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## Nuclear Medicine

UNIVERSITY... The University of Alberta .....DEGREE FOR WHICH THESIS WAS PRESENTED...*Ph.D. in Biomechanics*

YEAR THIS DEGREE GRANTED.....1971.....

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THE UNIVERSITY OF ALBERTA

The Potential Use of Zn-EDTA in Nuclear Medicine

by

 Donald Milburn Lyster

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH  
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE  
OF DOCTOR OF PHILOSOPHY

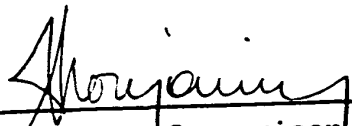
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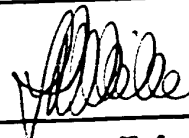
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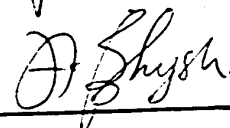
UNIVERSITY OF ALBERTA  
FACULTY OF GRADUATE STUDIES  
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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "The Potential Use of Zn-EDTA in Nuclear Medicine", submitted by Donald Milburn Lyster in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

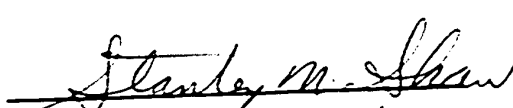
  
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To Carol, Tracy and Lauri,  
who make everything worthwhile

## ABSTRACT

The possibility of using Zn-EDTA in nuclear medicine has been investigated. It was found that zinc-63 could be produced by the reaction  $^{64}\text{Zn}(n,2n)^{63}\text{Zn}$  after bombardment with 14 Mev neutrons, using a Cockroft-Walton Accelerator. The bombardment of Zn-EDTA directly did not appear to alter the chemical composition of the chelate.

Toxicity studies were performed on mice after the intravenous injection of  $\text{ZnCl}_2$ ,  $\text{ZnNa}_2\text{EDTA}$  and  $\text{ZnNa}_2\text{EDTA}$  plus  $\text{CaNa}_2\text{EDTA}$ . The  $\text{LD}_{50}$  for  $\text{ZnCl}_2$  was found to be 9.3(8.2 to 10.6) mg/Kg, the  $\text{LD}_{50}$  for  $\text{ZnNa}_2\text{EDTA}$  was found to be 1.32 (1.02 to 1.70) g/Kg, and the  $\text{LD}_{50}$  for  $\text{ZnNa}_2\text{EDTA}$  plus  $\text{CaNa}_2\text{EDTA}$  was found to be 2.3(1.91 to 2.77) g/Kg.

The excretion and biological turnover of injected  $^{65}\text{Zn-EDTA}$  was examined in mice and the elimination of activity was found to occur in three components. There is a fast component with a half-life of 10.4 minutes consisting of about 86.4 per cent of the administered dose, a medium component with a half-life of 185.1 minutes consisting of about 13.8 per cent of the administered dose and a slow component with a half-life of 12624.5 minutes consisting of about 3.7 per cent of the administered dose. It was also found that after administration, 93.7 per cent of the injected  $^{65}\text{Zn-EDTA}$  was excreted in the urine within two days and 3.1 per cent in the feces. The fast and medium compartments consisted of undissociated  $^{65}\text{Zn-EDTA}$  and all elimination of

activity occurred in the urine within 24 hours post-injection. The slow component was probably due to zinc-65 that was no longer chelated to the EDTA and excretion occurred via the feces.

Tissue distribution of injected  $^{65}\text{Zn-EDTA}$  in mice was analyzed and no organ appeared to concentrate the chelate for any prolonged periods of time. The muscle, blood, kidney, bone, liver, lung and pancreas were found to be the organs of major uptake of the zinc-chelate, but much of the activity in these organs was thought to be due to the blood. The kidney was the only organ which appeared to accumulate significant activity during the period when the majority of the  $^{65}\text{Zn-EDTA}$  was eliminated from the body.

Elimination of the  $^{65}\text{Zn-EDTA}$  was not as rapid from the dog and a renogram study revealed a biological half-life of 38 minutes in the kidney based on the "drainage segment" of the renogram. Elimination of activity from the blood occurred with a fast component having a half-life of 1.7 minutes and a slow component having a half-life of 36 minutes. The decrease in activity from the urine gave a half-life of 17.9 minutes.

It was found that satisfactory scans could be obtained by detection of  $^{63}\text{Zn-EDTA}$  using a Pho/Gamma III camera or an Ohio Nuclear dual probe rectilinear scanner.

Absorbed dose calculations were computed for the kidney and whole-body.  $^{65}\text{Zn-EDTA}$  gave a relative absorbed dose of 8.36 mrad/10  $\mu\text{Ci}$  for the kidney and 1.06 mrad/10  $\mu\text{Ci}$

for the whole-body.  $^{63}\text{Zn-EDTA}$  gave a relative absorbed dose of 76.74 mrad/10  $\mu\text{Ci}$  for the kidney and 0.34 mrad/10  $\mu\text{Ci}$  for the whole-body.

## ACKNOWLEDGEMENTS

I would like to express my gratitude to Dr. A.A. Noujaim who has guided me throughout this project. During this period he has not only been a source of encouragement and guidance but also a friend.

I should also like to express my sincerest thanks to Mr. Chris Ediss to whom I shall always be indebted for help given throughout this investigation.

The members of my committee have always been extremely willing to assist me in any way and I would like to thank them at this time.

The assistance of Dr. P. Heslip, Dr. J. Scrimger, Dr. S.R. Usiskin and Mrs. O. Tomasky is also appreciated.

The financial assistance in the form of a Medical Research Council of Canada Scholarship and the Pfizer Research Scholarship is gratefully acknowledged.



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## **I. INTRODUCTION**



The use of radioactive pharmaceuticals for various function or diagnostic tests or in vivo "visualization" of body organs has greatly increased in the last few years (1). One of the reasons for this rapid increase is the availability of short-lived isotopes from isotope generators or "cows". After the elution of these short-lived isotopes, various radio-pharmaceuticals can be prepared and these preparations are then injected into the patient to evaluate various parameters. However, due to the fact that the isotope must be used rapidly after elution, little time is available to test for sterility or pyrogenicity except in retrospect. Although this problem does not appear to be significant at the present time due to meticulous care in compound preparation (2, 3, 4), the possibility of injecting non-sterile or pyrogen-containing compounds must be considered. Such concern would not be necessary if the pharmaceutical could be compounded and tested for sterility and pyrogenicity before it was injected. After preparation of the "cold" molecule, it could be bombarded with neutrons or other nuclear particles to render it radioactive, and this possibility has been considered by some investigators (5). However, tests would have to be initiated to ensure that no molecular decomposition had taken place.

Besides the requirements for sterility and pyrogenicity, a radio-pharmaceutical must satisfy other criteria and these have been outlined by Wagner and Rhodes (6), and by Charlton (7). They include the following: selection of

an isotope with suitable half-life and physical properties so that counting statistics will be adequate and the absorbed dose will be kept at a minimum; availability in a form that will exhibit the desired biological behavior and selectively localize in a desired organ; integrity of the molecule when injected in vivo; and radionuclide purity. Selection of an appropriate radioactive isotope can be made by consulting the literature. One of the purposes of this investigation was to search for a nuclide which decays by positron emission so that lower quantities of activity can be injected (8). The nuclide must also have a half-life of about one hour. The target material should be readily available and have a high cross section for 14 Mev neutrons which are generated by the Cockroft-Walton Accelerator. Zinc-63, formed from zinc-64 by the (n, 2n) reaction, appeared to fulfill these requirements. It decays by positron emission about 93 per cent of the time with a half-life of 38.4 minutes (9). It has a cross section of 167 millibarns for 14 Mev neutrons (10). However, before  $^{63}\text{Zn}$  can be deemed suitable for use in nuclear medicine, the answers must be found to many questions, such as the following:

1. Does the compound remain intact after neutron bombardment?
2. What amount of compound can be injected without harmful effects?
3. How rapidly is the injected radio-pharmaceutical

eliminated from the body?

4. Does the integrity of the injected compound remain unaltered until it is excreted?

5. Does the injected compound localize in any specific organ or organs?

6. What is the absorbed dose of the injected compound in the target organ and whole-body?

7. Can enough activity be prepared to obtain a scan?

Therefore, the following study was undertaken to explore the potential of  $^{63}\text{Zn}$  as a scanning agent.

## II. SURVEY OF THE LITERATURE

## A. ZINC

### 1. Historical

Zinc does not occur in the free state in nature, and thus was not discovered as early as the other metals. However, by accident, it was used in the making of brass and so was given the name, zinc, from the German "of unknown origin". It was first described as a metal in 1597 but it had probably been used by the Chinese much before this date (11).

It is estimated that 0.004 per cent of the earth's crust is zinc, and its chief ores are the sulfide and carbonate known as sphalerite and calamine, respectively (12). Zinc ranks twenty-fifth in order of abundance, which is greater than copper or manganese, and in the same order of magnitude as that of iron (12). There is a wide variation in the zinc content of soils (13) and it is present in practically all plants (14, 15).

Elemental zinc has an atomic weight of 65.38 and an atomic number of 30. The stable isotopes of zinc are  $^{64}\text{Zn}$  (50%),  $^{66}\text{Zn}$  (27%),  $^{67}\text{Zn}$  (4%),  $^{68}\text{Zn}$  (18%) and  $^{70}\text{Zn}$  (1%). It is a bluish-white metal with a hexagonal lattice, has a melting point of 419.4°C, a boiling point of 907°C, and a specific gravity of 7.1 (16). There are ten other isotopes of zinc ranging from zinc-60 to zinc-72, and these have half-lives varying from 1.5 minutes to 245 days (17). Of these isotopes,  $^{65}\text{Zn}$  has been the most used in biological studies. The organic compounds of zinc which were prepared

by Frankland (18) led to the first clear theory of valency (19).

## 2. Pharmacological and Toxicological Aspects of Zinc

Many studies were done to observe the effects of zinc deficiency in animals. However, many of the early experiments performed on mice were unsuccessful due to improper diets (20). Also, some experimenters neglected to take adequate steps to eliminate differences in eating patterns between zinc-deficient and control animals (21). When controlled diets were used, it was found that zinc was a necessary element for the growth and survival of the mouse. Those animals on low-zinc diets failed to grow and died with symptoms of ataxia and posterior column disease (22). Similar experiments on rats (23) showed that the zinc-deficient rats resumed their normal appearance when given adequate zinc. Shanklin et al. (24) were also able to produce a deficiency syndrome in baby pigs. Due to the rapid appearance of signs of zinc deficiency, Mills et al. (25) suggested that zinc must either have a rapid turnover or that it is located at a site where it is freely exchangeable. The same authors preferred the latter explanation. Hove et al. (26) showed that rats receiving 22 micrograms of zinc chloride per day demonstrated symptoms of deficiency while those receiving only 40 micrograms of the element appeared to be normal. This was also shown by Shanklin et al. (24) who found that a daily diet of 10 ppm or less of zinc in a special diet led to a deficiency syndrome whereas 14 ppm or

greater of zinc led to normal growth of the animal. Some of the symptoms of zinc deficiency noted by Shanklin were parakeratosis, reduced growth rate, fall in serum zinc, calcium, and alkaline-phosphatase levels, and reductions in bone size and strength. This implied that there was interference with the metabolic balance of zinc and calcium. Zinc-deficiency was shown to delay absorption of glucose from the gastrointestinal tract and to disturb protein metabolism (26). Hove et al. (27) showed that in zinc-deficient rats, the bone levels of the element were decreased while the soft tissue concentrations remained normal. Hyperkeratinization, thickening of the epidermis and intra- and intercellular edema of the skin and mucous membranes of the esophagus and mouth were observed in zinc-deficient rats (28, 29). Millar et al. (30) noticed the atrophy of the testicles and accessory sex organs. This was thought to be due to inadequate reproduction of gonadotrophins (31). Zinc-deficiency also disrupted the female reproductive process (32), and this deficiency effect was quite apparent in the offspring (33). Because parakeratosis was correlated to zinc-deficiency (34), a great deal of research was initiated to study the role of zinc in the body (35, 36, 37, 38). Hoekstra (39), while investigating parakeratosis in swine, noted a correlation between the lesions of bone and skin, and the "connective tissue diseases" in man. He suggested that further investigation in this area was needed. Prasad and Oberleas (40) have observed in people from the

Middle East, where there is a zinc deficiency, many of the same symptoms as those seen in animal experiments. However, the fetal abnormalities, impaired behavior, and decreased learning ability recorded in animals have not yet been observed in man. Since some of the above symptoms were also common in patients suffering from liver cirrhosis, the above authors suggested that the latter condition might be related to zinc-deficiency. On the other hand, it has been shown that hepatic damage led to a decreased uptake of zinc-65 in rats (41), and Vallee et al. (42) demonstrated a relationship between concentration of zinc and the severity of cirrhosis. However, Kahn et al. (43) reported an increased uptake and suggested that the low levels of zinc in the liver, reported by others, may be due to a dietary deficiency of zinc. The protective action of zinc against liver injury due to manganese and carbon tetrachloride is well known (44). Condon and Freeman (45) noted that the symptoms of zinc-deficiency were similar to those of uremia. Although they noted a decrease in plasma zinc, they concluded that it was due to redistribution rather than to total body deficiency. Trethewie (46) found that the histamine response of isolated guinea-pig jejunum was abolished in the presence of zinc sulfate or zinc chloride and suggested that this may be useful in allergic skin conditions. Studies were performed on experimental wound healing (47) and although it was found that zinc accumulated in the acute healing stage, there was low retention in the scar tissue.



The toxicity of zinc has long been a problem for industrial workers and many experiments were performed to determine the toxicity of this element in animals. Acute administration of zinc chloride to cows caused a dramatic fall in milk production and many animals died (48). They were found to have pulmonary emphysema, bleeding in the cortex and medulla of the kidneys, and liver degeneration. It is noteworthy to mention that there appears to be an interrelationship between copper and zinc metabolism (49, 50, 51). A diet containing one per cent zinc, as zinc carbonate, caused severe anemia and many animals died. However, additional feeding of copper prevented this (51). Fibrosis of the pancreas was noted after the injection of zinc oxide into cats and dogs (52, 53). Excess zinc as zinc oxide was shown to reduce the fat content of the liver and to increase fecal nitrogen, phosphorus and sulfur. The urinary nitrogen, uric acid and creatinine contents were elevated while urinary phosphorus and sulfur was decreased (54, 55, 56). Vallee (11) noted that dogs tolerated the intravenous administration of two milligrams per kilogram body weight of zinc gluconate. However, four milligrams per kilogram caused lassitude, enteritis and paresis of the hind legs with electrocardiographic changes similar to those of potassium intoxication. In humans, the symptoms of excess zinc are malaise, dizziness, tightness of the throat, emesis, colic and diarrhea (57). Hamdi (58) found increased concentrations of zinc in blood cells, whole blood, gastric

juice, and an elevated urinary zinc output in workers exposed to zinc oxide fumes in a brass foundry. After absorption, the zinc was rapidly eliminated from the plasma and some was stored in the blood cells, while the rest was eliminated via the intestinal and urinary tracts.

### 3. Absorption

The normal human zinc intake is 10-15 mg per day, most of which is excreted in the stools (59, 60). The absorption of zinc chloride by the gastro-intestinal tract was found to be variable in rats (61), and this variability was also noted by Spencer et al. (62). Spencer et al. (62) observed that the average absorption of zinc chloride in man was 50.8%, that absorption did not differ significantly during low and high calcium intake, and that increased protein intake caused decreased zinc absorption. Wasserman (63) showed that the probable site of zinc absorption was the duodenum. About forty to sixty per cent of zinc as the glycine complex was absorbed in the intestine of the rat (64). This was higher than that observed by other investigators (65, 66, 61). It has been shown in animal experiments that an antagonism exists between zinc and calcium during absorption (67, 68, 69, 70), but this could not be shown in man (62). O'Dell (71) suggested that this may have been due to the fact that the patients were fed diets low in phytate which must be present for calcium to exert its effect (72, 73, 74, 75). Huber and Gershoff (76) found that calcium decreased zinc absorption in rats fed zinc-deficient diets

only but had no effect on rats fed control diets. It had no effect on animals given zinc chloride by intraperitoneal injection, but differences noted were entirely dependent on zinc concentration. It has also been shown that absorption of zinc in animals was affected by the type of protein in the diet (77, 78, 79, 80). Spencer et al. (62) found a similar correlation in man but stated that too few patients were examined to make any sound conclusions. Chelating agents such as EDTA were shown to increase the absorption of zinc in rats (81) and turkey poults (82). It was found that the stability constants for zinc had to be from 13 to 17 to be effective (83, 84). O'Dell (71) stated that phytate must be present for EDTA to have its beneficial effect but it is not clear how EDTA acted to make zinc available for absorption. He suggested that the soluble complex was more available to the intestinal mucosa and that it exchanged the zinc ion in this region. Bohne et al. (85) found that the absorption of zinc did not increase with simultaneous parenteral administration of DTPA, but this only led to increased excretion of the absorbed fraction. Ruminants exhibited a homeostatic control mechanism for the absorption of zinc (86). In a zinc-deficient diet, a greater percentage of zinc was absorbed and less was excreted in the feces. Decreased absorption with age seemed to be determined by tissue changes and not by changes in ability to absorb. This homeostatic mechanism was also shown to be present in mice (87).

