml. aliquots of this solution containing 624 mg. of malonic acid were Millipore (R) filtered into individual 6 ml. vials. Each vial was then purged with sterile nitrogen for 5 minutes via two needles placed through the multi-dose sealer.

A stannous chloride solution was made up by dissolving 25.2 mg. of stannous chloride-dihydrate in 3 drops of concentrated hydrochloric acid and subsequently making the volume up to 10.0 ml. with nitrogen-purged distilled water. This solution was rapidly dispensed in 0.1 ml. aliquots into the 6 ml. elution vials containing the purged malonic acid solution. The solutions of malonic acid and stannous chloride were mixed for 20 to 30 seconds and then quick-frozen in a dry ice-acetone mixture. The frozen vials were stored at -20° C until used. Each vial contained 1.1 ml. of solution and could be made up to a final volume with 4.9 ml. of pertechnetate eluant. The final concentrations of malonic acid and stannous chloride were 1.0 M and 1.9 x 10^{-3} M respectively when made up to 6 ml.

6. Chromatography:
   a) Dibasic Acids

   The chromatographic behavior of the dibasic acids was investigated at various pH's to determine how the acids behaved on the Gelman (R) I.T.L.C. silica gel chromatography media. All the dibasic acids in this study were made up in distilled water as saturated solutions using 1.0N sodium hydroxide to adjust the pH. All the solutions were equilibrated overnight at room temperature before use. Using a Hamilton syringe, be-
tween 10 and 20 λ's of each acid were applied to two pieces of Gelman\textsuperscript{(R)} chromatography media. The chromatography strips were air dried at room temperature for 30 minutes. The strips were then run; one in N-butanol (A.C.S. Fischer Scientific) and one in 0.9% saline (Abbott Sodium Chloride for Injection U.S.P.) to a height of 12 to 14 cm. The solvent fronts were marked and the strips were allowed to dry at room temperature for 2 hours. The chromatograms were developed by spraying with a 10% solution of bromocresol green (Fischer Scientific) in 95% ethanol. The spots indicating the location of the acid were yellow while the background was a blue-green colour. The colours were enhanced by brief exposure to concentrated ammonium hydroxide vapours (Fischer Reagent Grade). The Rf values were noted at the leading edge of the developed spots.

b) Technetium Labelled Dibasic Acids

In order to determine the amount of radioactivity attached to the acids, or radiochemical purity, a two part chromatography system was employed identical to the ones used for the unlabelled acids. Ten λ's of the solution being tested were spotted to each of two pieces of Gelman\textsuperscript{(R)} chromatography media. The two strips were air-dried at 4° C for one hour and then one strip was run in N-butanol and the other in normal saline, both at 4° C. The solvents were allowed to run a distance of 12 to 14 cm. and then the solvent fronts were marked and the strips air-dried at room temperature for one hour.

The basis for measuring the radiochemical purity or percentage label, is the assumption that the water soluble labelled complex of dibasic acid and technetium migrates to the solvent front in normal saline and remains at the origin in the N-butanol system. Free, or uncomplexed pertechnetate is known to migrate to the solvent front in both systems.
To obtain a measure of the radiochemical purity for the soluble Tc-acid complex, the percentage of the total activity present at the butanol solvent front, assumed to be a measure of free pertechnetate only, was subtracted from the percentage of the total activity at the saline solvent front.

Two methods were used to determine the location of the activity on the chromatography media. The first method consisted of cutting the chromatography strip into 1 cm. pieces widthwise beginning at the origin and proceeding up the strip to at least 2 cm. past the solvent front. Each piece was numbered and placed in an R.T.U. Disposable Culture Tube (Beckton-Dickinson and Co.). The tubes were then counted in a Picker Nuclear Autowell II 100 sample gamma counter for 1 minute or 200,000 counts, whichever occurred first. Total activity from all the separate pieces was summed for each chromatogram and expressed as 100 percent of the activity for that system (saline or butanol). The three most active pieces of media from the solvent front were then totaled for the saline system and expressed as a percentage of the total activity. This was also done for the N-butanol system. The difference between the percentage of the total activity located at the solvent front in the butanol and the percentage located at the solvent front in saline was called the percentage radiochemical purity.

The second method of detecting the activity involved placing the dried chromatograms on the Picker Nuclear Dyna camera (R) detector head. The camera was set up to consider activity arising only from the area of the chromatogram. After allowing the Dyna camera to accumulate counts, a histogram representation of the activity was displayed on the camera oscilloscope. By using the integrating systems on the camera,
the amount of activity present at the solvent fronts of the two chromatograms could be determined and expressed as a percentage of the entire activity present. As in the previous method, the percentage activities at the solvent fronts could be subtracted to calculate the tag.

The camera method has the advantage of being much faster than the first method described, requiring less than 5 minutes as opposed to nearly an hour for cutting and counting the individual chromatogram pieces. The only advantage to using the Autowell II in the first method is that it can measure much lower levels of activity than the Dyna camera, making it possible to measure chromatograms which contain little activity.

A study comparing the reproducibility of the two methods for determining the location of the activity showed that they agree quite closely (171).

7. Toxicity Study:

The toxicity study was done to determine the $LD_{50}$ of the malonate kit, without the technetium, when given by intravenous injection. Various concentrations of malonic acid with a constant amount of stannous chloride were made up in distilled water and injected into Swiss strain mice. The pH of the solutions was adjusted to pH 7.4 with 1 N sodium hydroxide. The solution was injected as a constant volume of 0.2 ml. into a tail vein. The measured parameter was death within 5 minutes of injection. It was noted that if the mouse lived for 5 minutes after injection, it would survive the next 24 hours, although no specific study was made of this observation. The $LD_{50}$ was determined after the method of Litchfield and Wilcoxon (172). This method determines the 95% confidence limits of the $LD_{50}$ as well as
LD_{16} and LD_{84} limits.

8. Whole Body Excretion:

The whole body excretion curve was done to determine the rate at which mice excreted the Tc-99m-malonate complex. The complex was injected as 100 uCi. of activity in 0.1 ml. of solution via a tail vein. Immediately upon injection, the animal was placed in a 4½ oz. Falcon \textsuperscript{(R)} drink cup and then into a Tobor \textsuperscript{(R)} Nuclear Chicago Large Sample Counting System. The mice were counted immediately after injection and then at 5, 10, 15, 20, 25, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, 240, 270, 300, 330, 360, and 1440 minutes post-injection for intervals of 1 minute. A background was obtained and subtracted from all the observed counts.

Between each counting interval, the mice were removed from the counter and the Falcon \textsuperscript{(R)} drink cup. They were placed on an absorbent pad and induced to void if possible. If the mouse voided in the drink cup, a new cup was used for the next counting interval. Because this study was designed to study the loss of activity due only to excretory processes, all counts were corrected for radioactive decay. All data points were the mean of 5 or more mice.

9. Compartmental Analysis:

The values for the whole body excretion curve were plotted on semi-logarithmic paper as a function of time after correcting for decay. For each time interval, the activity was expressed as a percentage of the total activity present at t=0 min.

Computer curve stripping was employed using a program written for the Digital \textsuperscript{(R)} pdp 8/f computer (173). The program requires that the biological decay constants be widely separated and that the
data should contain sufficient points for each portion of the curve. The data was fed into the computer starting with the longest time value and proceeding to the shorter time periods. Initially, the last three data points were entered into the computer and a line of best fit was derived employing the weighted least squares method. The weighting was necessary due to the logarithmic nature of the data (174). The standard deviation of the slope was also calculated for these three points and then another data point was entered into the computer. With the addition of a fourth point, a second curve was computed with a new slope and standard deviation. The 't' value and its corresponding degrees of freedom were calculated by the computer and compared by the operator using students 't' test. The significance in the change of slope between the two lines was compared and if no difference in slope was noticed, a new data point was entered into the computer and compared. When the 't' test showed significant variation in the slope for N data points, a curve was computed for the slope of N-1 points. The ordinate intercept, half-life and standard deviation was calculated for the first curve and the values were subtracted from all the data at shorter time periods. The new set of points were considered in a similar manner until all the data was analyzed.

10. Excretion Analysis:

To determine whether the activity excreted by the mice remained complexed after excretion, a study was done of the mouse urine after the animals had been injected with the tagged complex. Five mice were injected with 20 uCi. of activity in 0.1 ml. of malonate kit via a tail vein. Immediately after injection, the animals were placed in 600 ml. beakers with wire mesh 1 cm. above the bottom of the beaker. The wire
mesh allowed the urine to fall through while maintaining the feces and the animal above the screen. The urine from each mouse was collected separately over a 2 hour period. Ten λ's of each urine sample were spotted onto 2 pieces of Gelman (R) chromatography media. The strips were dried and then run in N-saline and N-butanol at 4° C in the manner previously described for analysis of labelling efficiency.