#### 4. Distribution

It is felt that a complete review on the distribution of zinc is beyond the purpose of this investigation. The literature contains many excellent articles on zinc distribution under various physiological conditions. The following articles by Sheline et al. (88), Gilbert and Taylor (89), Wakeley et al. (90), Banks et al. (91), Miller (86), Parisi and Vallee (92), Prasad et al. (93), Stand et al. (94), Vallee (11) and Pekas (95) are of special mention. For the present discussion, an outline of the various organs and enzymes in the body which require zinc will be given.

##### a. Zinc Metalloenzymes

Vallee (11) and Parisi and Vallee (92) have considered the various zinc metalloenzymes and their role in the body. Some of those which have been characterized include carbonic anhydrase of bovine erythrocytes, alcohol dehydrogenase of yeast and of equine liver, glutamic dehydrogenase of bovine liver, lactic dehydrogenase of rabbit skeletal muscle, carboxypeptidase, and alkaline phosphatase. There are many other zinc metalloenzymes which have been identified and it is apparent that the element plays an important role in carbohydrate, lipid and protein metabolism (96). Prasad et al. (93) found that a zinc-deficient diet in pigs caused a decrease in various zinc-dependent enzymes. They also found that bone and pancreas were very sensitive to this deficiency and this may be the reason for retarded growth. Reinhold and Kfoury (97) also showed alterations in enzyme behavior in zinc-

depleted rats.

b. Male Genital Tract

That zinc is important to the male genital tract is evident by the fact that a deficiency leads to degeneration of the testes, hypoplasia of the coagulating glands, seminal vesicles and prostate, and a decrease in the number of sperms in the epididymes (30). The uptake of zinc in the prostate was extensively investigated using  $^{65}\text{Zn}$  and it was apparent that zinc was concentrated in this organ (98, 99, 100, 90). This has led some investigators to attempt a prostate scan with mixed success. Johnston et al. (101) were successful in scanning with  $^{65}\text{Zn Cl}_2$  and  $^{69\text{m}}\text{Zn Cl}_2$  and Lorber et al. (102) also obtained prostate scans using  $^{69\text{m}}\text{Zn Cl}_2$ . Verrilli et al. (103), however, claim that it is impossible to distinguish between the prostate and surrounding tissue. The possibility of using  $^{65}\text{Zn}$  for therapy of the prostate seems unlikely as Wakeley et al. (90) calculated that a dose of 80 rads to the prostate would yield approximately 50 rads to the whole body. In vitro studies by Saito et al. (104) led to the conclusion that the zinc content of epididymal sperm is a linear function of zinc concentration in the medium and that zinc in the canine or rat spermatozoa is bound in different ways. Although the male genital tract has an increased zinc content there is no apparent functional explanation for this. There is, however, much speculation as to its role (11).

### c. Pancreas

It has been known for years that there is a relationship between zinc and insulin (105, 106) and that the pancreas contains sufficient zinc to allow crystallization of insulin (107). Lowry et al. (108) showed that the zinc uptake by the pancreas of a diabetic rat was reduced. In animals which were laparotomized or whose pancreas was damaged by desoxycholate-induced pancreatitis (109), or which were exposed to ionizing radiation (110), a decrease of zinc in the pancreas was exhibited. Huber and Gershoff (76) found that when high-zinc diets were given to rats, the largest increase noted was in the pancreas. However, it has not been proven that a zinc-insulin complex must be formed in vivo before insulin becomes active (11). Although there are numerous theories in the literature, the role of zinc in the action of insulin has not been determined (11). Doubt concerning the importance of a zinc and insulin relationship was raised by Sherrill and Wick (111) who injected enough  $^{65}\text{Zn}$  to inactivate the mechanism responsible for the production of insulin, but were unable to detect any changes in blood sugar.

Because of the apparent localization of zinc in the pancreas, Meschan et al. (112) were moved to utilize this for pancreas scanning. Their scanning attempts were unsuccessful but they did succeed in obtaining a biphasic curve which represents organ concentrations in the liver and pancreas. However, this curve was not shown to be clinically useful.

Czerniak et al. (113) also expressed the possibility of using  $^{69m}\text{Zn}$  or  $^{65}\text{Zn}$  for in vivo diagnosis of pancreas disorders due to the rapidity with which it reached its maximum turnover rate after injection.

d. Blood

Extensive studies concerning the concentration of zinc in blood were reported (12, 42, 114, 115). Whole-blood zinc seems to be distributed as follows: 12% in serum, 3% in leucocytes, and 85% in the erythrocytes (11). Craig and Siegel (116) noted that zinc in the red blood cells was protein-bound. Vallee (11) also noted that the zinc levels in serum and plasma were equivalent, that there were no seasonal or diurnal variations, and that there was no difference between the sexes.

Vikbladh (115) reported that serum zinc existed in at least two fractions, 34% as firmly bound and 66% as loosely bound. Craig and Siegel (116) further stated that plasma zinc became protein-bound within three hours following its injection and remained non-dialyzable for up to forty days. Okunewick (117) showed that 90% of the zinc was bound to protein within the first three minutes after injection. English (118) also stated there was a component in blood plasma which accounted for 88% of the zinc-binding capacity and the complex had a half-life of 45 minutes. Gilbert and Taylor (89) showed that the clearance of zinc from plasma followed four exponential terms and these authors postulated the existence of a large soft tissue zinc pool that was

freely exchanging with the plasma zinc. Feaster et al.

(66) demonstrated that zinc readily entered the erythrocyte with no apparent increase in carbonic anhydrase activity, and that equilibrium between serum and erythrocytes was attained in 6 hours (61). Vallee and Gibson (119) reported that leucocytes contained 25 times more zinc than erythrocytes. The zinc in the leucocytes existed 81.1% as a soluble fraction and the rest as an insoluble fraction at pH 7.2 (120).

e. Other Organs

Mice and dogs have been extensively used to study the distribution of zinc in the body (121, 88, 94). There has also been some work done using ruminants (86) and man (122) to study the distribution of this element. The most rapid turnover of zinc appears to be in the liver, pancreas, kidney and pituitary gland. The least activity is associated with the red blood cells, brain, skeletal muscle, ovaries, testes, gallbladder and skin. The amounts in the spleen, gastrointestinal tract, adrenals, lymph nodes, bone, heart, lung and thymus are intermediate (88, 94). Iranzo et al. (123) found slightly different results using guinea pigs. The greatest accumulation of zinc was in the carcass and osseous system, followed by the skin and hair, and then the kidneys and adrenals. Strain et al. (124) reported that 10 days after administration, the aortic tissue in rats attained greater zinc concentration per gram than the most active visceral organs, liver and pancreas. The most rapid turnover of zinc was found to be in the kidney, liver, spleen



and pancreas, while the slowest turnover was in the lungs, stomach, myocardium and skeletal muscle (125). Condon and Freeman (45) showed that although the plasma zinc levels were low in patients who died of uremia, the zinc levels in hair, heart, liver and testes were normal. The uptake of zinc in the normal and diseased liver has drawn attention and Vallee et al. (42) found less zinc in patients with cirrhosis of the liver than in the normals. Nishi (126) also found that the uptake of zinc in rats with hepatic disturbance was less than control animals. Because of the concentration of zinc chloride in the liver, Johnston et al. (127) were inspired to utilize this for a scanning agent and they obtained good liver scans using  $^{69m}\text{Zn Cl}_2$ . Pekas (95) studied the distribution of zinc in pigs under continuous infusion and found that the homeostatic mechanisms for clearance of excess zinc are exceeded by the infusion of 0.25 mg Zn/Kg per hour, as zinc chloride or zinc sulphate. Also, tissues such as muscle, which are rapidly "saturated" with zinc, translocate their zinc to "unsaturated tissues" such as liver, pancreas, kidney and lung. Therefore, the zinc in saturated tissues remained constant while the zinc in unsaturated tissues increased.

##### 5. Excretion and Biological Turnover

It would appear that zinc is mainly excreted in the feces (59, 122, 88, 65, 94). Feldstein et al. (128) found that the cumulative excretion of zinc over a month in man averaged 3.5 per cent of a dose of  $\text{ZnCl}_2$  in urine and

12.5 per cent of the dose in feces. The amount of excretion was proportional to the state of tissue saturation which suggested the existence of a homeostatic control mechanism (86). This was also demonstrated by Cotzias et al. (87), who showed that when zinc was used as a metabolic load, it accelerated the elimination of  $^{65}\text{Zn}$ . Zinc is eliminated from the body through the feces mainly via the pancreatic juice, bile or duodenal secretions (129, 121) and Birnstingl (129) has shown that elimination by the pancreatic juice is the most extensive. The biological half-life of zinc in man has been estimated at from 322 to 334 days (130, 122). However, Richmond et al. (131) reported the effective half-life in man to be about 154 days, using  $^{65}\text{Zn}$ . Gilbert and Taylor (89) have administered  $^{65}\text{Zn}$  labelled plasma and discovered that the clearance curve could be divided into four exponential terms with half-lives of 0.74, 6.05, 195 and 12180 minutes, respectively. They found that the last term correlated with the fecal elimination. Czerniak et al. (113) mentioned that different organs exhibited different turnover rates for zinc. In the adrenals and pancreas, the maximum was reached shortly after injection. As mentioned previously, this fact may be utilized for in vivo exploration of the pancreas.

## B. ETHYLENEDIAMINETETRAACETIC ACID (EDTA) - CHELATES

### 1. Chemistry

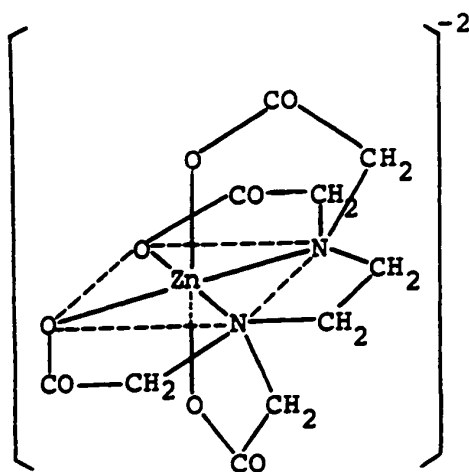
The chemistry of chelation is presented by several

authors (132, 133, 134, 135). Some of the general characteristics one should consider when choosing a chelating agent will be briefly presented. As the above authors have stated, the general behavior of chelating ligands is determined by factors such as negative charge of the ligand, and number of chelate rings formed. Selectivity is influenced by electronegativity of metal ions, and rigid positioning of donor groups. In general, stability is proportional to the number of negative charges of the ligand that associate with the metal ion and the number of chelate rings formed with the metal ion. The most stable chelate rings have five or six members (133, p. 108). Selectivity is greatest when the ligand only has nitrogen donors, and is least when it only has oxygen donors (136). Some chelates can be designed to take advantage of the small differences in ionic radii of metal ions, and this is best done using aromatic compounds as they are more stable. Another factor which must be considered is competing ions. These may be hydrogen and hydroxyl ions, cations, or chelating anions (133, pp. 114-116). Hydrogen ions compete with metal ions for donor groups at low pH, and hydroxyl ions compete with donor groups for metal ions at high pH. Metals compete with each other to form a chelate and the extent of displacement depends on the relative stabilities of the two chelates. In the same way, various chelates will compete with each other for metal ions.

ZnEDTA forms a very stable chelate and this fact is used in its titrimetric determination (137, 138). It has been

shown by isotopic exchange studies that zinc exchanges with its EDTA chelate (139, 140). The infrared spectrum has been studied extensively for zinc-EDTA, and the metal chelate bonding is reported to be primarily ionic (141, 142, 143). Figure 1 shows the structure of the complex (134).

Figure 1  
Structure of ZnEDTA



## 2. Pharmacological and Toxicological Aspects

It was demonstrated relatively early that the sodium salt of EDTA can be very toxic (144), and the rapid intravenous administration of Na<sub>2</sub>EDTA can lead to hypocalcemic tetany. However, this effect could be prevented by adding calcium chloride to the chelating agent before injection or by giving calcium salts intravenously or intracardially after injection of the compound. Popovici (144) also showed that

the rate with which blood calcium is lowered depends upon the mode and rate of administering the chelate. Intravenous, intraperitoneal, intramuscular and subcutaneous injections gave successively slower rates of lowering blood calcium levels, and by administering the chelate by slow intravenous drip, it was possible to give very large doses of the chelate. Spencer et al. (145) and Spencer (146) showed that slow intravenous infusion of  $\text{Na}_2\text{EDTA}$  produced excess calciuria without lowering the serum calcium levels which indicates a ready availability of calcium from bone. Even a relatively rapid infusion of  $\text{Na}_2\text{EDTA}$  decreased the serum calcium levels only temporarily, indicating a rapid mechanism for the maintenance of calcium homeostasis (146). Spencer et al. (147) found that administration of  $\text{Na}_2\text{EDTA}$  in quantities able to chelate only the excess calcium of hypercalcemic patients, did not cause hypocalcemia or compensatory release of bone calcium.  $\text{Na}_2\text{EDTA}$  has found clinical use in treatment of cardiac arrhythmias (148, 149). Spencer et al. (145) and Spencer et al. (150) also reported that the calcium chelate of EDTA could be given in relatively large quantities with no apparent changes in calcium balance or untoward effects. Attempts were made to correlate urinary calcium excretion with changes in urinary phosphorus excretion to see if regulation of calcium levels was due to parathyroid stimulation (146). However, the results were not consistent and the authors felt the rise in urinary phosphorus may be due to a mobilization of phosphorus from

the bone along with the calcium. With the knowledge that  $\text{CaNa}_2\text{EDTA}$  would not disrupt the calcium balance of the body, this chelate was employed to study the behavior of other metals in the body. Perry and Perry (151) found after its administration that the urinary clearance of zinc was greatly increased. Frantisek (152) and Truhaut et al. (153) also found an increased mobilization of zinc and the latter authors suggested that supplements of zinc should be given, especially when using DTPA in metal poisoning. Catsch et al. (154), however, reported that the zinc depletion is transitory and the depleted stores are quickly refilled with zinc absorbed from the intestine. This transitory effect was also found by Havlicek (155) who stated that zinc was mobilized from the extracellular and partially from the intracellular space. Because of the ability to mobilize metals in the body, EDTA was used in the treatment of heavy metal poisoning.

It has been found that some other toxic effects are apparent besides the risk of hypocalcemia which has been mentioned. Many authors have reported changes in animals and humans after prolonged administration of EDTA (156, 157, 158, 159). The most apparent change noted by these authors was the production of hydropic degeneration of the proximal convoluted tubules. Foreman et al. (157) stated that the development of lesions and their severity depended on both the dose given, and the number of days the drug was administered. They also showed that the lesions produced in the

kidney were reversible when the drug was withdrawn. Other changes noted included engorgement of the reticuloendothelial cells of the liver, spleen, bone marrow and lymph nodes; albuminuria, oliguria, fatigue, nausea, chills and muscular pain. Meltzer et al. (160) found no serious side effects or toxicity when they administered  $\text{Na}_2\text{EDTA}$  as a 3 g dose, which was given in a 0.5 per cent solution over 2.5 to 3 hours on alternate days. The etiology of the renal changes is not known but several theories have been proposed. It has been suggested that metal chelates partially dissociate in the kidney due to pH changes or competition with other chelating agents (161) and this increased metal ion concentration is toxic. However, Foreman et al. (157) found that the renal toxicity in rats was not diminished when the pH of the urine was raised by administering bicarbonate. These authors (157) suggested that a depletion of a required metal from enzymes in the renal tubular cells started a chain reaction which led to the formation of lesions. Catsch et al. (154) implied that zinc and cobalt might play a role in the development of these lesions since no lesions were observed when the chelates of those metals were administered. Similarly, manganese depletion has also been implicated as the cause of renal lesions since  $\text{MnEDTA}$ , when administered to animals, caused no lesions (162).