To investigate any effect which the urine alone may have had upon the labelled compound, 20 uCi. of active compound in 0.1 ml. of solution was mixed with 0.5 ml. of the urine obtained from five control animals. The urine and labelled complex were incubated in a Buchler (R) Instrument water bath at 37° C for 2 hours. The five samples were then spotted on chromatography media and the percentage label of the complex was determined.

A standard was also run consisting of 10 λ's of active complex spotted on Gelman (R) chromatography media after 2 hours of sitting in the 6 ml. vial at room temperature. The excreted complex and the complex incubated with urine were compared with control percentage label for the complex.

11. Tissue Distribution:

To determine the areas of localization within the mammalian body, mice were injected with the malonate kit solution containing 100 uCi. of Tc -99m in 0.1 ml. of solution. Each mouse in this study was injected with 0.1 ml. of solution, weighed, and returned to its cage where it was allowed food and water until time for dissection. A 0.1 ml. standard was prepared at the time of injection to compare with the activity retained by the mouse at the time of analysis. By counting the samples and the standard at the same time, decay could be neglected. The time
intervals used were: t=0, 5, 60, 180, 360, 720, and 1440 minutes. Five mice were used for each time interval, with mean and standard deviation calculated for each group of data.

The animals were sacrificed at the time of analysis by rapid decapitation and an immediate blood sample was collected and measured. The organs analyzed were: the brain, lungs, liver (with gallbladder), kidneys, large and small bowel, muscle, bone, stomach, spleen and tail. For the muscle, a sample was taken of the quadriceps extensor muscle and weighed. The bone sample consisted of the femur, scraped clean and weighed. All organs were washed twice to remove traces of blood and then wet-weighed. The stomach and bowels were emptied of contents before weighing to obtain consistent organ weights. All the samples were placed in R.T.U. tubes after weighing. The carcass was weighed and dissolved in 100 ml. of concentrated nitric acid (Fischer Reagent Grade) for 12-16 hours. While the carcass was dissolving, the samples were kept refrigerated at -20°. After the carcass had completely dissolved, a 5 ml. aliquot of the nitric acid solution was placed in an R.T.U. tube. All the samples and the standards were counted for 1 minute or to a minimum of 10,000 counts, whichever was the greater. A background count was obtained and subtracted from the total counts for each animal.

The total dose remaining in each animal was taken to be the sum of the counts taken from all the organs plus the carcass. The carcass counts were extrapolated for the volume of nitric acid used to dissolve the entire carcass. The counts from the tail were not included. The tail was found to contain a variable amount of activity, particularly over the shorter time intervals when compared with the standard. The activity in each organ was expressed as a percentage of the total
activity injected. The activity in the bone, muscle, and carcass was expressed as percentage-per-gram of tissue while the blood sample was expressed as a percentage-per-ml.

In order to facilitate comparison of the relative concentration of activity in each organ's tissue, the percentage of total activity - per-organ was also expressed as a percentage-per-gram of tissue for the liver, kidneys, large and small bowels, brain, and spleen. Comparisons were made between the percentage activity per gram of kidney to the percentage-per-gram activities in the blood, bowels and liver.

12. Radionuclide Static Scintiscans:

The scans were all done on a Picker Nuclear Dyna camera with an ultrafine parallel hole collimator. The camera had an on-line Digital (R) pdp 8/e computer to provide image enhancement as well as video playback via a Sony (R) Videotape recorder. Output consisted of a conventional x-ray film (Cronex (R) 4 Medical x-ray film) as exposed by a Dunn (R) Multiple Image camera, and as a colour-coded Polaroid (R) print. The Dunn camera could be set for single or multiple images of variable exposure length. The computer-enhanced Polaroid (R) pictures were corrected for non-uniform detector response and adjusted for colour range and for background subtraction. For the rabbit scans, no background subtraction, and as wide a colour range as would allow representation of all activity, was used.

All the dibasic acids were labelled and used for static scans of rabbits. These preliminary scans were used to evaluate each acid's potential for use as a renal scanning agent. The criteria for evaluation included intensity of kidney image, lack of relative liver and bone uptake and general background intensity. The degree of activity in the
stomach and thyroid areas was also noted.

The rabbits were injected with the labelled solution via an ear vein and then returned to their cages until time for the examination. Five to ten minutes before the scintiscan was to be performed, the rabbits were removed from their cages and anesthetized with sodium Nembutal (Abbott Laboratories) 50 mg. per kg., given intravenously via an ear vein. The animals were titrated with further doses of Nembutal as required to ensure continuing narcosis. Once unconscious, the rabbit was placed upon a movable table and positioned beneath the detector head of the Dyna camera so a posterior view could be obtained. With the detector over the animal, the left and right sides were located on the field of view by the use of a technetium -99m point source held close to the detector head for a few seconds. When positioning was complete, the camera was activated and counts were accumulated until satisfactory images were obtained, usually 3 to 5 minutes, depending upon the activity remaining within the animal.

All radioactive excreta were collected during the course of the scan and stored until safe for disposal. The mobile table was covered with absorbent pads to protect it from contamination. Upon completion of the scintiscan, the rabbits were returned to their cages and allowed to recover consciousness.

13. Radionuclide Dynamic Scintiscans:

This study also employed the use of the Picker Nuclear Dyna camera\(^{(R)}\). The images were displayed sequentially by the Dunn camera\(^{(R)}\) for 5 second exposures. Only the Tc -99m - malonate complex was used for the dynamic study.

The rabbit was rendered unconscious with sodium Nembutal 50 mg.
per kg., just prior to placing the animal supine beneath the detector head. Once positioned, 0.1 ml. of the malonate complex was injected rapidly via an ear vein. The flow of activity within the animal was monitored on videotape from the moment of injection until 20 minutes post-injection, at which time no further change in the radioactive pattern was evident. When the scan was completed, any radioactive excreta was gathered up and stored for later disposal, while the animal was returned to its cage for recovery.

As part of the play back system on the camera, areas of interest within the field of activity may be monitored. Using this system, the pattern of radioactive buildup and elimination was followed for the heart and both kidneys. The obtained flow of activity could be displayed as activity vs time, analogous to a renogram study. Measurements of the loss of activity through the kidneys were made from this data.
C. RESULTS & DISCUSSIONS
C. RESULTS AND DISCUSSIONS

1. Chromatography of the Dibasic Acids:

The chromatographic behavior of the dibasic acids was investigated after a preliminary study showed that their Rf values were pH-dependent. It was felt necessary to find a pH at which the chromatographic behavior of the acids could be reliably determined.

The acid solutions were made up as described earlier and the solutions were adjusted to pH = 1, 3, 5, and 7 respectively. The Rf values were measured as the leading edge of the developed colour spot. In normal saline, all the acids showed Rf values of 1.0. The values for the various acids run in N-butanol are listed in Table 6.

<table>
<thead>
<tr>
<th>Acid</th>
<th>Rf Values a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH 1</td>
</tr>
<tr>
<td>Maleic</td>
<td>1.0</td>
</tr>
<tr>
<td>Oxalic</td>
<td>1.0</td>
</tr>
<tr>
<td>Malonic</td>
<td>1.0</td>
</tr>
<tr>
<td>Succinic</td>
<td>1.0</td>
</tr>
<tr>
<td>Glutaric</td>
<td>1.0</td>
</tr>
<tr>
<td>Adipic</td>
<td>1.0</td>
</tr>
</tbody>
</table>

a values are mean of 2 experiments  
b poorly defined leading margin of exposed colour

From this data it appears that the undissociated acids are more lipophilic and therefore migrate with the solvent front in the butanol. The stronger acids do not move with the butanol solvent front when the pH is low, as they are already dissociated. Adipic acid, a very weak acid, does not ionize sufficiently to remain at an Rf = 0 for butanol until the higher pH values are reached. The other acids all appear to be quite well dissociated at pH3.
Calculations were done based upon the dissociation constants, to show the percentage dissociation of the acids at various pH's (Table 7).