### 3. Absorption

Downes and McDonald (163) used  $^{51}\text{Cr}$ -EDTA as a marker to test gastro-intestinal function of sheep. They found that

after oral administration of the chelate, and in one case, direct insertion into the rumen, that 85 - 91 per cent of the dose was recovered in the feces during the first 10 days, and only a slight amount was found in the urine with a maximum of 4.7 per cent. Because of this low absorption, Rask-Madsen and Schwartz (164) use  $^{51}\text{Cr}$ -EDTA to test for ulcerative colitis. Foreman et al. (165) and Foreman and Trujillo (166) also noted the low absorption of EDTA when given orally. They found a maximum of 18 per cent absorbed in the rat and 5 per cent in the human. After oral administration of  $\text{CaNa}_2\text{EDTA}$ , Spencer (146) found no increase in calcium excretion in the urine which indicated that the chelate was not absorbed. Since there was also no increase of calcium in the stools, no calcium was attracted from the body for binding with EDTA. Foreman et al. (165) explained the low absorption on the basis of stomach acidity. At low pH, the chelate dissociates and the free acid is precipitated. Foreman and Trujillo (166) also reported the chelate to be very poorly absorbed through the skin. The authors noted these results came as a surprise as Popovici et al. (144) found a fall in serum calcium levels after application of EDTA to the skin, and yttrium and lead excretion was increased after oral administration of this chelate (167, 168). Spencer (146) reported that the calcium salt of EDTA was well absorbed when given intramuscularly. This route of administration was very painful and the addition of procaine to the solution only partially alleviated the pain. Although



EDTA itself is poorly absorbed, it apparently aids in the absorption of zinc (71). As mentioned previously, it competes with phytate for zinc and, as suggested by the author, it then exchanges the zinc ion at the intestinal mucosa. This enhancement of zinc absorption by the addition of EDTA to the diet is also reported by Vohra and Gonzales (82).

#### 4. Distribution

Because of the increasing use of EDTA in medicine, Foreman et al. (165) undertook a study which included the determination of the distribution of carbon-14 labelled  $\text{CaNa}_2\text{EDTA}$  in the rat. They found that no organ concentrated the chelate after 6 hours except the skin which retained about 0.5 per cent of the injected dose. The only organs with detectable  $\text{CaNa}_2\text{EDTA}$  levels were the kidneys, liver, skeleton and gastrointestinal tract. Foreman et al. (165) also found that no chelate entered the red blood cell compartment and that it did not readily pass the blood brain barrier. This fact is utilized in scanning for abnormalities in the brain as the chelate will only pass if the barrier is disrupted and then it appears to concentrate in the tumor tissue (169, 170). When Krasnai et al. (171) injected  $^{197}\text{Hg-EDTA}$  into the rat they found, contrary to the results reported by Foreman and coworkers (165), that the kidneys retained their activity from 5 to 24 hours even though the activity in the other organs had decreased with time. Autoradiography was also done on cross sections of the kidney 3 hours post injection and the epithelial cells of the convoluted tubules in the

renal cortex were found to contain the activity.  $^{60}\text{Co}$ -EDTA has been used as an extracellular marker in pharmacology because it distributes itself into a volume equivalent to the total water content (172, 173). The distribution of chelated- $^{65}\text{Zn}$  has been studied in rats (174) and mice (94). Brahmanandam et al. (174) have found that  $^{65}\text{Zn}$ -EDTA was preferentially deposited in skin, bones and muscles. The authors also point out that once a firm chelate is formed, the distribution and tissue localization is determined by the chelate moiety rather than the metal. Volf et al. (175), however, found that the chelated metal did influence the distribution pattern of EDTA in blood, plasma and bones. Brahmanandam et al. (174) also stated that other factors such as metal-to-chelating agent ratio and time played important roles because of the laws of mass action. This fact has also been pointed out by Greenberg and Dudley (176) who employed yttrium-90 chelates for therapy. They found that the greatest skeletal concentrations were achieved when four to five times the amount of chelate necessary to prevent precipitation of  $\text{Y}(\text{OH})_3$  was employed. They further stated that this excess protected the yttrium from being split away from the chelate by other physiological competitors. Stand et al. (94) determined the  $^{65}\text{Zn}$ -EDTA deposition in the pancreas, intestine, liver, spleen, kidney, bone, lung and heart at 24 hours post-injection. When comparing the distribution for one and twenty-four hours, the authors reported an increase with time in the pancreas and liver, a marked decrease

in the kidney, and no change in the blood, muscle, bone, lung and spleen.

##### 5. Excretion and Biological Turnover

Foreman et al. (165) and Foreman and Trujillo (166) were among the first to investigate the excretion of EDTA complexes. Using  $^{14}\text{C}$ -labelled  $\text{CaNa}_2\text{EDTA}$ , they determined the turnover time from the blood, after intravenous injection, to be about 50 minutes in the rat and 1 hour in humans. After parenteral administration, 95 to 98 per cent of the injected dose appeared in the urine of the rat by 6 hours and 90 per cent was recovered in the urine of humans after 7 hours. Spencer (146) also found the chelate to be quantitatively excreted and only up to 80 per cent of the EDTA complex was excreted in 8 hours. Foreman et al. (165) found a small portion of the administered chelate was turned over slowly and postulated that this moiety resulted from chelation with a metal strongly fixed in the body such as iron. They also reported about 2 to 4 per cent of the parenterally administered dose was excreted in the feces and less than 0.1 per cent in the respiratory  $\text{CO}_2$ . Foreman et al. (165) and Foreman and Trujillo (166) felt that the elimination of chelate, which was mainly via the kidney, was by both glomerular filtration and tubular excretion. However, Forland et al. (177), studying renal excretion of  $\text{CaNa}_2\text{EDTA}$  in dogs, by using various clearance techniques and competitive and selective tubular transport inhibition, reported that EDTA was excreted only by glomerular filtration. Bröchner-Mortensen et al. (178)

compared the clearance of inulin, which is excreted only by glomerular filtration, with that of  $^{51}\text{Cr-EDTA}$  and found the two values to correspond closely. They also showed that some chelate was retained for several days which suggested tubular reabsorption had taken place. Heath et al. (179) have also suggested that  $^{51}\text{Cr-EDTA}$  undergoes tubular reabsorption to a limited extent. Eide (180), however, has demonstrated by using stop flow technique that  $^{51}\text{Cr-EDTA}$  was neither secreted nor reabsorbed by the renal tubules in the dog. He suggested that the low values in glomerular filtration rate when compared to inulin, also noted by Chantler et al. (181), Heath et al. (179) and Stacy and Thorburn (182), might be due to partial dissociation of the complex. This dissociation might have taken place in the bottle, even before the injection was made. Brugsch (183) reported that 1 to 3 per cent of lead EDTA was dissociated in the tubular system of the kidney. In spite of these apparent difficulties, many investigators, employing various radioactive metals chelated with EDTA, have taken advantage of the rapid renal clearance of EDTA to determine glomerular filtration rate or scan the kidneys.

Another way in which investigators have utilized the fact that EDTA chelates are rapidly eliminated from the body, is through their use in treatment of heavy metal poisoning or removal of radioisotopes from the body. Ethylenediamine-tetraacetic acid has been shown to increase the excretion of lead (184), manganese and zinc (185), lanthanum, yttrium,

scandium, cesium and zinc (186), and zinc (187, 128). Sugiura and Tanaka (188) have found a linear correlation between the quantity of zinc removed from protein and the formation constants of zinc chelates. EDTA was also useful in removing strontium although the element was not removed as the EDTA chelate (186). As mentioned previously, it has also been employed for the removal of excess calcium in the treatment of hypercalcemia. Many other elements have been investigated with respect to elimination after EDTA therapy and these are reviewed by Catsch and Kavin (189).

### C. PRODUCTION OF POSITRON EMITTERS

#### 1. General Methods

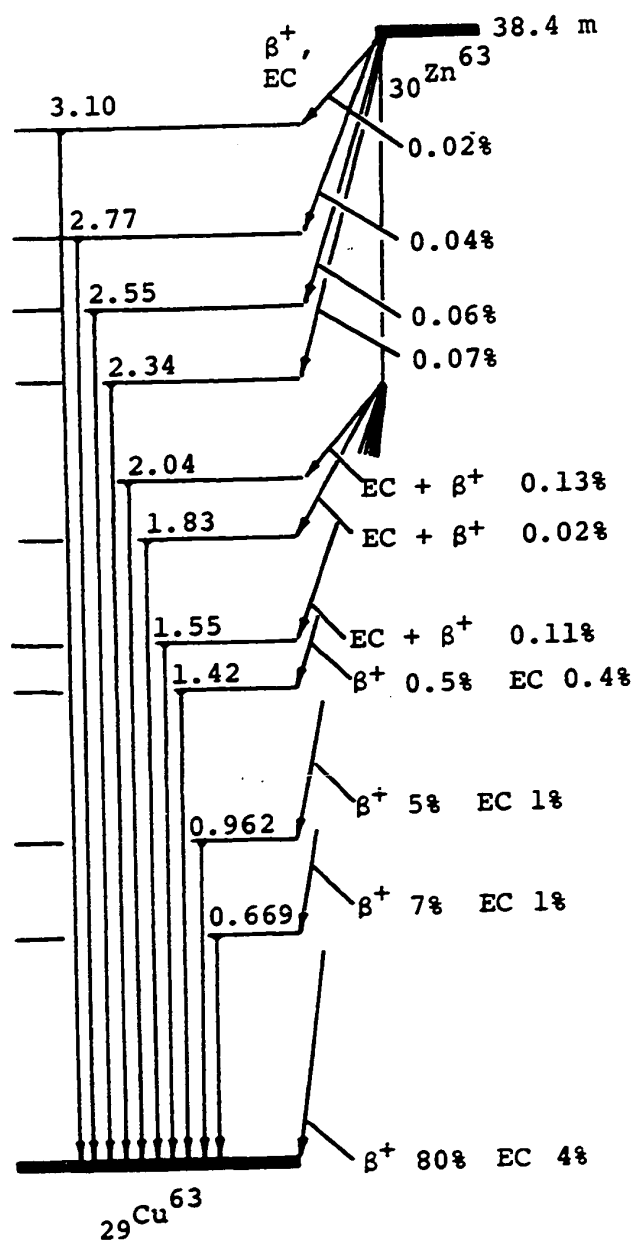
The production of isotopes is discussed by Wilson (190), Wang (191) and Moe et al. (192), and only the general principles will be presented in this discussion. Positron emitters are produced by the same methods as other isotopes and in general, the isotope produced depends on the target material, the impinging particle and the energy of the impinging particle. The irradiation time is governed by the amount of product needed, cross section of the target atom for the type and energy of the impinging particle, flux of impinging particles and percent abundance of target atoms. Radioisotopes result when the neutron-to-proton ratio is too high or too low and positrons result from neutron-deficient nuclides. Two basic types of machines, commercially used to produce these various isotopes, are reactors and particle

accelerators. The common types of neutron reactions are  $(n,\gamma)$ ,  $(n,\text{fission})$ ,  $(n,p)$ ,  $(n,\alpha)$ ,  $(n,\gamma) \xrightarrow{\text{decay}}$ , and  $(n,2n)$ . The  $(n,\gamma)$  type reaction involves thermal neutrons whereas the others usually involve fast neutrons. There are many types of accelerators available and these are more useful for the production of positrons. Cockcroft-Walton Accelerators, Van de Graaff Electrostatic Generators, Linear Accelerators and Cyclotrons can all be used to accelerate particles and they differ only in the mechanics involved to accomplish this task. The more common charged-particle activation reactions are  $(p,n)$ ,  $(p,2n)$ ,  $(d,n)$ ,  $(d,2n)$ ,  $(p,\alpha)$ ,  $(p,\alpha n)$ ,  $(p,pn)$ ,  $(p,2p)$ ,  $(d,p)$ ,  $(d,\alpha)$ ,  $(p,d)$  and  $(\alpha,n)$ . Radioisotope generator systems are also available for the production of positrons and  $^{68}\text{Ga}$  can be obtained by milking  $^{68}\text{Ge}$  with EDTA (193). It is possible for hospitals that are equipped with a cyclotron to produce their own positron-emitting isotopes for use in nuclear medicine (194) and some of the positron emitters produced by cyclotrons and used routinely are  $^{15}\text{O}$  produced by  $^{14}\text{N} (d,n)$ ,  $^{13}\text{N}$  produced by  $^{12}\text{C} (d,n)$ ,  $^{11}\text{C}$  produced by  $^{10}\text{B} (d,n)$  or  $^{11}\text{B} (d,2n)$ , and  $^{18}\text{F}$  produced by  $^{16}\text{O} (d,pn)$ . These isotopes are often preferable to reactor produced isotopes for a variety of reasons such as more convenient half-life, better target localization, higher specific activities, or more suitable radiation.

## 2. Production of $^{63}\text{Zn}$

The decay spectrum of  $^{63}\text{Zn}$  has been extensively examined (195, 196, 197, 198, 199) and the decay scheme shown in Fig. 2

Figure 2  
Decay scheme of  $^{63}\text{Zn}$  <sup>a</sup>



<sup>a</sup> from Lederer (9).

has been compiled.  $^{63}\text{Zn}$  decays by positron (93%) and electron capture (11%) with a half-life of about 38.3 minutes. Besides the positron peak its major gamma radiations are at 0.669 Mev (8%), 0.962 Mev (6%) and 1.42 Mev (0.9%) (17). There are four principal means of production of  $^{63}\text{Zn}$  and these are as follows:  $^{60}\text{Ni}$  ( $\alpha, n$ ) (200, 201);  $^{63}\text{Cu}$  (p, n) (202, 203, 197);  $^{63}\text{Cu}$  (d, 2n) (204, 199); and  $^{64}\text{Zn}$  (n, 2n) (205, 206).

#### D. STUDY OF THE KIDNEY IN NUCLEAR MEDICINE

##### 1. The Renogram

The renogram has progressed a great deal from its first trial in humans in 1956 (207, 208) to its present day form. Several reviews are presented on the history of its development (209, 210, 211). The basic equipment needed to perform a renogram study is appropriately collimated, matched scintillation detectors with dual rate meters and a dual chart recorder. Winter (212) also suggested the utilization of a third probe placed over a highly vascular area to reduce false interpretation of renograms. This third probe would represent clearance of the radioactive agent from the blood due to combined kidney function. Although these are the basic requirements for the renogram, there are many sophisticated instruments which may also be employed. Split crystal scintillation camera techniques have been used (213) which allow for simultaneous renogram curves and sequential images, small changes in patient position, minimum renal "crosstalk"



and greater reproducibility. Data obtained from scintillation cameras has been fed into computers for rapid and impartial interpretation of the renogram (214), and computer programs have been written to simulate normal or abnormal renograms (215). Instead of chart recorders, output may be transferred to magnetic tape or displayed on an oscilloscope using a multichannel analyzer (216). The technique of obtaining a renogram involves five basic steps (217, pp. 22-26). These involve selection of the radiopharmaceutical, determination of the dose to be injected, positioning of the patient, locating the kidneys and finally fasting or hydrating the patient. There are many isotopes which have been utilized for renograms or kidney function tests and these will be considered in a later section. The dosage of isotope given is determined by physical characteristics such as quality and type of equipment and size of patient although these are not critical. The most important factor is to inject enough activity to avoid statistical fluctuations. The position of the patient does not appear to be too critical and it usually depends on the opinion of the doctor doing the test. Kidneys can be located by an x-ray or preliminary injection of another isotope. The latter is preferred and by choosing the proper discriminator levels, the contribution from this isotope can be eliminated. Opinion on whether a patient should be fasted or hydrated is also varied and it would appear that as long as a standard technique is followed at all times,

valid renograms can be obtained. There are three distinct segments observed in the renogram and initially they were referred to as the vascular segment, the secretory segment, and the excretory segment (207). This terminology was found to be imperfect and new terminology for the various segments was suggested (218, 219). The first segment is referred to as the phase of "tracer appearance", the second as the segment of "blood flow", and the third is referred to as the "drainage segment". The time elapsed from injection of the isotope to disappearance of the tracer from the field of view of the counter is referred to as the "renal transit time". Interpretation of the renogram has varied from direct qualitative analysis of the tracings, which is the fastest method, to mathematical treatment of data to obtain quantitative information such as glomerular filtration (220, 221). Various models for the excretion of compounds have been proposed (222, 223). When one peruses the literature, it becomes apparent that many problems exist concerning the correct interpretation. Numerous factors must be met before a valid renogram is obtained, and this by no means is a settled question (224, 225, 217, pp. 32-36, 211). Each investigator chooses his own set of parameters to be met and no standard procedure is apparently in sight. In spite of the extensive controversy and apparent shortcomings of the renogram, it continues to play a significant role in nuclear medicine and is a valuable tool in the diagnosis of renal abnormalities. Screening for kidney disease (226),

safe study of kidney during pregnancy (227, 228), study of hypertension (229, 230), and postoperative management of kidney transplants (231, 232) are a few areas in which the renogram is playing a significant role.