**TABLE 7:**

<table>
<thead>
<tr>
<th>Acid</th>
<th>$K_a$</th>
<th>pH=1</th>
<th>pH=3</th>
<th>pH=5</th>
<th>pH=7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxalic</td>
<td>$5.4 \times 10^{-2}$</td>
<td>35.1%</td>
<td>98.2%</td>
<td>99.8%</td>
<td>greater than 99.9%</td>
</tr>
<tr>
<td>Malonic</td>
<td>$1.4 \times 10^{-3}$</td>
<td>1.4%</td>
<td>58.3%</td>
<td>99.3%</td>
<td>greater than 99.9%</td>
</tr>
<tr>
<td>Succinic</td>
<td>$6.4 \times 10^{-5}$</td>
<td>less than 0.1%</td>
<td>6.0%</td>
<td>86.5%</td>
<td>99.8%</td>
</tr>
<tr>
<td>Glutaric</td>
<td>$4.5 \times 10^{-5}$</td>
<td>less than 0.1%</td>
<td>4.3%</td>
<td>81.8%</td>
<td>99.8%</td>
</tr>
<tr>
<td>Adipic</td>
<td>$3.7 \times 10^{-5}$</td>
<td>less than 0.1%</td>
<td>3.6%</td>
<td>78.7%</td>
<td>99.7%</td>
</tr>
<tr>
<td>Maleic</td>
<td>$1.0 \times 10^{-2}$</td>
<td>9.1%</td>
<td>90.9%</td>
<td>99.0%</td>
<td>greater than 99.9%</td>
</tr>
</tbody>
</table>

These calculations show that 99% of each acid was dissociated at pH 7, which supports the result of all the acids having an Rf=0.0 in N-butanol at this pH. It was concluded that if the pH was adjusted to pH 7 or above, the chromatography systems would show Rf=1.0 in normal saline and Rf=0.0 in N-butanol.

2. Chromatography of the Technetium Labelled Dibasic Acids:

The six acids were each labelled with technetium 99m using stannous chloride as the reducing agent. For each acid, the pH was kept at 7.4 as the solution would be buffered to this pH when injected. The percentage radiochemical purity was measured using the two-part chromatography system and the results are given in Table 8.

**TABLE 8:**

<table>
<thead>
<tr>
<th>Acid</th>
<th>Percentage Radiochemical Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxalic acid</td>
<td>94.0 ± 1.0%</td>
</tr>
<tr>
<td>Malonic acid</td>
<td>91.2 ± 6.1%</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>16.0 ± 5.0%</td>
</tr>
<tr>
<td>Glutaric acid</td>
<td>5.7 ± 7.6%</td>
</tr>
<tr>
<td>Adipic acid</td>
<td>Negligible</td>
</tr>
<tr>
<td>Maleic Acid</td>
<td>60.0 ± 8.3%</td>
</tr>
</tbody>
</table>

a Mean of 4 experiments
Each of these labelled acids was subsequently injected into a rabbit to yield scintiscans which are discussed later.

In the study to find the optimum conditions for labelling each of the dibasic acids, it was found that the solubility of the stannous ion was important. If the stannous ion came out of solution as a white precipitate, then the majority of the activity would attach to the precipitate quite firmly. In an attempt to measure the stability of the labelled precipitate, 0.1 ml. of labelled adipic acid – stannous precipitate suspension was placed upon a 0.22 μ Millipore(R) filter. The filter was washed with 500 ml. of normal saline. After washing, 70 percent of the initial activity was still retained upon the filter. This indicated that the activity could not be easily removed from the precipitate by washing.

Aqueous stannous +2 ions are unstable at any pH greater than 2 or 3 (170), so a ligand is required to give this oxidation state stability. The strength of the dibasic acids as ligands is probably measured by the radiochemical purity of the soluble labelled fraction. The dibasic acids in descending order of stability as shown by their radiochemical purity with technetium is as follows: oxalic acid greater than malonic acid greater than maleic acid greater than succinic acid greater than glutaric acid greater than adipic acid.

Because of the solubility of stannous 2+ ions, it would be desirable to use a minimal amount of this ion. However, the amount cannot be reduced too severely or efficient labelling will not occur. The usual amount of stannous 2+ added (greater than 10^-5 moles to 6 ml. of solution) to a pertechnetate solution contains a great excess of stannous ions when compared with pertechnetate. One millicurie
of pertechnetate contains only $1.9 \times 10^{-12}$ moles (175). In a recent study using pyrophosphate it was shown that only a very small amount of the stannous ion reduced the pertechnetate, while the remainder of the stannous ions appeared to reduce the oxidizing species present in the solution. These oxidizing species, mainly in the form of dissolved oxygen, if left unaltered would prevent the technetium from being reduced (176). The minimum amount of stannous chloride was determined for pyrophosphate and it was shown that any increase in this amount did not affect the chelation of the technetium by the pyrophosphate (176).

3. Preliminary Radionuclide Scintiscans:

These scans depicted in Figure 3 were done with the various acids to determine the areas of distribution in the rabbit, as well as to evaluate potential usefulness of the acids as scanning agents.

The oxalic acid scintiscan showed high body background with the body outline quite clearly visible. The nuclide accumulated in the bladder to a greater degree than in any other organ. The skeleton showed an activity level somewhat higher than the general background but the definition was not sharp. The kidneys showed some definition of shape and were second only to the bladder in overall activity level. The activity in the chest area was probably due to blood activity in the heart and lung region. There was no clearly defined liver image.

The scan done with malonic acid (Figure 3b) also showed significant activity in the bladder region but this acid demonstrated the lowest apparent body background of all the acids used. The kidney images were quite evident, with the kidneys second only to the bladder in overall activity. There was some heart-lung activity but
FIGURE 3:
Radioscintiscans of Six Technetium Labelled Dicarboxylic Acids

3A Oxalic Acid

3B Malonic Acid

3C Succinic Acid

3D Glutaric Acid

3E Adipic Acid

3F Maleic Acid
no apparent liver image. The skeleton activity was slight with poor definition. There was no thyroid or gut activity to indicate the breakdown of the compound in vivo to form the pertechnetate anion.

Succinic acid (Figure 3c) showed a well defined liver image when compared with either oxalic or malonic acid. The liver image was probably due to the large amount of colloid present in the preparation i.e.: 79.7% ± 14.6. The soluble succinic acid -Tc complex, was visible as activity in the kidneys and bladder region, similar to the oxalic and malonic acid scans. As can be seen from the scan, (Figure 3) if a predominant liver image is present it obscures the right kidney.

Technetium - labelled glutaric acid (Figure 3d) showed a more predominant liver image than does the succinic acid scintiscan. The level of colloid in this preparation was measured at 89.5% ± 11.3. In the glutaric acid scan, a greater amount of bone uptake was present than with the other agents. Colloid does localize in the bone marrow reticulo-endothelial system (111), but there is a possibility of a specific attraction of the bone for glutaric acid, as the bone localization appears greater than with other agents with more colloid e.g.: Adipic Acid, 91.8 ± 6.5 percent.

Adipic acid (Figure 3e) showed no significant soluble labelled complex with technetium. Upon injection this preparation had the most predominant liver uptake of all the saturated acids. A small focus of activity was seen above the left kidney and below the left lobe of the liver and was probably due to the spleen. Some bone activity was present, as was a small amount of kidney activity. The kidney activity may have been due to the slow dissolution of insoluble colloid lodged within the liver, or to the breakdown of the complex. As there
was no thyroid or gut uptake, slow dissolution is the likeliest explanation for the kidney activity.

Spleen activity was also present for the labelled complex of maleic acid (Figure 3f). The kidney and bone images in this scan were about equal in activity. The lack of activity in the bladder region could be due to a lack of breakdown of the complex or more likely, to the animal having voided just prior to the scanning. Other scans done with adipic acid complex did show bladder activity.

The large amount of liver activity for the maleic complex is interesting as the amount of colloid was only 24.7 ± 40.7 percent, indicating a specific attraction for the liver over and above that due to the colloid particles.

From these results it would appear the oxalic acid complex showed too little localization other than in the urine within the bladder and so was not considered further. The succinic, glutaric, adipic and maleic acid complexes showed varying amounts of liver activity which appeared to increase with the amount of colloid present in the preparation. None of these four acids showed localization which could be judged equal to or greater than technetium labelled sulfur colloid (111).

Of the six acids under consideration, the malonate complex showed the best localization in the kidneys as well as the least body background with little or no liver image. Malonic acid was therefore chosen for further investigation.

4. Technetium Labelled Malonic Acid Complex:

The malonate kit was developed in an effort to produce a quick-frozen, stable preparation which could be thawed and reproducibly labelled with pertechnetate solution. The limit of radiochemical
purity was set at greater than 90 percent. Experiments to determine the optimum concentration of the product gave the results shown in Table 9.

**TABLE 9:**

Concentration of Malonic Acid vs Radiochemical Purity of the Tc - Malonic Acid Complex 

<table>
<thead>
<tr>
<th>Concentration of Malonic Acid</th>
<th>Radiochemical Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01 M</td>
<td>9.1 ± 7.8%</td>
</tr>
<tr>
<td>0.50 M</td>
<td>21.8 ± 6.1%</td>
</tr>
<tr>
<td>0.64 M</td>
<td>44.4 ± 10.2%</td>
</tr>
<tr>
<td>1.00 M</td>
<td>91.0 ± 8.1%</td>
</tr>
<tr>
<td>1.90 M</td>
<td>91.5 ± 7.7%</td>
</tr>
<tr>
<td>2.50 M</td>
<td>93.2 ± 4.0%</td>
</tr>
<tr>
<td>3.20 M</td>
<td>90.7 ± 5.9%</td>
</tr>
</tbody>
</table>

\(a\) Mean of 4 trials ± standard deviation  
\(b\) Stannous ion concentration constant at 1.9 \(x 10^{-3}\) M

Initially, the concentration of stannous chloride was fixed at 1.9 \(x 10^{-3}\) M. The minimum concentration of malonic acid which gave the required radiochemical purity was 1.0 M. This concentration of malonic acid was then used while the concentration of stannous chloride was varied. The results, depicted in Table 10, show that a minimum of 1.8 \(x 10^{-3}\) M stannous ion was necessary to achieve a satisfactory radiochemical purity.

**TABLE 10:**

Concentration of Stannous Chloride vs Radiochemical Purity of the Tc - Malonic Acid Complex 

<table>
<thead>
<tr>
<th>Stannous Chloride Concentration</th>
<th>Radiochemical Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>(7.3 \times 10^{-4}) M</td>
<td>89.1 ± 2.8%</td>
</tr>
<tr>
<td>(1.8 \times 10^{-3}) M</td>
<td>94.5 ± 6.1%</td>
</tr>
<tr>
<td>(3.6 \times 10^{-3}) M</td>
<td>94.3 ± 7.2%</td>
</tr>
<tr>
<td>(7.2 \times 10^{-3}) M</td>
<td>94.6 ± 4.3%</td>
</tr>
</tbody>
</table>

\(a\) Mean of 4 experiments ± standard deviation  
\(b\) Malonic acid concentration constant at 1.0 M
On the basis of the Tables 9 and 10 a kit was formulated containing 1.0 M malonic acid and $1.8 \times 10^{-3}$ M stannous chloride. The pH was adjusted as previously mentioned. The kits were found to be $94.7 \pm 4.1$ percent radiochemically pure when measured immediately after addition of the pertechnetate solution. The radiochemical purity measured $97.3 \pm 2.1$ percent after 24 hours storage at $4^\circ$ C. Due to the physical half-life of technetium-99m, stability for a 24 hour period is well in excess of what is desirable.

5. Toxicity Study:

The toxicity study was done after the manner of Litchfield and Wilcoxon (172) and involved the use of 34 mice of both sexes. Malonic acid in concentrations of 100mg./ml., 110mg./ml., 120mg./ml., 130mg./ml., 140mg./ml., and 150mg./ml. was used. The solutions all contained 0.42mg./ml. of stannous chloride dihydrate, the same amount as was present in the malonate kits. The injection size was 0.2 ml. per animal. The results of the toxicity study are given in Table 11.

The value of $(\text{Chi})^2$ for the 5 degrees of freedom was found to be 11.1 (172). As 1.78 is less than 11.1, the data may be concluded to be not significantly heterogeneous. The $LD_{16}$ was = 658mg./kg., the $LD_{50}$ was = 800mg./kg. and the $LD_{84}$ was = 840mg./kg. The slope function can be computed as being equal to:

$$S = \frac{LD_{84}/LD_{50} + LD_{50}/LD_{16}}{2} = \frac{840/800 + 800/658}{2} = 1.13$$

The total number of animals tested between the expected effects of 16 and 84 percent was $N^- = 15$. The factor for the $LD_{50}$, $f(LD_{50}) = S 2.77/ N^- = (1.13)(7158) = 1.09$. This figure computes the 95% confidence limits as: lower limits $= LD_{50} / f(LD_{50}) = 800/1.09 = 734$ mg./kg. and
TABLE 11:

Determination of the Intravenous LD$_{50}$ for the Stannous Malonate Complex in Mice

<table>
<thead>
<tr>
<th>Dose mg/kg</th>
<th>No. Dead/No. Tested</th>
<th>Observed% Dead</th>
<th>Expected% Dead</th>
<th>No. Observed-Expected</th>
<th>Contribution to (Chi)$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>555</td>
<td>0/5</td>
<td>0</td>
<td>5.5%</td>
<td>5.5</td>
<td>0.055</td>
</tr>
<tr>
<td>612</td>
<td>1/5</td>
<td>20</td>
<td>12.0</td>
<td>8.0</td>
<td>0.055</td>
</tr>
<tr>
<td>722</td>
<td>1/5</td>
<td>20</td>
<td>26.0</td>
<td>6.0</td>
<td>0.019</td>
</tr>
<tr>
<td>812</td>
<td>2/5</td>
<td>40</td>
<td>55.0</td>
<td>15.0</td>
<td>0.058</td>
</tr>
<tr>
<td>862</td>
<td>4/5</td>
<td>80</td>
<td>72.0</td>
<td>8.0</td>
<td>0.03</td>
</tr>
<tr>
<td>929</td>
<td>4/5</td>
<td>80</td>
<td>87.0</td>
<td>7.0</td>
<td>0.04</td>
</tr>
<tr>
<td>1026</td>
<td>4/4</td>
<td>100</td>
<td>89.0</td>
<td>11.0</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Total No. of Animals = 34
No. of Doses $K$ = 7
Animals/dose = 4.86
Degree of freedom $N = K - 2 = 5$

$(\text{Chi})^2 = 4.86 	imes 0.367 = 1.78$
the upper limit = 800 x 1.09 = 872 mg./kg. Therefore, the intravenous LD$_{50}$ dose for the stannous malonate complex is 800 (734 to 872) mg./kg.

This complex cannot be considered a toxic substance as defined by Christensen et al (51), who states that a substance can be considered non-toxic to mice if it has an LD$_{50}$ of greater than 750 mg./kg. given intravenously. The published values for the LD$_{50}$ of malonic acid given intraperitoneally to rats is 1540 mg./kg. (51). The sodium salt (not specified as to mono or dibasic) is listed as 2500 mg. per kg. (51).

The literature values are higher than the ones reported here but this may be attributed either to species differences or to the presence of the stannous ion in the solution. The different route of injection may also be significant. The literature values are for intraperitoneal injections while the route of injection in this study was intravenous. Because the cause of death appeared to be due to tetany, the intraperitoneal route might be expected to be less lethal as the body would have time to mobilize calcium ions into the blood stream.

The rate of malonate complex injection was found to significantly affect response to the complex. If the highest dose of 150 mg. per ml. was given slowly over a period of one minute or longer, the animal would invariably survive. The injection rate was therefore kept at 0.2 ml. per 10 seconds throughout the toxicity study.

6. Excretion Study:

The excretion study was designed to determine first, the rate at which the technetium -99m leaves the body and secondly, the chemical composition of the activity in the urine. Because of the
short half-life of technetium, all the counts were corrected for decay and were compared with the counts at t=0. A minimum of five mice were counted for each data point. The whole body excretion curve is plotted in Figure 4, from the data listed in Table 12.

**TABLE 12;**

**Percentage of Total Injected Activity Remaining in Mice vs Time**

<table>
<thead>
<tr>
<th>Time (hr.)</th>
<th>Mean Percentage$^a$</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.083</td>
<td>92.8</td>
<td>4.8</td>
</tr>
<tr>
<td>0.167</td>
<td>91.3</td>
<td>4.3</td>
</tr>
<tr>
<td>0.250</td>
<td>84.7</td>
<td>6.5</td>
</tr>
<tr>
<td>0.333</td>
<td>83.0</td>
<td>7.4</td>
</tr>
<tr>
<td>0.417</td>
<td>82.4</td>
<td>6.7</td>
</tr>
<tr>
<td>0.500</td>
<td>51.8</td>
<td>5.8</td>
</tr>
<tr>
<td>1.000</td>
<td>28.9</td>
<td>9.8</td>
</tr>
<tr>
<td>1.500</td>
<td>21.7</td>
<td>2.0</td>
</tr>
<tr>
<td>2.000</td>
<td>19.7</td>
<td>3.7</td>
</tr>
<tr>
<td>3.000</td>
<td>15.0</td>
<td>1.4</td>
</tr>
<tr>
<td>4.000</td>
<td>13.6</td>
<td>4.1</td>
</tr>
<tr>
<td>5.000</td>
<td>11.7</td>
<td>3.0</td>
</tr>
<tr>
<td>6.000</td>
<td>8.3</td>
<td>2.1</td>
</tr>
<tr>
<td>24.000</td>
<td>0.4</td>
<td>0.1</td>
</tr>
</tbody>
</table>

$^a$ Mean values from five mice

From the data it can be seen that 91.7 $\pm$ 2.1 percent of the activity is eliminated from the mouse body at 6 hours after injection without taking the physical decay of technetium $^{99m}$Tc into account.