## 2. The Kidney Scan

Like the renogram, scanning of the kidney has progressed rapidly since its inception (233). The basic requirements are a detector, electronic analyzing system and image output. As was mentioned before, the degree of sophistication can be varied for each system. The output may be in the form of a dot recording, color coded, or placed on magnetic tape to facilitate computer analysis (234, pp. 42-46). The detector head may also be in two basic forms. In one form the detector head is mobile over the area to be examined while in the other, the detector head is stationary. The terminology for the two types of "scans" resulting from the various detectors is quite unsettled (234, p. 42). Taplin et al. (235), Anger (8) and Timmermans and Merchie (234) have reviewed the various types of detector heads which are used to accomplish these procedures. The stationary or camera type devices are capable of performing dynamic studies and this is of advantage when doing renograms as it is then possible to "see" the passage of the isotope through the kidney and relate this to the various segments of the renogram (236, 237). Freeman et al. (238) have also obtained rapid sequential scintiphotographs of renal blood flow and Loken et al. (214) have combined the scintillation

camera with computer output to help interpret renal function. Visualization of the kidney has allowed the detection of alterations in size, shape and position of the kidneys, space-occupying disease, renal insufficiency, amount of functioning renal parenchyma in various diseases, and renal vascular diseases (239, 240, 241). Recent applications in the postoperative management of renal transplants have also been found useful (232, 242). As is the case with renograms, there have been various attempts to quantitate the information obtained in scanning (243, 244) and with improvements in equipment and tissue localization, this could add significantly to the clinical application of scanning.

### 3. Isotopes Used in the Study of the Kidney

Many isotopes are utilized in the study of the kidney. These isotopes have been incorporated in various forms to improve localization in the kidney or enhance their excretion. Timmermans and Merchie (245) have reviewed these various aspects, and for the present discussion, the most important radiopharmaceuticals used in this respect will be reviewed.

#### a. Iodine-131 and Iodine-125

As noted by Timmermans and Merchie (245, p. 13), iodine radioisotopes have been extensively used for functional examinations. Although iodine-131 has been the most utilized isotope, it has two serious faults which are radio-toxicity due to  $\beta$ -emission and its relatively hard gamma

energy. Iodine-125 does not emit any  $\beta$ -radiations and its gamma energy is very low, hence may be preferred due to these advantages. Hippuran (246), which is ortho-iodo-hippurate of sodium, is the most extensively used form and is available with the  $^{131}\text{I}$  and  $^{125}\text{I}$  label. Inulin (247) and serum albumin (248) have also been used extensively and are available with an  $^{131}\text{I}$  or  $^{125}\text{I}$  label. Johns et al. (249) prepared  $^{131}\text{I}$ -3'-iodoaminopterin which is an enzyme agent that localizes in the kidney and recently, human fibrinogen labelled with  $^{125}\text{I}$  has been utilized to detect rejection of human renal transplants (250).

b. Mercury-203 and Mercury-197

Mercury has been the isotope most utilized for scanning purposes (245, p. 13). Mercury-203 is considered highly toxic due to its  $\beta$ -radiation and long half-life. Thus, mercury-197 is preferred in clinical medicine, as it has a short half-life, weak  $\gamma$ -radiation and no  $\beta$ -radiations. Mercury dichloride (251) and chlormerodrin (230) are the most common forms employed.  $^{197}\text{Mercury}$ -labelled EDTA has also been employed in kidney scanning (171).

c. Technetium-99m

Technetium-99m has been extensively used in the last few years due to its ready availability from an isotope generator. It is a pure  $\gamma$  emitter of medium energy (140 Kev) and short half-life (6 hours). A few of the forms in which  $^{99\text{m}}\text{Tc}$  have been injected to scan the kidneys or evaluate their function are as follows:  $^{99\text{m}}\text{Tc}$ -DTPA (252,

253);  $^{99m}\text{Tc}$ -Fe-EDTA or  $^{99m}\text{Tc}$ -Fe-DTPA (235, 254);  $^{99m}\text{Tc}$ -Fe-Ascorbic acid complex (255);  $^{99m}\text{Tc}$ -Gelatin (256); and  $^{99m}\text{Tc}$ -Penicillamine Acetazolamide complex (257). As is apparent, a great deal of research is presently taking place using  $^{99m}\text{Tc}$  in various forms to scan the kidneys or determine their function.

d. Chromium-51

$^{51}\text{Cr}$ -labelled EDTA (258, 259) and  $^{51}\text{Cr}$ -labelled inulin (260, 261) have been used to determine glomerular filtration rate.

e. Gallium-68

$^{68}\text{Ga}$  which decays mainly by positron emission has been prepared as  $^{68}\text{Ga}$ -EDTA (262) and  $^{68}\text{Ga}$ -polymetaphosphate-Mg-polymetaphosphate (263) and both have been used in kidney scanning.

f. Indium-113m

Indium-113m has also been extensively investigated in the past few years. It is readily available from a  $^{113}\text{Sn}$ - $^{113m}\text{In}$  isotope generator and has a 393 Kev gamma ray with a 1.7 hour half-life. For injection it may be prepared as  $^{113m}\text{In}$ -DTPA (264);  $^{113m}\text{In}$ -Fe-DTPA-Ascorbic acid (265); or  $^{113m}\text{In}$ -EDTA (254, 266).

g. Carbon-14 and Carbon-11

Carbon-14 has been used to label inulin (267) but this method of measuring renal clearance requires a liquid scintillation counter. Sodium  $^{11}\text{C}$ -benzoate (268) and  $^{11}\text{C}$ -carboxylates (269) have been used for kidney scanning.

Carbon-11 is a positron emitting isotope but its half-life is only 20.4 minutes.

h. Cobalt-57 and Cobalt-60

$^{57}\text{Co}$ -labelled vitamin  $\text{B}_{12}$  (270, 271) and  $^{57}\text{Co}$  or  $^{60}\text{Co}$ -labelled vitamin  $\text{B}_{12}$  (272) have been used to measure glomerular filtration rates.

i. Krypton-85

$^{85}\text{Kr}$  as inert gas has been used for the determination of renal blood flow in man (273, 274).

j. Rubidium-82 and Rubidium-86

Rubidium-82 has been used to visualize the kidneys in animals (275). Its positron emission (96%) has an ultra-short half-life (75 seconds). Therefore, it is infused directly after elution from a  $^{82}\text{Sr}$  generator with 0.3 M ammonium acetate. Blandy et al. (276) have found that renal blood flow could be measured satisfactorily using rubidium-86.

k. Xenon-133

$^{133}\text{Xe}$  is dissolved in sterile saline and it can be used to determine the intrarenal distribution of blood flow (277, 278).

l. Ytterbium-169

$^{169}\text{Yb}$ -DTPA was used in the visualization of renal structure and function (264, 279).

m. Gold-198

Colloidal-gold-198 has been used to measure the renal output in dogs (280).

n. Thulium-170

Burke (281) has used a thulium-170 capsule as an energy source for taking radiographs of renal calculi during surgery.

Although this list may not cover all isotopes used to study the kidneys, it shows the extensive research being done in an attempt to find the ideal isotope in the ideal form suitable for the various kidney tests.



### **III. EXPERIMENTAL METHODS AND MATERIALS**

#### A. ANIMALS

Male mice of the ALAS strain weighing 25-30 g on arrival were used for the initial part of this investigation. No more than five animals were housed in plastic cages with bedding of spruce and poplar wood shavings. The mice were allowed Purina Laboratory Chow and tap water ad libitum.

Mongrel male dogs weighing 17 to 20 kilograms were used for the renogram study. They were allowed food (Dr. Ballards Champion Dog Food) and water ad libitum until 24 hours before the experiment. At this time all food was withdrawn. The animals were housed in individual cages and after injection of isotope, all urine and feces were collected until the excreted activity was safe.

#### B. PREPARATION OF SOLUTIONS

All chemicals used in the present investigation were of ACS standard.

##### 1. ZnCl<sub>2</sub>

Solutions of zinc chloride (Fisher Scientific Co., Fair Lawn, N.J.) were made by dissolving various quantities in distilled water. If a precipitate was formed, the addition of a few drops of 0.1 N HCl was found to eliminate this.

##### 2. ZnNa<sub>2</sub>EDTA

Solutions of ZnNa<sub>2</sub>EDTA were prepared by dissolving 0.2726 g of ZnCl<sub>2</sub> and 0.7545 g of Na<sub>2</sub>EDTA·2H<sub>2</sub>O (Fisher Scientific Co., Fair Lawn, N.J.) in 5 ml of distilled water. Sufficient NaHCO<sub>3</sub> (10%) was added until complete dissolution

and the pH was adjusted to 7.0 using 0.1 N HCl and a Beckman pH meter (Zeromatic II). The volume was then adjusted to 10.0 ml with distilled water. The solution was filtered through a 0.45  $\mu$  millipore filter using a Swinnex<sup>®</sup>-25 filter unit (Millipore Corp., Bedford, Mass.) into a pre-sterilized 20 cc serum bottle (Wheaton Glass Co., Millville, New Jersey).

### 3. ZnNa<sub>2</sub>EDTA plus CaNa<sub>2</sub>EDTA

Solutions were prepared by dissolving 0.6814 g ZnCl<sub>2</sub> and 1.9112 g Na<sub>2</sub>EDTA·2H<sub>2</sub>O in 5 ml of distilled water. To this solution was added 0.0149 g CaCl<sub>2</sub> (J.T. Baker Chemical, Phillipsburg, N.J.) which was the amount calculated to chelate the excess Na<sub>2</sub>EDTA. One ml of 10 N NaOH (Fisher Scientific Co., Fair Lawn, N.J.) was added and the solution was then made slightly basic using 1 N NaOH. The pH was adjusted to 7.0 using 0.1 N HCl and the total volume made up to 10 ml with distilled water. This preparation was filtered through a 0.45  $\mu$  millipore filter using a Swinnex<sup>®</sup>-25 filter unit into a pre-sterilized multi-dose container.

### 4. Preparation of Radioactive Zinc Chelates

The preparation of the <sup>65</sup>Zn-EDTA chelates was identical to the methods previously outlined. However, 1 ml of <sup>65</sup>ZnCl<sub>2</sub> (Zinc-65 HSA, I.C.N., Nuclear Science, Pittsburg, Pa.) was added to the solution. The activity was 1 mCi per ml and the quantity of ZnCl<sub>2</sub> per ml was no more than 2 mg. Therefore, when the radioactive zinc chelate was made, the amount of "cold" ZnCl<sub>2</sub> added was 2 mg less than previously outlined.

To assure that all zinc-65 was chelated, samples of the chelate solution were spotted on ChromAR<sup>®</sup>-500 sheets (Mallinckrodt Chemical Works Laboratory Products, Montreal) and the chromatogram developed using a solvent system consisting of ethyl alcohol:water (1:1). A sample of  $^{65}\text{ZnCl}_2$  was also spotted on the chromatogram. After development, the strips were exposed to Medical X-ray film (CEA, non screen, safety blue base, Sweden) for 12 hours and an autoradiogram obtained. The X-ray film was developed in Kodak X-ray developer (Eastman Kodak Co., Rochester, N.Y.) for 5 minutes, washed for 30 seconds, fixed in Kodak liquid X-ray fixer and replenisher (Eastman Kodak Co., Rochester, N.Y.) for 10 minutes and then washed for 1 hour. It was found that the  $^{65}\text{Zn-EDTA}$  solution produced only one spot with an  $R_f = 0.79$ . No activity was detected at the origin which corresponded to the  $^{65}\text{ZnCl}_2$ . It was therefore assumed that all  $^{65}\text{Zn}$  was chelated.

##### 5. Check for Chelation

Samples were checked for chelation using Infra Red Spectrometry (I.R.), Nuclear Magnetic Resonance Spectrometry (N.M.R.) and Carbon, Hydrogen and Nitrogen Analysis (C,H,N). In the case of I.R. and N.M.R. analysis, samples were obtained by evaporating the chelate solutions to dryness. The I.R. study was performed on a Beckman I.R. 10 Infrared Spectrophotometer (Beckman Instruments Inc., Fullerton, California) using 1-3 mg of material and 500 mg of KBr as outlined by Sawyer and Paulsen (282). The N.M.R. analysis

was performed on a 60 MC NMR (Varian model A-60D) by dissolving 80 mg of material in  $D_2O$ . Analysis for C, H and N was done after precipitation of the Zn-EDTA from solution by the addition of absolute alcohol (282). The precipitate was recrystallized four times by precipitation from water and then dried for 72 hours before analysis.

#### 6. Preparation of Solutions for Direct Bombardment

Empty ten-milliliter ampoules were boiled in 0.1 per cent sodium hexametaphosphate (Hooker Chemical Corp., New York) for 1 hour. They were then rinsed in sterile, pyrogen-free water, covered with aluminum foil, and placed in an oven at  $250^{\circ}C$  for 1 hour. The  $ZnNa_2EDTA$  plus  $CaNa_2EDTA$  was prepared as outlined before using water for injection. The solution was transferred to the ampoules in an aseptic room by filtering through a  $0.45\ \mu$  millipore filter and the ampoules sealed. An additional 0.5 ml was placed in each ampoule to allow extraction of 10 ml. The ampoules were then autoclaved at  $121^{\circ}C$  for 20 minutes. No change in pH was found after autoclaving the solution. The chelated solutions were also checked by I.R., N.M.R., and C,H,N analysis before and after terminal sterilization and no change was noticed.

#### C. SAMPLE ACTIVATION

Activation of samples was accomplished using a Cockroft-Walton Accelerator (Texas Nuclear, Austin, Texas, Series 9900). Samples to be activated were taped directly on the

water cap portion of the target assembly and as near as possible to the tritium target that was to be bombarded. For the determination of  $^{63}\text{Zn}$  production, samples were bombarded for 5 minutes at 120 KEV and a beam current of 1 milliamper (ma). After bombardment, the samples were removed from the target assembly and a 1 ml aliquot transferred to a gamma counting vial. This sample was then placed in front of a lead-shielded 1 1/2 inch by 1 inch NaI (Tl) crystal and a 5-minute count spectrum stored in a Nuclear Chicago 512 Multi-Channel Analyzer (Model 25601). The multi-channel analyzer was calibrated using standard  $^{137}\text{Cs}$  and  $^{60}\text{Co}$  sources (ICN Tracerlab Gamma Spectrometer Standards, Serial No. 308, Model R-35, Waltham, Mass.). One minute counts were also obtained at various times after bombardment by counting the 0.511 Mev energy region using a single channel analyzer. For the production of  $^{63}\text{Zn}$  for scanning, samples were bombarded with neutrons for 1 hour at 120 Kev and 1 ma. The total neutron flux during bombardment was determined using a calibrated proton recoil monitor. A fresh target was always used for prolonged bombardment in order to obtain the maximum neutron flux.

#### D. TOXICITY DETERMINATION

Toxicity studies were done on mice to determine the  $\text{LD}_{50}$  after intravenous injection. Mice were given various concentrations of  $\text{ZnCl}_2$ ,  $\text{ZnNa}_2\text{EDTA}$  plus excess  $\text{Na}_2\text{EDTA}$  or  $\text{ZnNa}_2\text{EDTA}$  plus excess  $\text{CaNa}_2\text{EDTA}$  by tail vein injection.