The data in Table 12 was also plotted on semi-log paper (Figure 5) and a two-part excretion curve became apparent. This data was then subjected to curve stripping after the method of Lyster and Ediss (173). The curve stripping showed the two components to have biological half-lives of 46.8 minutes ($\pm$ 25.8) and 196.4 min. ($\pm$ 8.8)
FIGURE 4:

Percentage of Total Injected Activity Remaining in Mice vs Time
Figure 5:
Log percentage of total injected activity remaining in mice vs. time

\[ t_{\frac{1}{2}} = 0.78 \pm 0.42 \text{ hr.} \]

\[ t_{\frac{1}{2}} = 3.27 \pm 0.15 \text{ hr.} \]
respectively. The biological half-life can be computed as:

\[ \frac{1}{T_{2\text{ Bio.}}} = \frac{1}{T_1} + \frac{1}{T_2} \]

\[ T_1 = 46.8 \pm 25.8 \]

\[ T_2 = 196.4 \pm 8.8 \]

\[ T_{2\text{ Biol.}} = 37.8 \text{ minutes (± 15.8)} \]

The first excretion component is less precise because urinary excretion is difficult to achieve in a mouse over short time intervals and catheterization was not attempted. A faster excretion component was suspected at this time and is demonstrated in the renogram studies of rabbits.

From the intercepts calculated by the curve stripping analysis, it was demonstrated that 29.7 percent of the injected dose was cleared at the slower rate, while 70.3 percent was eliminated at the faster rate.

An attempt was made to determine the amount of activity which was excreted in the feces. The feces of five mice were collected over 6 hours and individually counted. Less than 0.1% of the excreted activity per mouse was detected in the feces. It was concluded that the majority of the activity was eliminated via the kidneys.

The urine was chromatographed in the same manner as the labelled complex. Urine was collected over a period of 2 hours from each of 5 animals, with feces kept separate in the manner mentioned. Ten microliters of each urine sample were spotted upon two pieces of Gelman\(^{(R)}\) chromatography media which were developed and measured in the manner of the Tc - malonate complex.

The radiochemical purity of the malonate complex as it was excreted from the mice measured 76.8 ± 9.3% compared with the kit radiochemical purity of 94.7 ± 4.1%. To account for any effect that some
undefined component within the urine might have had upon the stability of the complex, 0.05 ml. of labelled malonate kit was incubated with 0.2 ml. of urine and chromatographed. The measured radiochemical purity was 95.5 ± 8.0%. To evaluate the effects of both temperature and time upon the stability of the complex in urine, a mixture of 0.05 ml. of complex and 0.2 ml. of urine was incubated at 37°C for 2 hours. The radiochemical purity after incubation was 90.0 ± 5.4 percent.

The excretion study demonstrated that the labelled malonate kit distributed throughout the blood stream with 70.3 percent of the activity remaining within the blood stream and being rapidly cleared. The remaining 29.7 percent possibly diffuses into the intracellular spaces under the effect of high blood concentration. The extracellular portion subsequently diffuses slowly back into the blood stream where it is cleared by the kidneys, thus giving the slow component of excretion mentioned above.

Disregarding physical decay, 99.6 ± 0.1 percent of the injected activity left the bodies of the mice after 24 hours and if physical decay of Tc -99m is included this percentage increases to greater than 99.9 percent of the initial activity.

7. Radioisotopic Renography performed in Rabbits with Tc -99m Labelled Malonic Acid:

The renogram curve depicts the pattern of activity as it accumulates in, and flows through, the kidney (134, 135). By monitoring on the Dyna camera (R) the activity arising from the rabbit's kidneys only, a renogram curve was obtained which is depicted on Figure 6. The curves were measured and the time taken to achieve half the peak activity of the kidneys was noted. For the left kidney to decrease its peak
FIGURE 6:
RADIOISOTOPIC RENOGRAMS IN RABBITS WITH Tc-99m LABELLED MALONIC ACID

6a Left Kidney

6b Right Kidney

6c Heart
activity by one-half required 5.3 minutes, while the right kidney re-
quired 5.5 minutes. Monitoring over the heart region (Figure 6c) yielded
a similar disappearance curve with a half-value of 5.7 minutes. Because
of the similarity between the clearance values over the heart and the
two kidneys, it appears that in the rabbit there is a fast clearance
component with a mean half-value of 5.5 minutes. If this data should
also apply to mice, then there may have been an undetected faster ex-
cretion component to the earlier excretion study. The Dyna camera \(^{(R)}\) has
insufficient resolution to give accurate renogram curves for mice.

8. Distribution Study in Mice of Technetium -99m Labelled Malonic Acid:

Tissue distribution was done for mice according to the pro-
cedure previously described. The results of the distribution study,
measured as "percentage of total activity per organ vs time", are listed
in Table 13.
The organs with "percentage activity per organ" of less than 5 percent are
depicted in Figure 7 while those organs with a higher percentage of the
total activity are shown on Figure 8.
The "percentage activity per organ" for the brain and spleen
stayed almost constant over the 24 hour study period. The complex did
not show any marked affinity for these organs. The lungs showed a peak
activity at 3 hours with 3.47 ± 1.68 percent of the injected activity
present at this time. The activity in the brain, spleen and lungs was
presumably due to whatever blood which was present in the organ at
the time of sacrifice. The organ with the greatest accumulation of the
complex appeared to be the liver, followed in descending order by the
kidneys, large and small bowels, lungs, stomach, spleen and brain. The
liver showed peak activity relative to the whole body at 6 hours post-
injection, although this is not significant as greater than 90 percent
TABLE 13:

Percentage of the Whole Body Activity residing in the Lungs, Spleen, Brain, Liver, Kidneys, Large and Small Bowels and Stomach of Mice

<table>
<thead>
<tr>
<th>TIME (Hours)</th>
<th>LUNGS</th>
<th>SPLEEN</th>
<th>BRAIN</th>
<th>LIVER</th>
<th>Kidneys</th>
<th>L &amp; S BOWELS</th>
<th>STOMACH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>7.20±1.5%</td>
<td>0.14±0.10%</td>
<td>0.92±0.41%</td>
<td>8.94±5.21%</td>
<td>2.04±1.12%</td>
<td>1.55±2.42%</td>
<td>0.21±0.18%</td>
</tr>
<tr>
<td>0.083</td>
<td>2.38±0.73</td>
<td>0.39±0.16%</td>
<td>0.24±0.07</td>
<td>6.30±1.56</td>
<td>10.30±2.37</td>
<td>7.49±1.46</td>
<td>3.11±3.22</td>
</tr>
<tr>
<td>1.00</td>
<td>2.91±1.66</td>
<td>0.49±0.15</td>
<td>0.20±0.04</td>
<td>11.81±2.51</td>
<td>7.84±0.74</td>
<td>6.70±1.14</td>
<td>1.54±0.35</td>
</tr>
<tr>
<td>3.00</td>
<td>3.47±1.68</td>
<td>0.69±0.08</td>
<td>0.25±0.07</td>
<td>13.36±1.32</td>
<td>9.44±1.44</td>
<td>7.63±2.04</td>
<td>2.50±0.93</td>
</tr>
<tr>
<td>6.00</td>
<td>2.56±0.71</td>
<td>0.78±0.29</td>
<td>0.41±0.11</td>
<td>21.72±8.09</td>
<td>9.86±1.19</td>
<td>5.86±0.79</td>
<td>1.97±0.78</td>
</tr>
<tr>
<td>12.00</td>
<td>2.05±0.84</td>
<td>0.64±0.32</td>
<td>0.29±0.13</td>
<td>15.94±2.52</td>
<td>11.91±3.80</td>
<td>5.64±1.48</td>
<td>2.25±1.42</td>
</tr>
<tr>
<td>24.00</td>
<td>1.53±0.34</td>
<td>0.82±0.37</td>
<td>0.34±0.13</td>
<td>14.62±5.57</td>
<td>7.82±153</td>
<td>4.22±0.96</td>
<td>2.35±1.10</td>
</tr>
</tbody>
</table>

*Mean values for five mice ± standard deviation*
FIGURE 7:

PERCENT OF TOTAL BODY ACTIVITY RESIDING IN THE LUNGS, SPLEEN AND BRAIN OVER A 24 HOUR PERIOD

Lungs
Spleen
Brain

Percent of Injected Dose

Time (hours)
FIGURE 8:
PERCENT OF TOTAL BODY ACTIVITY RESIDING IN THE LIVER, KIDNEYS, 
L & S BOWELS, & STOMACH OF MICE
OVER A 24 HOUR PERIOD

Percent of Injected Dose

Liver
Kidneys
Large and Small Bowel
Stomach

Time (hours)
TABLE 9:
PERCENTAGE OF TOTAL BODY ACTIVITY PER GRAM OF TISSUE (L & S Bowels, and Brain) IN MICE OVER A 24 HOUR PERIOD

![Graph showing the percentage of total body activity per gram of tissue over time for large and small bowel and brain. The graph includes data points for each tissue type at various time intervals (1 to 24 hours).]
of the injected dose has already left the mouse by this time. The kidneys showed a fairly constant level of activity throughout the study with high values at 0.083 hours (10.30 ± 2.37 percent) and at 12 hours (11.91 ± 3.80 percent). The large and small bowels showed slightly decreasing levels of activity, from 7.49 ± 1.46 percent at 0.083 hours to 4.22 ± 0.96 percent at 24 hours, through the duration of the study. This would add support to our findings that negligible amounts of activity were eliminated via the feces in the time period measured.

In order to determine relative tissue accumulation of activity, the percentage of the activity in each organ was expressed as a "percentage of the total activity per gram of tissue". The exception was the blood activity which was expressed as "percentage of the total activity per ml. of blood". The data is listed in Table 14 and on Figures: 9, 10, 11, 12.

Comparing percentage activity per gram (or ml.), of the organs considered, the blood and the kidneys showed the highest levels of activity. The similar concentrations of activity shown by the kidneys and the blood indicates that the complex is largely retained in the blood, with the kidneys acting as the excretion route rather than as an organ for which the complex has specific affinity. The lungs, liver and stomach are all comparable in activity concentrations with peak values being observed at 6 hours post-injection. The spleen shows a sharp increase in activity at 24 hours post-injection, with a value of 7.04 ± 3.29 percent per gram of tissue. This rise is probably due to the spleen's slower equilibrium with the remainder of the blood pool. The carcass and muscle levels are consistently low. This is a desirable characteristic as they make up the background level of activity
TABLE 14:

Percentage of the Whole Body Activity per Gram of Tissue (Large and Small Bowels, Brain, Spleen, Carcass Muscle, Lungs, Stomach, Liver, Kidneys, Blood and Bone) in Mice over a 24 Hour Period

<table>
<thead>
<tr>
<th>TIME (HOURS)</th>
<th>LARGE &amp; SMALL BOWELS</th>
<th>BRAIN</th>
<th>SPLEEN</th>
<th>CARCASS</th>
<th>MUSCLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>0.66±1.14</td>
<td>2.51±1.04</td>
<td>0.93±0.77</td>
<td>1.63±0.60</td>
<td>1.18±0.53</td>
</tr>
<tr>
<td>0.083</td>
<td>2.40±0.48</td>
<td>0.64±0.16</td>
<td>2.33±0.48</td>
<td>3.24±0.89</td>
<td>2.69±0.64</td>
</tr>
<tr>
<td>1.00</td>
<td>3.13±0.75</td>
<td>0.46±0.09</td>
<td>3.05±1.87</td>
<td>2.25±0.40</td>
<td>1.10±0.29</td>
</tr>
<tr>
<td>3.00</td>
<td>3.04±0.37</td>
<td>0.62±0.15</td>
<td>4.26±1.93</td>
<td>1.82±0.25</td>
<td>1.27±0.41</td>
</tr>
<tr>
<td>6.00</td>
<td>3.10±0.68</td>
<td>1.03±0.35</td>
<td>4.17±0.66</td>
<td>2.46±0.26</td>
<td>1.01±0.16</td>
</tr>
<tr>
<td>12.00</td>
<td>2.16±0.47</td>
<td>0.65±0.28</td>
<td>3.58±1.33</td>
<td>1.84±0.25</td>
<td>0.76±0.18</td>
</tr>
<tr>
<td>24.00</td>
<td>2.28±0.60</td>
<td>0.84±0.29</td>
<td>7.04±3.29</td>
<td>2.49±0.26</td>
<td>1.08±0.15</td>
</tr>
</tbody>
</table>

............Continued

\[86\]
### TABLE 14: Time (Hours)

<table>
<thead>
<tr>
<th>TIME (HOURS)</th>
<th>LUNGS</th>
<th>STOMACH</th>
<th>LIVER</th>
<th>KIDNEYS</th>
<th>BLOOD</th>
<th>BONE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>38.74±6.77</td>
<td>0.89±1.12</td>
<td>4.43±2.21</td>
<td>4.78±2.27</td>
<td>53.54±12.68</td>
<td>1.43±0.61</td>
</tr>
<tr>
<td>0.083</td>
<td>9.09±1.12</td>
<td>5.96±0.85</td>
<td>3.45±0.60</td>
<td>25.85±3.71</td>
<td>14.64±2.79</td>
<td>6.32±1.69</td>
</tr>
<tr>
<td>1.00</td>
<td>9.78±7.43</td>
<td>6.09±0.92</td>
<td>7.03±1.92</td>
<td>17.83±3.90</td>
<td>22.65±8.19</td>
<td>8.55±2.21</td>
</tr>
<tr>
<td>3.00</td>
<td>10.67±2.42</td>
<td>9.34±4.77</td>
<td>6.89±1.16</td>
<td>24.68±4.78</td>
<td>22.08±4.66</td>
<td>8.30±3.37</td>
</tr>
<tr>
<td>6.00</td>
<td>8.84±1.92</td>
<td>7.18±2.98</td>
<td>12.00±4.52</td>
<td>21.38±2.84</td>
<td>26.30±3.36</td>
<td>18.62±3.46</td>
</tr>
<tr>
<td>12.00</td>
<td>6.12±1.55</td>
<td>6.29±2.79</td>
<td>7.58±1.40</td>
<td>20.52±5.23</td>
<td>19.72±6.17</td>
<td>7.68±1.91</td>
</tr>
<tr>
<td>24.00</td>
<td>7.98±2.89</td>
<td>10.54±6.81</td>
<td>9.28±2.72</td>
<td>21.81±4.93</td>
<td>27.69±13.37</td>
<td>28.68±6.74</td>
</tr>
</tbody>
</table>

\(a\) Mean Values From Five Mice ± Standard Deviation
FIGURE 10: 
PERCENTAGE OF TOTAL BODY ACTIVITY PER GRAM OF TISSUE (SPLEEN, CARCASS, MUSCLE) IN MICE OVER A 24 HOUR PERIOD
Percent of Injected Dose
Per Gram of Tissue

Liver
Stomach
Lungs

Liver (in mice over a 24-hour period) Percentage of total body activity per gram of tissue (lungs, stomach).

FIGURE II:
Blood, Bone, in mice over a 24-hour period.

Percentage of total body activity per gram of tissue (kidneys).

FIGURE 12:
for the organs of interest.

In order to compare target-to-background activity concentration ratios of the "percentage total activity per gram" were calculated for the kidney and for various organs adjacent to, or overlying, the kidney. The data is listed in Table 15 and depicted in Figure 13.

**TABLE 15:**

*Ratio of Percentage Total Body Activity per Gram of Tissue in Mice of the Kidney Compared to the Large and Small Bowels, Liver and Blood over a 24 Hour Period*

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Kidney/L&amp;S Bowels</th>
<th>Kidney/Liver</th>
<th>Kidney/Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>7.24</td>
<td>1.08</td>
<td>0.09</td>
</tr>
<tr>
<td>0.083</td>
<td>11.19</td>
<td>7.49</td>
<td>1.77</td>
</tr>
<tr>
<td>1.00</td>
<td>5.70</td>
<td>2.54</td>
<td>0.79</td>
</tr>
<tr>
<td>3.00</td>
<td>8.12</td>
<td>3.58</td>
<td>1.12</td>
</tr>
<tr>
<td>6.00</td>
<td>8.13</td>
<td>1.78</td>
<td>0.81</td>
</tr>
<tr>
<td>12.00</td>
<td>10.01</td>
<td>2.71</td>
<td>1.04</td>
</tr>
<tr>
<td>24.00</td>
<td>9.57</td>
<td>2.35</td>
<td>0.79</td>
</tr>
</tbody>
</table>

*(Ratios calculated from mean values of the distribution data)*

Figure 13 shows the relative constancy of the kidney/blood ratio, the mean ratio being 0.91 percent over the 24 hour period. At 0.083 hours post-injection, the liver, an organ which interfered with the right kidney image for some of the other acids studied showed the least uptake relative to the kidney, with a kidney/liver ratio of 7.5:1. At this time period the large and small bowels showed the least uptake relative to the kidneys with a ratio of 11.2:1 (kidney/bowels). As the gut overlies both the kidneys, any large amount of activity in these organs would obscure the kidney images.