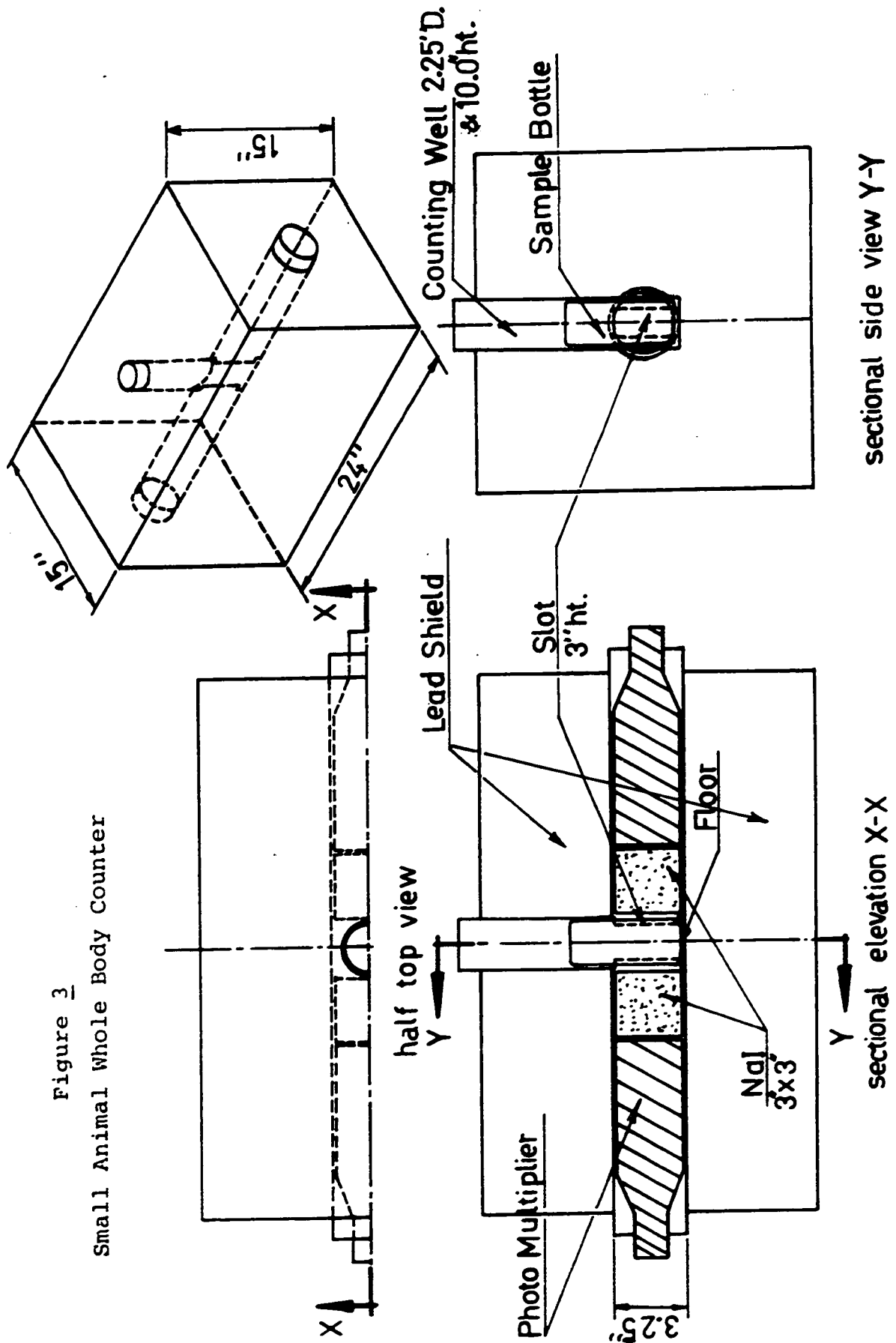
Results were recorded 5 days later. However, any deaths which occurred did so within 1 hour of injection. The  $LD_{50}$  was determined using the method described by Litchfield and Wilcoxon (283). This method is simple and fast to use. It helps determine the  $LD_{50}$  with its 95 per cent confidence limits, recognize heterogeneity of data by using the statistical parameter  $(\chi)^2$ , and uses zero and 100 per cent observations to their best effect.

#### E. WHOLE-BODY EXCRETION ANALYSIS

##### 1. Whole-Body Counting

Animals were given 0.8 g per Kg of  $^{65}\text{Zn-EDTA}$  by tail vein injection and placed in plastic bottles 5 inches long and 2.2 inches in diameter. The bottle containing the radioactive mouse was then lowered into a small animal whole-body counter (figure 3) and the activity recorded for 1 minute using two 3" x 3" NaI(Tl) crystals placed opposite to each other. As each count was completed, the bottle was withdrawn from the chamber and the mouse removed. The animal was then induced to excrete any urine or feces, transferred to a cleaned bottle and lowered into the counting chamber where activity was again recorded. This procedure was repeated for each counting period. Activity remaining in the body was determined every 4 minutes for a period of 1 hour, then every 30 minutes for a period of 6 hours, and finally once daily for 7 days. A standard sample was prepared at the time of injection of the animal and

Figure 3  
Small Animal Whole Body Counter





this was used to determine the relative quantity of isotope injected in each mouse. The standard solution was also lowered into the counting chamber and counted similarly to the mice.

It was necessary to determine if location of activity or movement during counting significantly influenced the detection of activity. Sealed glass tubes were prepared which contained  $^{65}\text{Zn}$ . One glass tube was 1 inch long and 5/16 inches in diameter and the other was 1 7/8 inches long and 6/16 inches in diameter. A rectangle of Clean-Klay was made having the dimensions 1 1/2 inches in height, 1 inch in width and 2 inches in length. Holes were made 5/8 inch from the bottom of the rectangle and located in the center of the rectangle (cavity 3), 3/8 inch from the center (cavity 2) and 3/4 inch from the center (cavity 1). The small glass tube was placed in these cavities and it represented localized concentration at various positions in the chamber. The long glass tube was alternately positioned longitudinally in the phantom at a height 5/8 inch from the bottom and this was used to represent activity distributed throughout the chamber. The radioactivity in the phantom was examined at two different heights (1 1/2 inches and 5/8 inches) relative to the position of the NaI(Tl) crystals. This was thought to represent mice in both the standing and lying positions respectively. The bottle containing the radioactive phantom was then rotated through eight different positions in

the counting chamber with the activity located in each of the various cavities. Each study was repeated three times and the results are shown in figures 4, 5, 6 and 7. All results are expressed relative to the highest count obtained.

The small cylinder was then placed in cavity 2 and located  $5/8$ " from the bottom of the counting chamber. This was inserted in the whole body counter 50 times at random and 1 minute counts recorded. It was found that the standard deviation of the counts was only 1.04 per cent. A similar experiment was performed using the large cylinder located  $5/8$ " from the bottom of the chamber and the standard deviation of the counts was found to be 0.79 per cent. The small cylinder was also placed in cavity 2,  $5/8$ " from the bottom of the chamber and the bottle was rotated while 1 minute counts were taken. This was repeated 25 times and the standard deviation of the counts was found to be 0.52 per cent. Rotation of the large cylinder located  $5/8$ " from the bottom of the chamber gave a standard deviation of the counts of 0.45 per cent.

It was therefore felt that position and movement of the animal in the whole-body counter would not influence the detection of activity significantly under the conditions of our investigation. Although the detection of activity was about 18 per cent less at certain positions of the small cylinder these represented extreme conditions and it was felt that such conditions would not exist in our study.

Figure 4

Relative Efficiency of Detection of Activity in Small  
Cylinder Located  $5/8$ " from Bottom of Counting Chamber

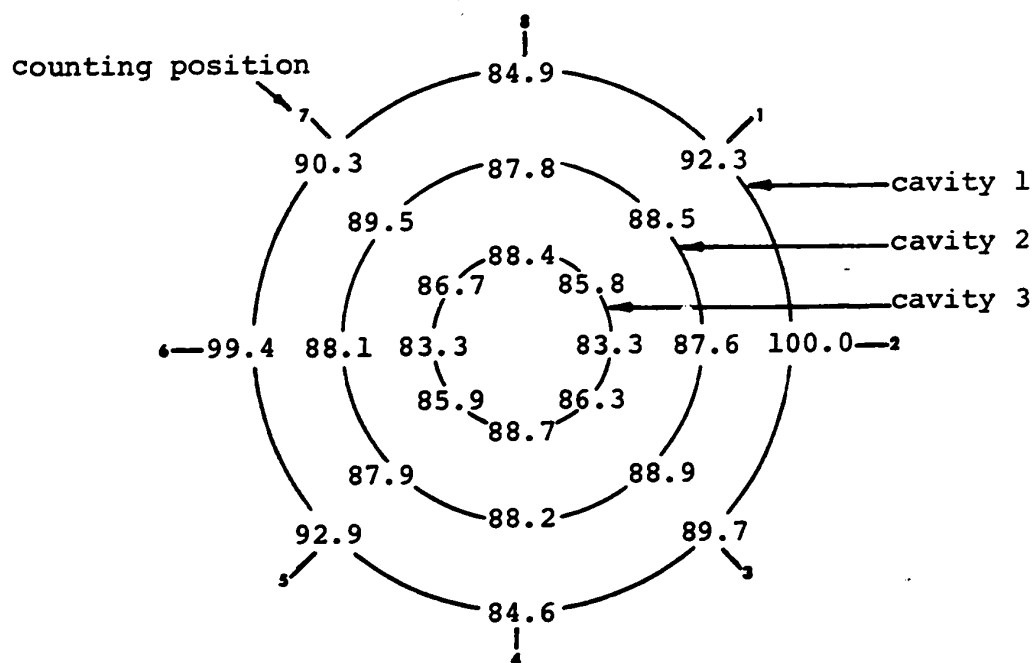


Figure 5

Relative Efficiency of Detection of Activity in Small  
Cylinder Located  $1\ 1/2$ " from Bottom of Counting Chamber

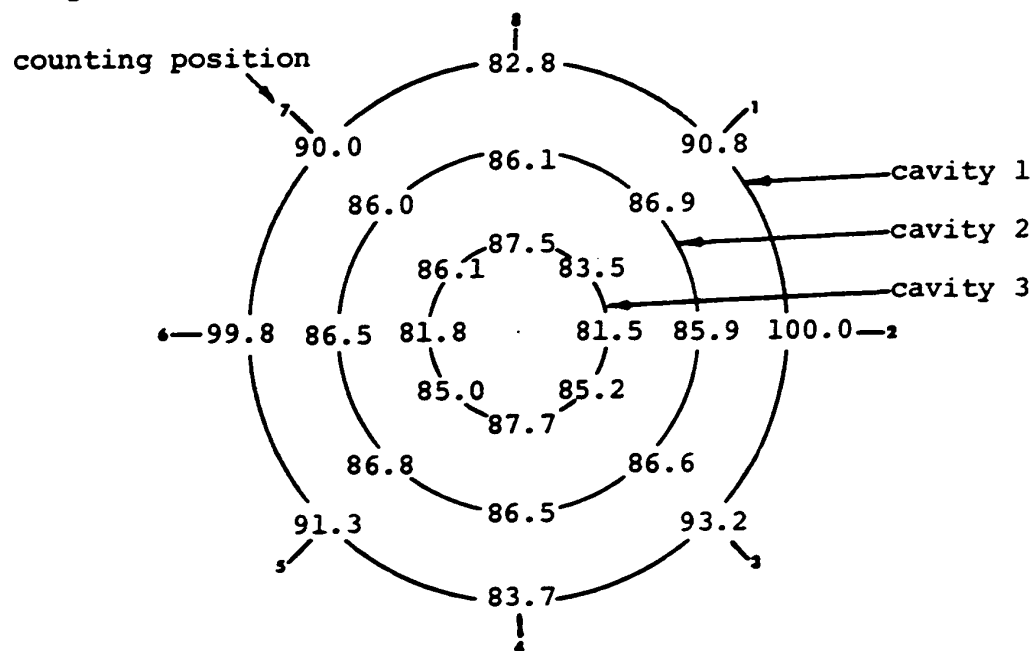


Figure 6

Relative Efficiency of Detection of Activity in Large  
Cylinder Located  $5/8$ " from Bottom of Counting Chamber

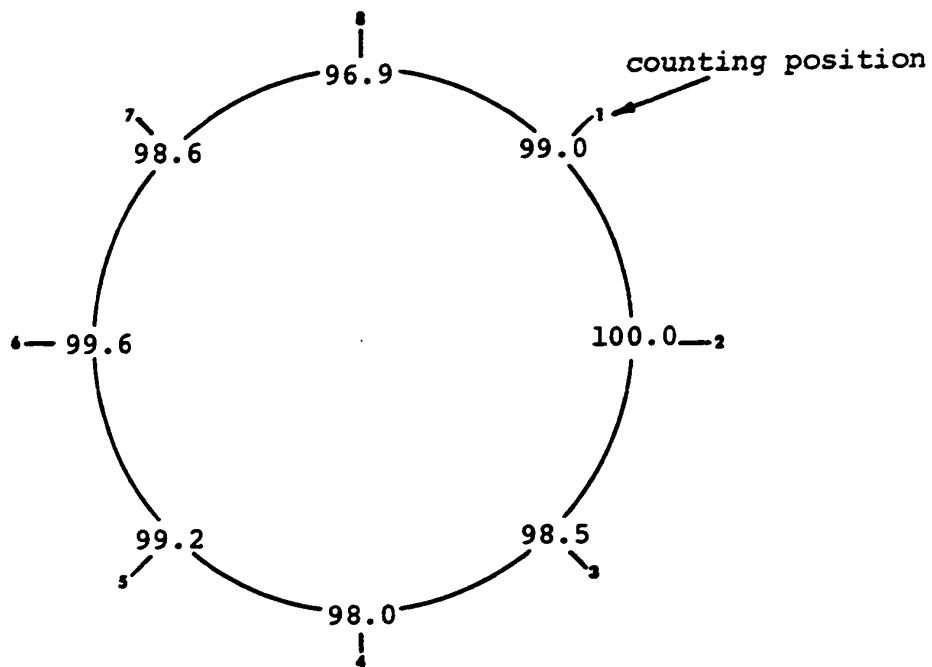
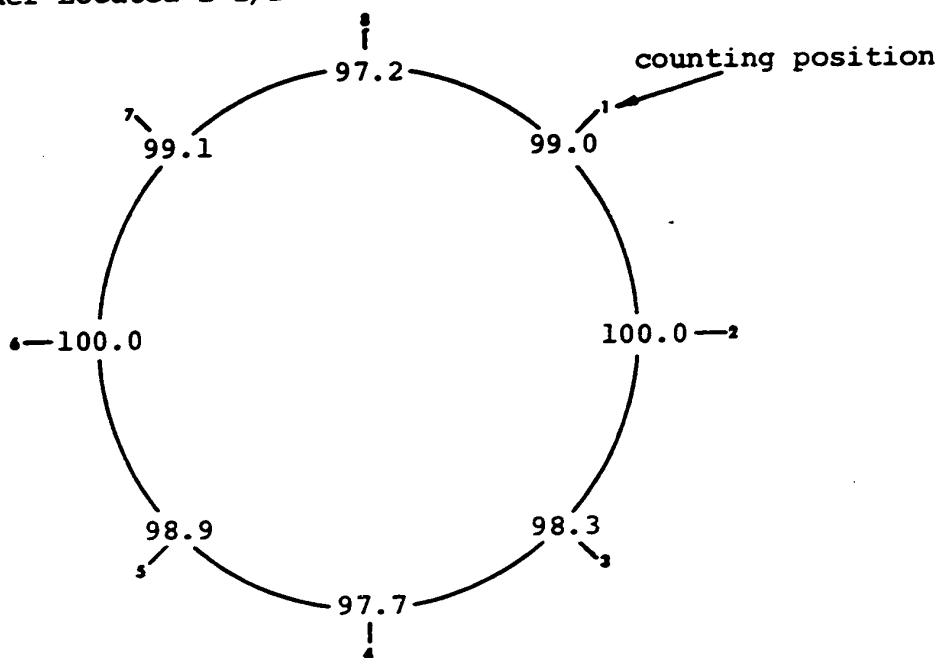


Figure 7

Relative Efficiency of Detection of Activity in Large  
Cylinder Located  $1\ 1/2$ " from Bottom of Counting Chamber



## 2. Compartmental Analysis

The activity remaining in each mouse was determined at the various times post-injection. These values were then corrected for background and plotted on semi-logarithmic graph paper as a function of time. The activity during the initial 6 minutes post-injection remained essentially constant but after this period, the excretion curve represented a series of exponential decays. Therefore, the data from the initial 6 minutes were ignored when analyzing the elimination of activity and zero time represents 6 minutes post-injection. As the excretion curve may be represented by a series of exponential decays, the activity remaining in the body at time "t" can be given by the equation

$$f(t) = \sum_{i=1}^n A_i e^{-\lambda_i t}$$

where  $A_i$  represents the activity at zero time

$\lambda_i$  represents the elimination rate constant

n represents the number of components

t represents time after injection (plus 6 minutes).