From an examination of Figure 13 it is apparent that the optimum time to scan a mouse would be 0.083 hr. post-injection as the kid-
FIGURE 13:
RATIO OF PERCENTAGE TOTAL BODY ACTIVITY PER GRAM OF TISSUE
IN MICE OF THE KIDNEY COMPARED TO THE L&S BOWELS,
LIVER AND BLOOD OVER A 24 HOUR PERIOD
ney to background ratio is then at its highest.

9. Distribution studies performed on the Dyna camera(R):

Radioisotopic scintiscans were done using the Dyna camera(R) to give non-destructive distribution studies of rabbits which could be compared qualitatively with the results obtained using mice. The first distribution series is given on Figures: 14 and 15 in the form of a series of pictures taken sequentially to follow the flow of activity within the animal. The images depicted on Figures 14 and 15 were recorded for 5 seconds each so that the study extends from t=0 to t=180 seconds. The animal is viewed in the posterior projection.

The first exposure in Figure 14 shows some initial blood pool activity due to the combined heart and lung blood content. Five seconds later, the heart area is more pronounced and the kidneys are just visible. The great arteries are also apparent. Over the next 75 seconds, the kidney images become more pronounced, while the activity in the heart and lung region diminishes. The overall background activity rises slightly during the first 75 seconds. At approximately 85 seconds into the series, bladder activity begins to be noticeable. The onset of activity in the bladder indicates a renal transit time of approximately 80 seconds. After 120 seconds, the bladder is the predominant area of activity and remains so for the remainder of the study.

A series of Polaroid(R) colour-coded static images were taken from the Dyna camera(R) at 10, 15, 20 and 120 minutes post-injection. The kidneys in these views show maximum activity at 10 minutes after the time of injection, while the bladder image is still relatively small. These images are depicted in Figure 16.

The body background remains constant for the series of pictures
FIGURE 14A:

DYNAMIC DISTRIBUTION OF Tc-99m-MALONATE IN RABBITS
EXPOSURES TAKEN AT 5 SECOND INTERVALS

1.0 cm. = 10.0 cm.
FIGURE 14B:
DYNAMIC DISTRIBUTION OF TC-99M-MALONATE IN RABBITS
EXPOSURES TAKEN AT 5 SECOND INTERVALS

1.0 cm. = 10.0 cm.
FIGURE 15A:
Static Distribution of Tc-99m-Malonate in Rabbits
Images taken at 10 and 15 Minutes

T = 10 MINUTES

T = 15 MINUTES
FIGURE 15b:
Static Distribution of Tc-99m-Malonate in Rabbits
Images taken at 20 and 120 Minutes

\[ T = 20 \text{ Minutes} \]

\[ T = 120 \text{ Minutes} \]
while the heart-lung activity decreases gradually over the 2 hour period. These static views show good visualization of the kidneys and support the mouse data, that the optimum time for scanning is within the first 10 minutes after injection.

10. Dosimetry:

The radiation dosimetry for Tc-99m labelled malonic acid was computed to compare it with other technetium-99m labelled compounds now in use. The radiation doses were calculated for the whole body, kidneys, liver skeleton and bladder. The bladder dose was computed twice first assuming catheterization, and secondly assuming that the bladder retained all the activity excreted over a 6 hour period. Certain other assumptions were also made in the dose calculations, as follows:

a) The distribution and excretion data as determined in mice apply to humans as well.

b) A biphasic excretion curve exists with $t_1^b = 0.78 \pm 0.42$ hours and $t_2^b = 3.27 \pm 0.15$ hours.

c) The kidneys and whole body excretion complies with the biphasic curve in (2).

d) The liver and skeleton follow the slow excretion curve component of (2).

The absorbed doses were calculated according to the general equation as proposed by Loevinger and Berman (177).

$$\bar{D}(r_1 + r_2) = \sum_{\Delta \phi} A_2 \Delta \phi_1 (r_1 + r_2) \text{ rad}$$

where:

$$\bar{D}(r_1 + r_2) = \text{The dose to the region from a second region where } r_1 \text{ is the target and } r_2 \text{ is the source region (rads).}$$
Equilibrium dose constant for radiation of type \( i = 1, 2, 3, 4 \), with a fractional frequency \( r_i \) per disintegration and a mean energy \( \bar{E}_i \) in MeV.

\[
D_i = 2.13 n_i \frac{E_i}{(\text{g-rads})(\text{uCi-hr})}
\]

\( \phi_i(r_1 + r_2) \) = The absorbed dose fraction in a region \( r_1 \). Values for \( \phi_i \) were obtained from Snyer et al (178).

\( C_2 \) = Cumulated uniform concentration of activity in region 2, (uCi - hr/g).

For the more common case where \( r_1 = r = \text{volume} \), the equation can be written as:

\[
\bar{D} (v - v) = \bar{C}_v \sum \Delta_i \phi_i (v - v) \text{ rad}
\]

where \( \Delta_i = \frac{C_v}{M_v} \)

\( \bar{C}_v \) = the cumulative activity in the volume considered

\( M_v \) = mass of the volume considered.

For technetium 99m, values of \( \Delta_i \) were obtained from the decay scheme and results outlined by Dilman (179). The \( \Delta_i \) and \( \phi_i \) values for the organs under consideration are shown in Table 16.

a) Theoretical Absorbed Dose from Tc -99m - Malonic Acid

Technetium decays according to the scheme depicted (179):

\[
\begin{array}{ccccc}
99_{\text{Tc}}^{43} & 6.0 \text{ hr.} & t_{\text{h}} & .1427 \text{ MeV} & \text{Photon} & \%\text{Disintegration} & \text{Energy(MeV)} \\
& & & & 1 & 98.6 & 0.0022 \\
& & & & 2 & 98.6 & 0.1405 \\
& & & & 3 & 1.4 & 0.1427 \\
\end{array}
\]

i) Whole Body Dosimetry

Values for \( \Delta_i \) and \( \phi_i \) for various emissions from Tc -99m of uniform distribution in the whole body (based upon the 70 kg. standard man).
TABLE 16:
Absorbed Dose Fractions for the Whole Body

<table>
<thead>
<tr>
<th>$E_i$ (MeV)</th>
<th>$n_i$</th>
<th>$\Delta_i$ (g-rad</th>
<th>$\phi_i$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0021</td>
<td>0.00</td>
<td>0.0000</td>
<td>1.000</td>
</tr>
<tr>
<td>0.0017</td>
<td>0.986</td>
<td>0.0036</td>
<td>1.000</td>
</tr>
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<td>0.1405</td>
<td>0.883</td>
<td>0.2643</td>
<td>0.370</td>
</tr>
<tr>
<td>0.1195</td>
<td>0.0883</td>
<td>0.0225</td>
<td>0.370</td>
</tr>
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<td>0.1377</td>
<td>0.0109</td>
<td>0.0032</td>
<td>0.370</td>
</tr>
<tr>
<td>0.1401</td>
<td>0.0036</td>
<td>0.0011</td>
<td>0.370</td>
</tr>
<tr>
<td>0.1427</td>
<td>0.0003</td>
<td>0.0001</td>
<td>0.370</td>
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<td>0.0009</td>
<td>0.370</td>
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<td>0.0003</td>
<td>0.370</td>
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<td>0.0184</td>
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<td>0.933</td>
</tr>
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<td>0.0183</td>
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<td>0.0206</td>
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<tr>
<td>0.0210</td>
<td>0.0018</td>
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<td>0.892</td>
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<td>0.0010</td>
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$\Sigma \Delta_i \phi_i = 0.1131339$ g-rad/uCi-hr

The cumulative activity $\tilde{\Lambda} = Ao \left( \frac{f_1^1 + f_2^2}{\lambda_1 \lambda_2} \right)$ uCi-hr.

$Ao =$ injected activity (uCi)
$f_1 =$ fraction of the dose excreted with the $t_1^1 = 0.78$ hr. = 0.703
$f_2 =$ fraction of the dose excreted with the $t_2^2 = 3.37$ hr. = 0.297
$\lambda_1 =$ decay constant for the fast component of excretion = 0.8885 hr$^{-1}$
\( \lambda_2 \) = decay constant for the slow component of excretion  
= 0.2119 hr.\(^{-1}\)

\( \lambda \) = physical decay constant for technetium 99m  
= 0.1155 hr.\(^{-1}\)

\( \tilde{A} \) = 16135.1 uCi.-hr.

\[ \sum \Delta_i \phi_i \] (total body) = 0.1131339 g-rad/uCi.-hr.

\( m \) = 70,000 gm. (Standard man body weight)

\[ \bar{D} = \frac{\tilde{A}}{m} (\sum \Delta_i \phi_i) \]

\( \bar{D} \) = 0.026 rads

= 26 mrads per 10 mCi. of Tc -99m activity

ii) Kidney Dosimetry

Similarly values obtained for the kidneys are outlined in Table 17.

\( A_{\text{Kidney}} \) = 15,100 uCi. - hr.

\( A_{\text{Total Body-Kidney}} \) = 16,135.2 ' '  
\( A_{\text{Liver-Kidney}} \) = 1,218.7 ' '  
\( A_{\text{Skeleton-Kidney}} \) = 1.849.7 ' '  
\( A_{\text{Bladder-Kidney}} \) = 79,653.7 ' '  

\( D \) = 158.9 mrads/10 mCi. with bladder component  

or \( \bar{D} \) = 138.2 mrads/10 mCi. assuming catheterization

iii) Liver Dosimetry

Refer to Table 18

\( A_{\text{Liver}} \) = 1,218.7 uCi. - hr.

\( A_{\text{Whole Body-Liver}} \) = 16,135.2 ' '  
\( A_{\text{Kidneys-Liver}} \) = 1,190.8 ' '  
\( A_{\text{Skeleton-Liver}} \) = 1,849.7 ' '  
\( A_{\text{Bladder-Liver}} \) = 79,653.7 ' '  

\[ \bar{D} = 103.4 \text{ mrads/10 mCi. with bladder component} \]

or \[ \bar{D} = 43.4 \text{ mrads/10 mCi. assuming catheterization} \]

iv) Bone Dosimetry

Refer to Table 19.

\[ A_{\text{Skeleton}} = 1,849.7 \text{ uCi. - hr.} \]
\[ A_{\text{Whole Body-Skeleton}} = 16,135.2 \]
\[ A_{\text{Kidneys-Skeleton}} = 1,190.8 \]
\[ A_{\text{Liver-Skeleton}} = 1,218.7 \]
\[ A_{\text{Bladder-Skeleton}} = 79,653.7 \]

\[ \bar{D} = 2064.1 \text{ mrads/10 mCi. with bladder component} \]

or \[ \bar{D} = 102.7 \text{ mrads/10 mCi. assuming catheterization} \]

v) Bladder Dosimetry

Refer to Table 20.

\[ A_{\text{Bladder}} = 79,653.7 \text{ uCi. - hr.} \]
\[ A_{\text{Whole Body-Bladder}} = 16,135.2 \]
\[ A_{\text{Kidneys-Bladder}} = 1,190.8 \]
\[ A_{\text{Liver-Bladder}} = 1,218.7 \]
\[ A_{\text{Skeleton-Bladder}} = 1,849.7 \]

\[ \bar{D} = 11.9 \text{ rads/10 mCi. with bladder component} \]

or \[ \bar{D} = 1.5 \text{ mrads/10 mCi. assuming catheterization} \]

The doses calculated with and without catheterization show the importance of frequent voiding or bladder catheterization to avoid exposing the patient to unnecessary radiation dosage.

The radiation doses from Tc-99m malonate complex appear to be similar to doses from other technetium-99m labelled compounds (Table 5). In general, technetium-99m gives lower radiation doses than do other nuclides.
TABLE 17:

Absorbed Dose Fractions for the Kidneys

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<tr>
<th>$E_i$ (MeV)</th>
<th>$n_i$</th>
<th>$\Delta_i$ (g-rad)</th>
<th>$\phi_1$</th>
<th>$\phi_2$</th>
<th>$\phi_3$</th>
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<td>0.00036</td>
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$\Sigma_{i} \phi_i = 0.000556 \quad 0.0263602 \quad 0.0012923 \quad 0.0002911 \quad 0.0001324$

1. Contribution from whole body to kidneys.
2. Contribution from the kidneys.
3. Contribution from the liver to the kidneys.
4. Contribution from skeleton to the kidneys.
5. Contribution from the bladder to the kidneys.


<table>
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<th>$\Delta (\text{g-rad})_1$ (uCi-hr)</th>
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<th>$\phi^2_1$</th>
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$\Sigma \Delta \phi_i = 0.0570025$ 

1. Contribution from the liver.  
2. Contribution from the body to the liver.  
3. Contribution from the kidney to the liver.  
4. Contribution from the skeleton to the liver.  
5. Contribution from the bladder to the liver.
**TABLE 19:**

Absorbed Dose Fractions for the Skeleton

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<tr>
<th>$E_i$ (MeV)</th>
<th>$n_i$</th>
<th>$A_i$ (g-rad)</th>
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<th>$\phi_3$</th>
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</table>

$\sum_i A_i \phi_i = 0.0593818 \quad 0.0245145 \quad 0.0196320 \quad 0.0147566 \quad 0.0125337$

1. Contribution from the skeleton.
2. Contribution from the whole body to the skeleton.
3. Contribution from the kidneys to the skeleton.
4. Contribution from the liver to the skeleton.
5. Contribution from the bladder to the skeleton.
## TABLE 20:

### Absorbed Dose Fractions for the Bladder

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<tr>
<th>$E_i$(MeV)</th>
<th>$n_i$</th>
<th>$\Delta L_i$(g-rad)</th>
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</tbody>
</table>

$$\sum \Delta L_i \phi_i = \frac{0.0762563}{0.0008287} = \frac{0.0001690}{0.0001144} = 0.0028331$$

1. Contribution from the bladder.
2. Contribution from the whole body to the bladder.
3. Contribution from the kidneys to the bladder.
4. Contribution from the skeleton to the bladder.
5. Contribution from the liver to the bladder.
D. SUMMARY & CONCLUSIONS
D. SUMMARY AND CONCLUSIONS:

1. Oxalic, malonic, succinic, glutaric, adipic and maleic acid were labelled in aqueous solution with technetium -99m. The relative radiochemical purities obtained are given.

2. A stable, radiopharmaceutically-acceptable kit using malonic acid was prepared.

3. The LD$_{50}$ of a stannous-malonate complex was investigated by intravenous injection into mice and found to be 800 (734-872) mg./kg.

4. The excretion of the Tc -99m-malonate complex was found to be entirely via the kidneys with a two-part excretion curve in mice. A fast component, measured at 46.8 ± 25.8 minutes, consisted of 70.3 percent of the administered dose and a slow component measured at 196.4 ± 8.8 minutes accounted for 29.7 percent of the administered dose. It was felt that the fast component reflected the clearance of the complex retained in the blood stream alone, while the slow component was a measure of the extravascular clearance of the complex.

5. Twenty-four hours after injection, 99.6 ± 0.1 percent of the activity had left the body of the mouse, not accounting for physical decay of the technetium -99m. Chromatography of the excreted urine revealed that 76.8 ± 9.3 percent of the injected dose was still in the form of the complex after two hours of collecting the urine. No measurable amount of activity could be determined in the feces after six hours.

6. Tissue distribution studies in mice revealed that no organ showed a high level of the injected complex. The kidney and blood showed the highest accumulation of all the organs. The other organs in-
vestigated were the brain, lungs, stomach, large and small bowel, liver, spleen, and bone. From consideration of the distribution pattern with time, and the scintiscans done on rabbits, it was determined that an optimum scan would be obtained at 5 to 10 minutes after injection of the labelled complex.

7. Rabbit renograms were obtained by monitoring the flow pattern of labelled complex as it passed through the kidney. Analysis of the renogram showed a fast component of excretion of 5.5 minutes.

8. Theoretical radiation dose was calculated based upon the excretion and distribution data obtained for mice and extrapolated to humans. The radiation dose was calculated at 26 mrads/10mCi. for the whole body, 159 mrads/10mCi. for the kidneys, 103 mrads/10mCi. for the liver, 2064 mrads/10mCi. for the skeleton and 11.9 rads/10mCi. for the bladder.

This investigation has attempted to evaluate the use of low molecular weight dibasic acids for use as technetium carrier molecules. The acids are capable of complexing technetium -99m to varying degrees and preliminary scans showed that malonic acids had the best localization in the kidney.

Examination of the malonate complex showed it to be relatively non-toxic and to have good stability in vitro when labelled with technetium -99m. The excretion study showed it to clear quickly via the kidneys, with minimal breakdown of the complex.

Enough activity localized in the kidneys to give good scintiscans at any time up to 6 hours post-injection, although optimum scans were obtained at 5-10 minutes post-injection. The radiation dose calculated from the distribution and excretion study showed the dose to
be comparable to other technetium-labelled complexes currently available.
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