The above equation assumes that all the exponentials are separate and unrelated but this does not occur in biological systems (284). Since only qualitative information was sought in this investigation, no correction was made and the data was analyzed as if the various compartments were independent. The method of Dick and Lea (285) was used to determine if the last portion of the curve consisted of a single exponential or an exponential plus a bound fraction. This method

is based on the fact that if the last portion of the curve is the sum of an exponential plus a bound fraction, then

$$f(t) = Ae^{-\lambda t} + B$$

where B represents the bound fraction.

Differentiating with respect to "t" gives

$$\frac{df(t)}{dt} = -\lambda Ae^{-\lambda t}$$

$$\begin{aligned} \text{then } \frac{df(t)}{dt} &= -\lambda[f(t) - B] \\ &= \lambda B - \lambda f(t) \end{aligned}$$

When  $\frac{df(t)}{dt} = 0$ , then  $\lambda B = \lambda f(t)$  or  $B = f(t)$ . Therefore, by drawing  $\frac{df(t)}{dt}$  vs  $f(t)$  (i.e. cpm/m vs cpm) on normal graph paper, a straight line is obtained whose X-intercept represents the amount of the bound fraction. If no bound fraction is present, the line will pass through the origin as when  $\frac{df(t)}{dt} = 0$   $f(t) = 0$ . If a bound fraction is present, its value must be subtracted from all the data and the curve redrawn. If no bound fraction is present, the number of components and their half-lives can be determined by employing curve peeling techniques.

For our investigation, a computer program was written for use on a digital PDP-8/L computer which would perform curve peeling of the data (Appendix, No. 3). The method relies on results being taken at time intervals extending over prolonged periods and also well separated decay constants. If these criteria are met then the data obtained for large

values of "t" are due to one exponential only (assuming no bound fraction).

Data was fed into the computer starting with the longest time period and progressing to the shorter time periods. To begin, the last three results were fed into the computer and a line of best fit calculated using the weighted least squares method. The weighting factor used was a combination of a weight which allowed for the log ordinate (286) and a weight which allowed for the spread in counting statistics (287). The standard deviation of the slope was also computed. An additional earlier point was then fed into the computer and a weighted least squares fit determined for all data now in the computer and a new slope and standard deviation of the slope recorded. This new slope was then compared to the last determined slope by using a Students "t" test. The t value and its corresponding degrees of freedom were printed out and a check for a significant change was effected by consulting a set of t tables. If no significant difference was noted in the slopes, the next earlier data point was then fed into the computer and the process repeated. When a significant difference occurred using n points, instructions were fed to use the slope obtained from n-1 points. This is followed by calculation of the ordinate intercept value (initial value of that component), the half-life of the determined component, and the standard deviation of the determined slope. This information is then used to remove the contribution of this long component,

thereby producing a modified set of data for the earlier time periods. Using this new data, the process is again repeated to determine the slopes of the remaining components until no data are left.

The values for the various compartment half-lives from each mouse along with their corresponding initial per cent activity were thus determined. Analysis was repeated for 6 mice and the mean and standard error of the mean was calculated for each component half-life and its corresponding initial activity.

### 3. Analysis of Excretion

To determine the quantity of injected zinc-65 excreted in urine and feces after intravenous administration of  $^{65}\text{ZnNa}_2\text{EDTA}$ , six mice were placed in individual siliconized glass beakers. Wire mesh was placed in the bottom to allow urine and feces to fall through. During the initial period of 6 hours post-administration, the feces was carefully removed to prevent any cross contamination from the highly radioactive urine. After this period, the urine was transferred to a separate beaker and then urine and feces were collected for two days post-injection. The combined urine samples and feces samples were then individually analyzed by counting aliquots in a Nuclear Chicago Automatic Gamma Well Counter (model 4216) and the per cent activity excreted by each route determined. A standard sample of the injected solution was also counted directly to determine the activity each animal received.



In order to examine the chemical form of the excreted material, 10  $\lambda$  samples of excreted urine were spotted on ChromAR<sup>®</sup> 500 sheets. Standard samples of  $^{65}\text{ZnNa}_2\text{EDTA}$  and  $^{65}\text{ZnCl}_2$  dissolved in urine were also spotted on the sheets and the chromatograms were developed in a solvent system of ethyl alcohol:water (1:1). The chromatograms were then exposed to medical non screen X-ray film for 1 day. The film was subsequently processed as described previously and after drying the Rf of the spot obtained from the urine sample was compared to those from the  $^{65}\text{ZnNa}_2\text{EDTA}$  and  $^{65}\text{ZnCl}_2$  standards.

#### F. TISSUE DISTRIBUTION ANALYSIS

Each mouse (25-30 g) received 0.8 g/Kg of  $^{65}\text{Zn-EDTA}$  by tail vein injection and was then placed in a cage until time for analysis. Animals were allowed food and water ad libitum during this period. At the time of injection, three 0.1 ml samples of the injected solution were added to counting vials and diluted to 3 ml with distilled water. These standards were counted concurrently with the tissue samples. This procedure eliminated the need for decay correction. Analysis was carried out after 2, 6, 12, 18, 24, 30, 40 and 50 minutes, 1, 3 and 5 hours, and 2, 4, 6 and 14 days. At the time of sacrifice, the animals were rapidly decapitated and exsanguinated. A quantity of blood was collected in a heparinized syringe for later analysis. The tissues analyzed were the liver, kidneys, bladder muscle, spleen, gallbladder,

heart, lung, muscle, bone (femur), brain, eyes, testicles, stomach, tail, pancreas and adrenals. All organs examined were blotted free of blood after being excised and placed in disposable weighing boats. The entire liver was excised and weighed. Then a portion was weighed and used for analysis. The small organs (bladder muscle, gallbladder, eyes, testicles, stomach and adrenals) were wet weighed and then dissolved directly in the gamma counting vials using 3.0 ml of concentrated nitric acid. The remaining organs were wet weighed and then dissolved in 8 ml of concentrated nitric acid. After 12 hours, sufficient nitric acid was added to yield 10.0 ml of solution. Three milliliters of this solution were then transferred to a gamma counting vial for counting. The blood samples were also dissolved in sufficient nitric acid to make 3 ml. All samples were then counted for a period of 40 minutes on a Nuclear Chicago Automatic Gamma Well Counter. Experiments were also done where alternate sample and background vials were counted and it was found that background counts did not change significantly throughout the counting period. Therefore, background counts were determined at the beginning and end of each run and the average was used to obtain corrected data counts. The per cent of injected dose was then determined for each tissue and the mean and standard error of the mean for six animals was calculated using standard techniques. Values which deviated more than normally expected were rejected using the Chauvenet Criterion (288) and new means

and their standard error were calculated neglecting this rejected data. For organs of major uptake (liver, kidneys, muscle, bone, lung, pancreas and blood) experiments were repeated to replace data that had been rejected so that all results were calculated using data obtained from six animals. All calculations were done using a digital PDP-8/L computer.

#### G. RENOGRAM STUDY

For the renogram study a male mongrel dog weighing 17 kg was used. The animal was allowed food and water ad libitum until the day of the study after which time all food was withdrawn. In this experiment, the animal was anesthetized using Sodium Pentobarbital Inj. U.S.P. (Abbott Laboratories Ltd., Montreal). A venocath<sup>®</sup>-18 (Abbott Laboratories, Chicago, Illinois) was inserted in the saphenous vein of the left hind leg and a bladder catheter (1550-Bardic 14) was also inserted. The animal was then transported to the Dr. W.W. Cross Cancer Institute (Edmonton, Alberta) and the renogram was performed in the Department of Nuclear Medicine. The dual probes were balanced using the  $^{65}\text{Zn}$ -EDTA solution that was to be injected and the discriminators adjusted to bracket the 1.114 Mev energy peak. For the study, the dog was positioned on its back and the probes were directed anterior to the kidneys as suggested by Winter (289). Location of the kidneys was determined by palpation and the probes positioned accordingly. For this study 100  $\mu\text{Ci}$  of  $^{65}\text{Zn}$ -EDTA in 2 ml of solution was injected into the

antecubital vein. Activity detected by each probe was recorded for 10-second intervals and the results obtained displayed on a Nuclear Chicago 400-channel analyzer. The activity was then recorded for another 10 second interval and the results displayed in the next channel. This process was repeated for a period of 30 minutes and at the end of this period all values were displayed on an oscilloscope. The activity obtained from the right kidney was displayed in the first 200 channels and the activity for the left kidney displayed in the next 200 channels. At the termination of the experiment a printout of the renogram results was made using a high speed printer (Series 1200, Franklin electronics inc., Bridgeport, Penna.). During the period of the study urine and blood samples were also obtained. The urine was allowed to drain into gamma counting vials for 1 minute periods beginning at the time of  $^{65}\text{Zn}$ -EDTA injection. Blood samples of 100  $\lambda$  were also withdrawn at 30 seconds post-injection and 1 minute post-injection. Samples were then withdrawn every minute for 34 minutes. These were transferred to gamma counting vials and diluted to 3.0 ml using concentrated nitric acid. All urine and blood samples were then counted on a Nuclear Chicago Automatic Gamma Well counter for a period of 40 minutes or until 100,000 counts were obtained.

#### H. SCAN USING $^{63}\text{Zn}$

Two vials, each containing 10 g of  $\text{ZnNa}_2\text{EDTA}$  were

positioned on the water cap of the tritium target assembly of the Cockroft-Walton accelerator and bombarded with 14 Mev neutrons for 1 hour at 120 Kev and 1 ma. At the end of bombardment, the vials were taken to the Dr. W.W. Cross Cancer Institute and sufficient activity was added to each of two thyroid phantoms (Picker Nuclear, 3602 Thyroid Phantom) to give reasonable count rates. Each phantom was then filled to capacity with distilled water. One phantom was scanned using a Nuclear Chicago Pho/Gamma III camera with a pinhole collimator and a depth of field of 3 cm. The time was recorded at the beginning of the scan and the picture exposed until 50,000 counts had been accumulated. The other phantom was scanned using an Ohio-Nuclear Inc. dual probe rectilinear scanner with an 8-inch crystal and a fine grain high energy collimator. The depth of field was 8 cm. The time was again recorded at the beginning of the scan. The scan was obtained using the following settings: intensity of 940, duration of 2, speed of 255 c/m and density information of 600 c/in.

At the completion of the scans, the activity in each thyroid phantom was determined. Five ml of solution were extracted from one of the thyroid phantoms and placed in a gamma counting vial. The counting vial was then positioned in front of a 1 1/2 inch by 1 inch NaI(Tl) crystal. A single channel analyzer was calibrated to count activity in the 0.511 Mev peak region. Activity from five milliliters was then accumulated for a period of 1 minute and the time

at the beginning of the counting period was recorded. This procedure was again repeated for five milliliters of solution obtained from the other thyroid phantom. A standard  $^{22}\text{Na}$  source containing  $2.8 \times 10^5$  dpm was then positioned in exactly the same position as the counting vials and activity accumulated for 1 minute. By allowing for decay of the standard since its calibration, and also that  $^{22}\text{Na}$  decays 90 per cent by positron emission (290) the theoretical activity of the  $^{22}\text{Na}$  standard could be determined. The background count rate was also determined for a period of ten minutes and this was subtracted from the activity obtained from the two thyroid phantoms and the  $^{22}\text{Na}$  standard source. By comparing the actual activity obtained from counting the  $^{22}\text{Na}$  standard with the theoretical activity the efficiency of detecting counts in the discriminator settings employed was determined. The counts recorded when counting the 5-ml phantom samples were then corrected using this efficiency factor and the actual activity in the 5-ml sample was determined by assuming that  $^{63}\text{Zn}$  decays by positron emission 93 per cent of the time (17). The actual activity in the total thyroid phantom was then calculated knowing the phantom volume to be 35 ml. Then, by employing the natural decay equation:

$$N = N_0 e^{-\lambda t}$$

and calculating the time between the beginning of each scan and the corresponding time for determination of activity in each phantom the activity present in each phantom used for

scanning was determined.

### I. AUTORADIOGRAPHY

Autoradiography was done on mouse kidneys at 30 seconds, 1 minute, 5 minutes and 1 hour after injection of 50  $\mu$ Ci of  $^{65}\text{Zn}$ -EDTA. The animals were decapitated, and the kidneys rapidly removed and blotted free from blood. The organ was then mounted on a holder containing O.C.T. (Temp. zone 0 to  $-15^{\circ}\text{C}$ , Lab-Tek, Illinois) and placed in an International-Harris Cryostat (model CT, International Equipment Co., Mass.) at  $-10^{\circ}\text{C}$ . When the kidney was sufficiently cooled, it was mounted on an International Minot Rotary Microtome (International Equipment Co., Mass.) and 8  $\mu$  sections taken. Sections were then transferred to Kodak Nuclear Track plates (Type NTB-3, 25 micron, Eastman Kodak Co., Rochester, N.Y.) in the dark and a precleaned and siliconized slide was clamped on top to assure that the section would remain flat. These slides were then transferred to light-tight boxes, placed in a refrigerator, and allowed to develop for 2 weeks. After this period, the exposed emulsion plates were developed in Kodak D-19 developer (Eastman Kodak Co., New York) for ten minutes, washed in water for 30 seconds, fixed in Kodak fixer (Eastman Kodak Co., New York) for 20 minutes and then washed for 1 hour.

#### **IV. RESULTS AND DISCUSSION**



#### A. PREPARATION OF ZINC-63

It was necessary to determine if zinc-63 could be prepared using the Cockroft-Walton accelerator, and if it could further be compounded in a form suitable for application in nuclear medicine. Two forms of zinc were considered for this study. The first form, zinc chloride, was selected since it had been previously used for liver and prostate scanning (291, 101, 102). Zn-EDTA was also selected as various EDTA chelates had been utilized in brain and kidney scanning (170, 171, 266, 169, 254) or kidney function tests (181, 259, 258).

##### 1. Production of $^{63}\text{Zn}$

For the production of  $^{63}\text{ZnCl}_2$ , it was possible to bombard the preparation directly. An ampoule was prepared containing 0.212 g of  $\text{ZnCl}_2$  dissolved in 1 ml of distilled water. This ampoule was taped on the back of the accelerator tritium target and bombarded with neutrons for a period of 5 minutes at a beam current of 1 milliamper (ma), and a high voltage of 120 Kev. After bombardment, the ampoule was detached and one milliliter removed by a syringe and placed in a gamma counting vial (this step was necessary to eliminate the possibility of analyzing any contamination due to activation of the glass ampoule). The counting vial was then placed in front of a lead-shielded 1 1/2 " x 1 " NaI(Tl) crystal. An energy spectrum (0 to 2.56 Mev) was stored for a counting period of 5 minutes in a Nuclear Chicago multi-channel analyzer. The results obtained are shown in figure 8.

One-minute counts, obtained by pulse height analysis of the 0.511 Mev energy peak region, were also recorded every five minutes for 115 minutes, and after subtraction of background, were plotted on semi-logarithmic graph paper (figure 9). Examining the energy spectrum obtained (figure 8), it was possible to detect a large peak corresponding to 0.511 Mev and two other peaks corresponding to 0.67 and 0.96 Mev, thus indicating that  $^{63}\text{Zn}$  was formed from the reaction,  $^{64}\text{Zn} (n, 2n) ^{63}\text{Zn}$  (196, 198, 292, 197). The expected peaks at 1.42, 1.55, 1.83, 2.04, 2.34 and 2.55 Mev were not apparent because of their low normal occurrence and the size of detector used. Since  $^{35}\text{Cl}$  has a cross section of about 4 millibarns for 14 Mev neutrons (compared to 167 for  $^{64}\text{Zn}$ ) (206), the probability of product formation from chlorine is minimal. Two other peaks were present at 75 Kev and 170 Kev. The 75 Kev peak was probably due to lead X-ray emission and the 170 Kev peak was probably due to backscattering from the 0.511 Mev peak. From the decay spectrum given in figure 9, it was apparent that only one major component existed and it had a half-life of 38 minutes which compares with the half-life of  $^{63}\text{Zn}$  reported by other investigators (196, 197, 198). It was therefore concluded that zinc-63 was the major isotope produced from the bombardment of  $\text{ZnCl}_2$ .

For the production of  $^{63}\text{Zn-EDTA}$ , two different approaches could be used. It is possible to bombard zinc in the form of its chloride, and then prepare the chelate; or the complex

Figure 8  
Energy Spectrum of 14 Mev Neutron-Bombarded  $\text{ZnCl}_2$

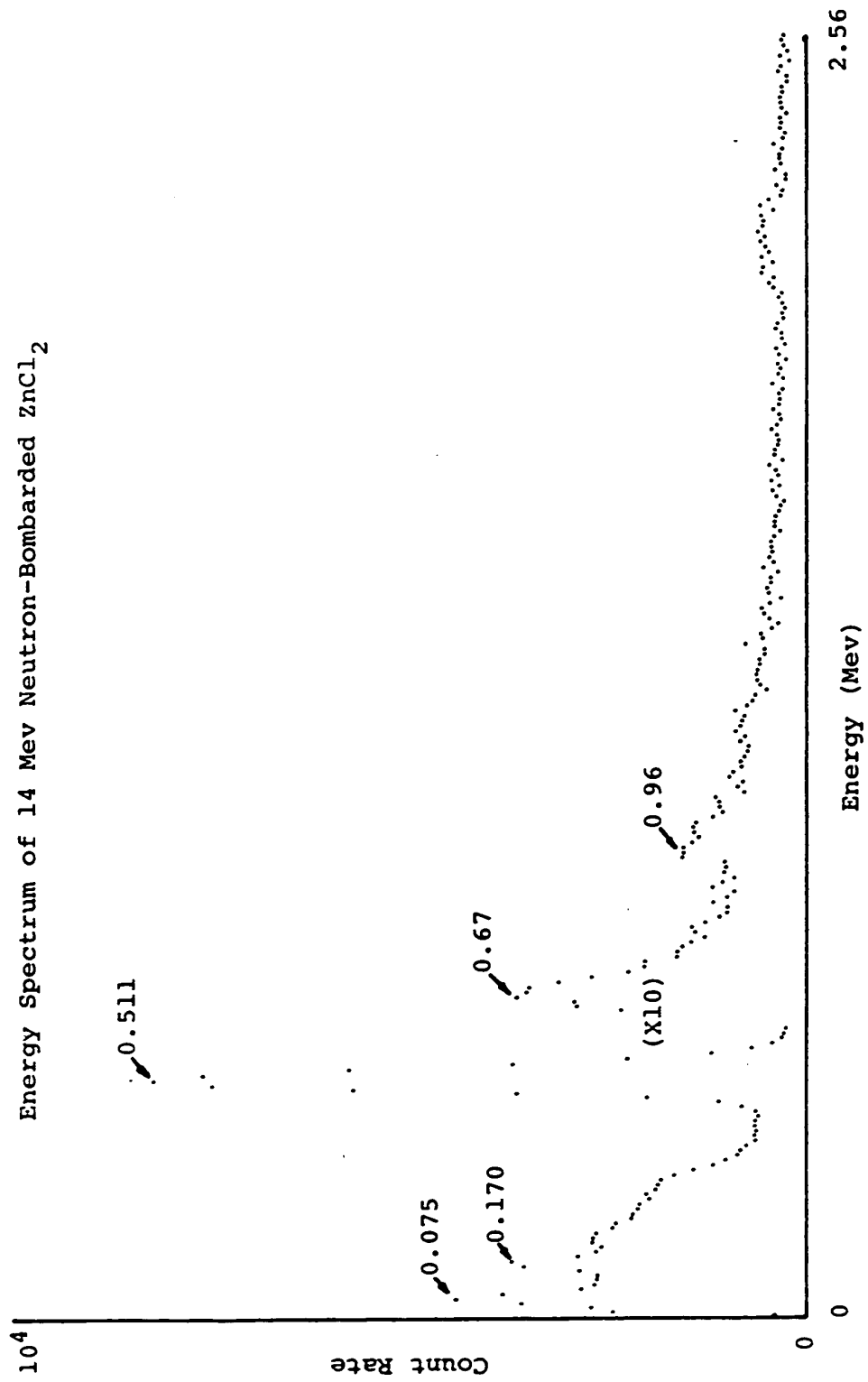
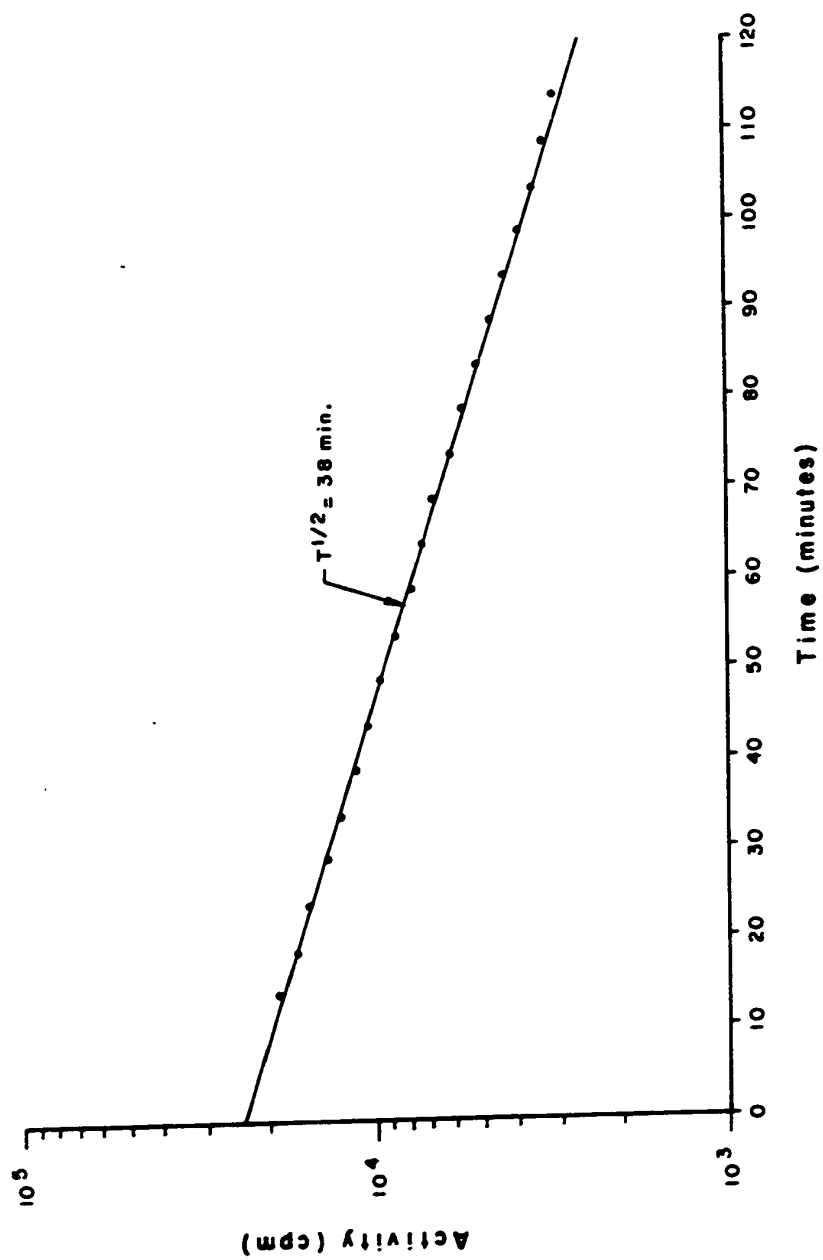


Figure 9  
Decay of Annihilation  $\gamma$ -Ray Peak After  
Bombardment of  $\text{ZnCl}_2$  with 14 Mev Neutrons



could be bombarded directly. Since it is highly desirable to avoid undue handling of the preparation in order to eliminate any possibility of contamination, the direct bombardment of Zn-EDTA was therefore preferred. Hence, a solution of the complex consisting of 0.6814 g of  $\text{ZnCl}_2$ , 1.9112 g of  $\text{Na}_2\text{EDTA}\cdot 2\text{H}_2\text{O}$  and 15 mg of  $\text{CaCl}_2$  dissolved in 10 ml of distilled water was prepared. This solution was sealed in an ampoule and taped to the front of the accelerator target assembly. After bombardment for 5 minutes at 1 ma and 120 Kev, 5 ml of solution were taken, added to a gamma vial and counted as before. The full energy spectrum (0 to 2.56 Mev) is shown in figure 10. One minute counts of the 0.511 Mev energy peak region were also taken at 2 minute intervals up to 90 minutes after the end of bombardment. The counting interval was then increased to 5 minutes until a total of 140 minutes had elapsed. The decay spectrum was then plotted on semi-logarithmic graph paper after subtraction of background (figure 11). It can be seen that the full energy spectrum obtained from bombardment of Zn-EDTA (figure 10) is identical to that obtained for  $\text{ZnCl}_2$  (figure 8) which would again indicate that  $^{63}\text{Zn}$  was formed. Again, the probability of product formation from chlorine was minimal due to its small cross section. The formation of  $^{23}\text{Na}$  would have also been insignificant due to its small cross section for fast neutrons (10). Two peaks are again present at 75 Kev and 170 Kev due to lead X-ray emission and back-scattering from the 0.511 Mev peak. From figure 11 it is

Figure 10  
Energy Spectrum of 14 Mev Neutron-Bombarded Zn-EDTA

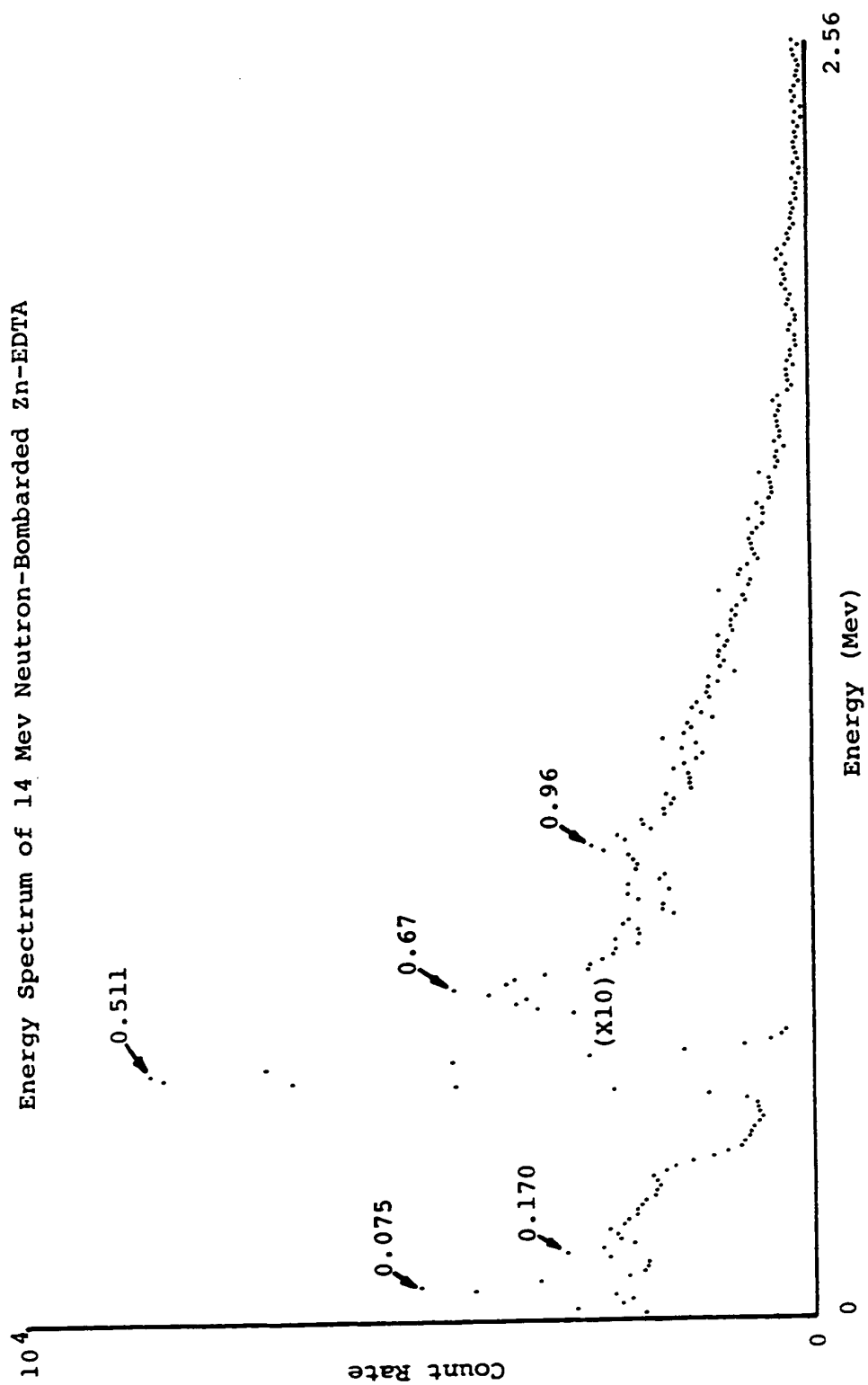
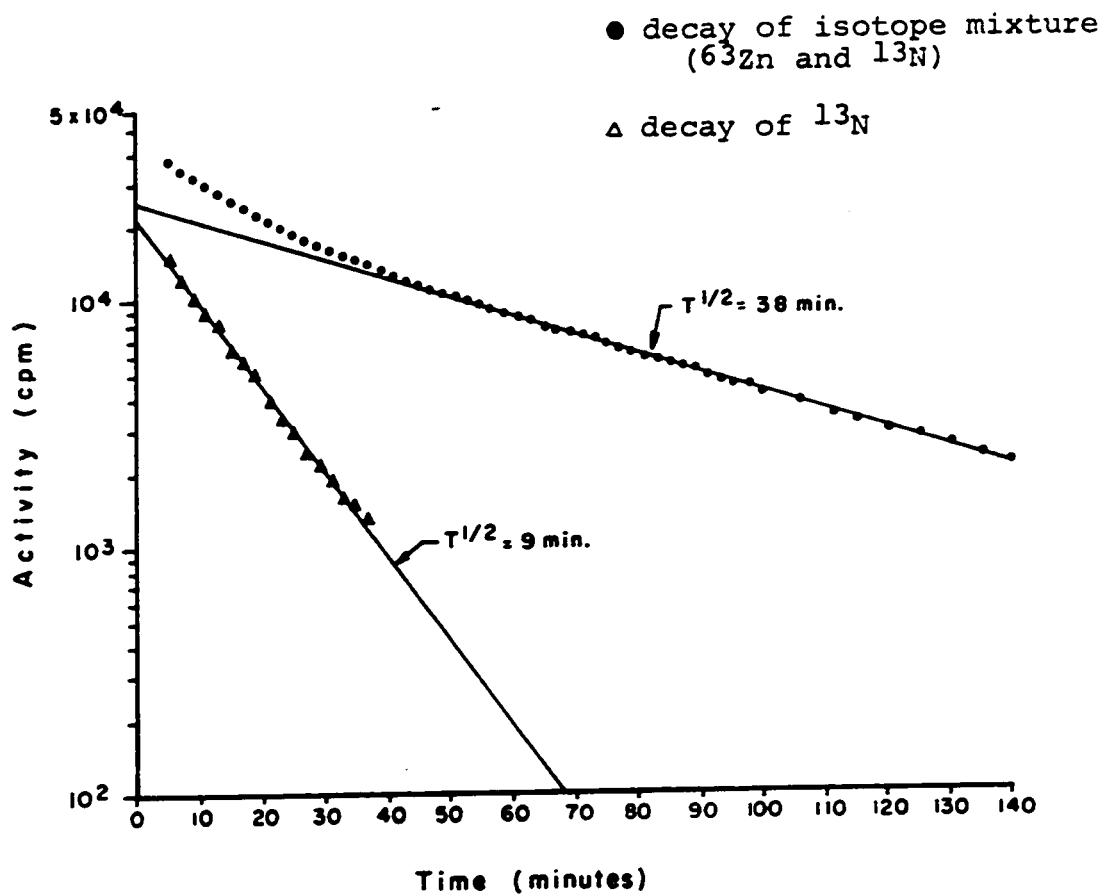


Figure 11

Decay of Annihilation  $\gamma$ -Ray Peak After 5 Minute  
Bombardment of Zn-EDTA with 14 Mev Neutrons



apparent that more than one component was decaying and by employing curve peeling, using manual spectrum stripping, two separate half-lives were determined. One half-life was found to be 38 minutes which was due to  $^{63}\text{Zn}$  and the other half-life was found to be 9 minutes. The 9 minute half-life was probably due to the decay of  $^{13}\text{N}$  produced from the reaction  $^{14}\text{N} (n, 2n) ^{13}\text{N}$ . The latter isotope has a reported half-life of 10 minutes (206), and although it has a small cross section (5 mb) for 14 Mev neutrons, it would appear more abundant as  $\text{ZnNa}_2\text{EDTA}$  contains two atoms of nitrogen for every one atom of zinc. No separate peaks were noticed on the full spectrum (figure 10) because  $^{13}\text{N}$  is a pure positron emitter.

## 2. Stability of $\text{ZnEDTA}$ Chelate

Although the direct bombardment of  $\text{ZnNa}_2\text{EDTA}$  solution offered the advantage that no manipulation of the preparation was necessary after sterilization, there was the possibility of decomposition. Some investigators have reported that the stability of drugs during neutron or gamma-ray bombardment is affected by radiation (293, 294). A series of ampoules were prepared, such that each 10 ml of solution contained 0.6814 g of  $\text{ZnCl}_2$ , 1.9112 g of  $\text{Na}_2\text{EDTA}$  and 15 mg of  $\text{CaCl}_2$  in distilled water. Two vials were bombarded for a period of 1 hour at 1 ma and 120 Kev. The optimum time for bombardment was calculated to be 85.8 minutes (see formula 2, appendix), knowing the half-life of the tritium target to be 100 minutes at 1 ma and 120 Kev (determined experimentally).



However, this time period was considered impractical for our purposes and a 1 hour bombardment time was chosen. After this, a further spectrum was recorded and it was found to be the same as that of figure 10. One of the solutions was evaporated to dryness under vacuum. The ZnEDTA complex was precipitated from the other solution by means of absolute ethanol as described by Sawyer and Paulsen (282). Control samples which were not exposed to neutron bombardment were treated in an identical fashion. Infra Red (I.R.) and Nuclear Magnetic Resonance (N.M.R.) spectra analyses were determined on the samples obtained by flash evaporation, and carbon, hydrogen and nitrogen analyses were performed on the samples obtained by alcohol precipitation. From the I.R. study (KBr disc) of the control preparations, peaks were noted at  $2920\text{ cm}^{-1}$  ( $-\text{CH}_2$ ),  $1600\text{ cm}^{-1}$ ,  $1400\text{ cm}^{-1}$ ,  $1320\text{ cm}^{-1}$ ,  $915\text{ cm}^{-1}$ ,  $840\text{ cm}^{-1}$  ( $-\text{C}=\text{O}$ ) and  $1105\text{ cm}^{-1}$  ( $-\text{C}-\text{N}$ ) and these values were comparable to those observed by Sawyer and Paulsen (143). This indicated the presence of  $\text{ZnNa}_2\text{EDTA}$ . After bombardment, no apparent change was noted in the I.R. spectrum and the previously mentioned peaks were evident. From the NMR study ( $\text{D}_2\text{O}$ ), two singlets were observed at 172 cps ( $-\text{CH}_2-\text{CH}_2-$ ) and 202 cps ( $-\text{CH}_2-\text{N}$ ). Integration of the spectrum revealed the singlet at 202 cps to be twice as large as the singlet at 172 cps of the control samples. After bombardment, no apparent change in the NMR spectrum was observed. Carbon, hydrogen and nitrogen analysis also revealed no change in

the chelate after bombardment. The chelate was assumed to have the following formula,  $\text{ZnNa}_2\text{EDTA} \cdot 3.5 \text{H}_2\text{O}$  (143). The percentage of C, H, N, calculated for the complex, were C, 25.95; H, 4.13; N, 6.06. The percentage of each found was C, 25.52; H, 4.00; N, 6.22.

From the above information it was felt that the Zn-chelate was indeed formed, and that no significant chemical changes occurred in the complex by exposing it directly to neutron bombardment.

#### B. TOXICITY STUDIES IN MICE

As mentioned previously, the method of Litchfield and Wilcoxon (283) was used to evaluate the  $\text{LD}_{50}$  of the injected solutions. This study was performed to determine the maximum intravenous dose of each form of injection that could be administered without harmful effects to the animal. Results for the tests were recorded 5 days post-injection although any deaths which occurred did so within one hour after injection.

##### 1. Intravenous $\text{LD}_{50}$ of $\text{ZnCl}_2$

The results for intravenous administration of increasing doses of  $\text{ZnCl}_2$  are shown in Table 1.

The value of  $(\text{Chi})^2$  for 5 degrees of freedom was found to be 11.1 (283). Since 1.0072 is less than 11.1, the data is not significantly heterogeneous. Therefore, the  $\text{LD}_{16} = 7.6 \text{ mg/Kg}$ , the  $\text{LD}_{50} = 9.3 \text{ mg/Kg}$ , and the  $\text{LD}_{84} = 11.8 \text{ mg/Kg}$ .

Table 1

Determination of Intravenous LD<sub>50</sub> for ZnCl<sub>2</sub> in Mice

Dose mg/Kg	Dead/ Tested	Observed % Dead	Expected % Dead	Observed - (minus) Expected	Contri- bution to (Chi) <sup>2</sup>
18.8	5/5	100 (99.7)	99.8	0.1	<0.001
14.0	5/5	100 (99.0)	97.0	2.0	0.0135
11.1	4/5	80	80.0	0.0	<0.001
9.9	3/5	60	60.0	0.0	<0.001
8.8	2/5	40	40.0	0.0	<0.001
7.8	0/5	0 (6.5)	22.0	15.5	0.13
7.0	0/6	0 (2.9)	9.0	6.1	0.05

Total animals = 36

Total = 0.1975

Number of doses, K = 7

(Chi)<sup>2</sup> = 0.1975 x 5.1 = 1.0072Animals per dose = 36/7  
= 5.1

Degrees of freedom, n = K-2 = 5

The slope function, s, is equivalent to

$$s = \frac{\text{LD}_{84}/\text{LD}_{50} + \text{LD}_{50}/\text{LD}_{16}}{2} = \frac{11.8/9.3 + 9.3/7.6}{2} = 1.24$$

The total number of animals tested at doses whose expected effects were between 16 and 84 per cent, N' = 20. The factor for LD<sub>50</sub>, fLD<sub>50</sub> = (s)<sup>2.77/√N'</sup> = (1.24)<sup>2.77/√20</sup> = 1.14.

Therefore, the lower limit = 9.3/1.14 = 8.2 mg/Kg and the upper limit = 9.3 x 1.14 = 10.6 mg/Kg. From the above data, the intravenous LD<sub>50</sub> of ZnCl<sub>2</sub> at the 95 per cent confidence level was found to be 9.3 (8.2 to 10.6) mg/Kg.

It is apparent that zinc chloride is very toxic when

administered intravenously and this has also been reported by Vallee (11) who found 2 mg of zinc gluconate per Kg of body weight was tolerated well but 4 mg per Kg produced lassitude, decreased tendon reflexes, bloody enteritis, diarrhea, and paresis of the hind legs in dogs. Gibson et al. (295) even reported that a dose as low as 1 mg/Kg of zinc gluconate temporarily depressed the leucocyte count in a dog. The Merck Index (296) lists the intravenous lethal dose of  $\text{ZnCl}_2$  in rats as 75 mg/Kg but does not list an  $\text{LD}_{50}$ . This value would seem high in view of our study; however, since mice were used in our experiment, this may account for the difference as the intraperitoneal injection of  $\text{ZnSO}_4$  is more toxic to mice than rats (297).

## 2. Intravenous $\text{LD}_{50}$ of $\text{ZnNa}_2\text{EDTA}$

The results for intravenous administration of increasing doses of  $\text{ZnNa}_2\text{EDTA}$  are shown in Table 2. Solutions containing 0.2726 g of  $\text{ZnCl}_2$  and 0.75450 g of  $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$  were prepared in various volumes of distilled water to make the required concentrations. The quantity of  $\text{Na}_2\text{EDTA}$  added was 50 mg in excess of the amount required to chelate the zinc in order to assure that all zinc ions would be chelated. The concentration of injection was calculated with respect to  $\text{ZnNa}_2\text{EDTA}$ . Solutions were administered at the rate of about 1 ml per 30 seconds.

The value of  $(\text{Chi})^2$  for 6 degrees of freedom was found to be 12.6 (283). Since 0.6451 is less than 12.6, the data is not significantly heterogeneous. Therefore, the

Table 2

Determination of Intravenous LD<sub>50</sub> for ZnNa<sub>2</sub>EDTA in Mice

Dose g/Kg	Dead/ Tested	Observed % Dead	Expected % Dead	Observed - (minus) Expected	Contri- bution to (Chi) <sup>2</sup>
2.00	2/2	100 (92.4)	73.0	19.4	0.2000
1.75	2/2	100 (91.0)	66.0	25.0	0.2900
1.50	3/5	60.0	58.0	2.0	0.0016
1.27	5/11	45.4	47.0	1.6	0.0010
1.01	4/12	33.3	34.0	0.7	<0.0010
0.90	3/10	30.0	28.0	2.0	0.0020
0.80	0/11	0 (6.6)	22.5	15.9	0.1400
0.33	0/17	0 (0.6)	1.9	1.3	0.0095

Total animals = 70

Total = 0.6451

Number of doses = 8

(Chi)<sup>2</sup> = 0.6451 x 8.75 = 5.6446

Animals/dose = 70/8

Degrees of freedom, n = 8-2 = 6

= 8.75

LD<sub>16</sub> = 0.68 g/Kg, the LD<sub>50</sub> = 1.32 g/Kg and the LD<sub>84</sub> = 2.60 g/Kg. The slope function, s, is equivalent to

$$s = \frac{\frac{LD_{84}/LD_{50} + LD_{50}/LD_{16}}{2}}{2} = \frac{\frac{2.60/1.32 + 1.32/0.68}{2}}{2} = 1.96$$

The total number of animals tested at doses whose expected effects were between 16 and 84 per cent, N' = 53. The factor for LD<sub>50</sub>, fLD<sub>50</sub> = (s)<sup>2.77/√N'</sup> = (1.96)<sup>2.77/√53</sup> = 1.29. Therefore, the lower limit = 1.32/1.29 = 1.02 g/Kg and the upper limit 1.32 x 1.29 = 1.70 g/Kg. From the above data, the intravenous LD<sub>50</sub> of ZnNa<sub>2</sub>EDTA at the 95 per cent confidence

level was found to be 1.32 (1.02 to 1.70) g/Kg.

### 3. Intravenous LD<sub>50</sub> of ZnNa<sub>2</sub>EDTA Plus CaNa<sub>2</sub>EDTA

The results for intravenous administration of increasing doses of ZnNa<sub>2</sub>EDTA with added CaCl<sub>2</sub> are shown in Table 3. Solutions containing 0.6814 g of ZnCl<sub>2</sub>, 1.9112 g of Na<sub>2</sub>EDTA·2H<sub>2</sub>O and 0.014 g of CaCl<sub>2</sub> were prepared in various volumes of distilled water to make the required concentrations. The amount of Na<sub>2</sub>EDTA added was 50 mg in excess of the quantity required to chelate the zinc ions. The quantity of CaCl<sub>2</sub> was calculated to chelate the excess Na<sub>2</sub>EDTA. The concentration of injection was computed with respect to ZnNa<sub>2</sub>EDTA, and solutions were administered at the rate of about 1 ml per 30 seconds.

Table 3  
Determination of Intravenous LD<sub>50</sub> for  
ZnNa<sub>2</sub>EDTA Plus CaNa<sub>2</sub>EDTA in Mice

Dose g/Kg	Dead/ Tested	Observed % Dead	Expected % Dead	Observed - (minus) Expected	Contri- bution to (Chi) <sup>2</sup>
2.42	5/5	100 (89.7)	55	34.7	0.5500
2.29	3/5	60	50	10.0	0.0400
2.16	2/5	40	46	6.0	0.0150
2.13	2/5	40	44	4.0	0.0065
2.01	3/5	60	38	22.0	0.2200
1.90	4/10	40	35	5.0	0.0110
1.79	0/5	0 (8.3)	31	22.7	0.2600
1.69	1/5	20	27	7.0	0.0250
1.60	0/5	0 (6.7)	23	16.3	0.1600

Total animals = 50

Total = 1.2875

Number of doses = 9

(Chi)<sup>2</sup> = 1.2875 x 5.6 = 7.210

Animals/dose = 50/9

Degrees of freedom = 9-2 = 7

= 5.6

The value of  $(\text{Chi})^2$  for 7 degrees of freedom was found to be 14.1 (283). Since 7.210 is less than 14.1, the data is not significantly heterogeneous. Therefore, the  $\text{LD}_{16} = 1.4$  g/Kg, the  $\text{LD}_{50} = 2.3$  g/Kg and the  $\text{LD}_{84} = 3.7$  g/Kg. The slope function,  $s$ , is equivalent to

$$s = \frac{\text{LD}_{84}/\text{LD}_{50} + \text{LD}_{50}/\text{LD}_{16}}{2} = \frac{3.7/2.3 + 2.3/1.4}{2} = 1.625$$

The total number of animals tested at doses whose expected effects were between 16 and 84 per cent,  $N' = 50$ . The factor for  $\text{LD}_{50}$ ,  $f\text{LD}_{50} = (s)^{2.77/\sqrt{N'}} = (1.625)^{2.77/\sqrt{50}} = 1.205$ . Therefore, the lower limit  $= 2.3/1.205 = 1.91$  g/Kg and the upper limit is  $2.3 \times 1.205 = 2.77$  g/Kg. From the above data, the intravenous  $\text{LD}_{50}$  of  $\text{ZnNa}_2\text{EDTA}$  plus  $\text{CaNa}_2\text{EDTA}$  at the 95 per cent confidence level was found to be 2.3 (1.91 to 2.77) g/Kg.

As is apparent, the  $\text{LD}_{50}$  for chelated zinc is much higher than that for zinc chloride. It can also be seen that the toxicity was reduced when the excess EDTA was administered as  $\text{CaNa}_2\text{EDTA}$  rather than  $\text{Na}_2\text{EDTA}$  and this is supported by the literature (298, 299, 145). It was also observed that rapid injection caused death whereas slow injection of the same dose showed no effect and this was probably due to the rapid lowering of serum calcium levels causing tetany (145, 147, 299). As mentioned by Foreman et al. (157), the  $\text{LD}_{50}$  for  $\text{CaNa}_2\text{EDTA}$  varies from 500 to 7,000 mg per Kg depending on the species and route of administration tested. Bauer et al.

(300) found that the acute intravenous administration of  $\text{CaNa}_2\text{EDTA}$  to rabbits gave an  $\text{LD}_{50}$  of less than 4 g per Kg but no deaths occurred at 2 g per Kg. However, the daily intravenous administration of 500 mg per Kg to dogs caused 50 per cent death in about 13 days. The authors also found that the acute intravenous administration of  $\text{PbEDTA}$  to rabbits gave an  $\text{LD}_{50}$  of 1 g/Kg. These acute dosages were much greater than the recommended therapeutic dosages of about 50 mg per Kg (160, 165, 157). The latter, however, were administered over a prolonged period. Foreman et al. (157) suggested that an acute injection of  $\text{CaNa}_2\text{EDTA}$  could possibly produce kidney lesions but the amount required would be lethal.

The relatively high  $\text{LD}_{50}$  found for  $\text{ZnNa}_2\text{EDTA}$  was expected in view of the report of Catsch (301) who found that  $\text{ZnDTPA}$  was less toxic than the corresponding calcium chelate. As mentioned previously, the formation of kidney lesions is thought to be related to the depletion of zinc or manganese (162), and by injecting the zinc chelate, this is avoided. The formation of  $\text{NaCl}$  during the production of the  $\text{ZnNa}_2\text{EDTA}$  may also have contributed to the low toxicity as Foreman and Nigrović (162) have noted that its presence afforded a marked protective action at high toxicity doses.

As a result of the toxicity studies, it was decided not to pursue the investigation of  $\text{ZnCl}_2$  any further. Although it has been used by other investigators in the form of  $^{69\text{m}}\text{ZnCl}_2$  or  $^{65}\text{ZnCl}_2$  for scanning (101, 291, 102), the



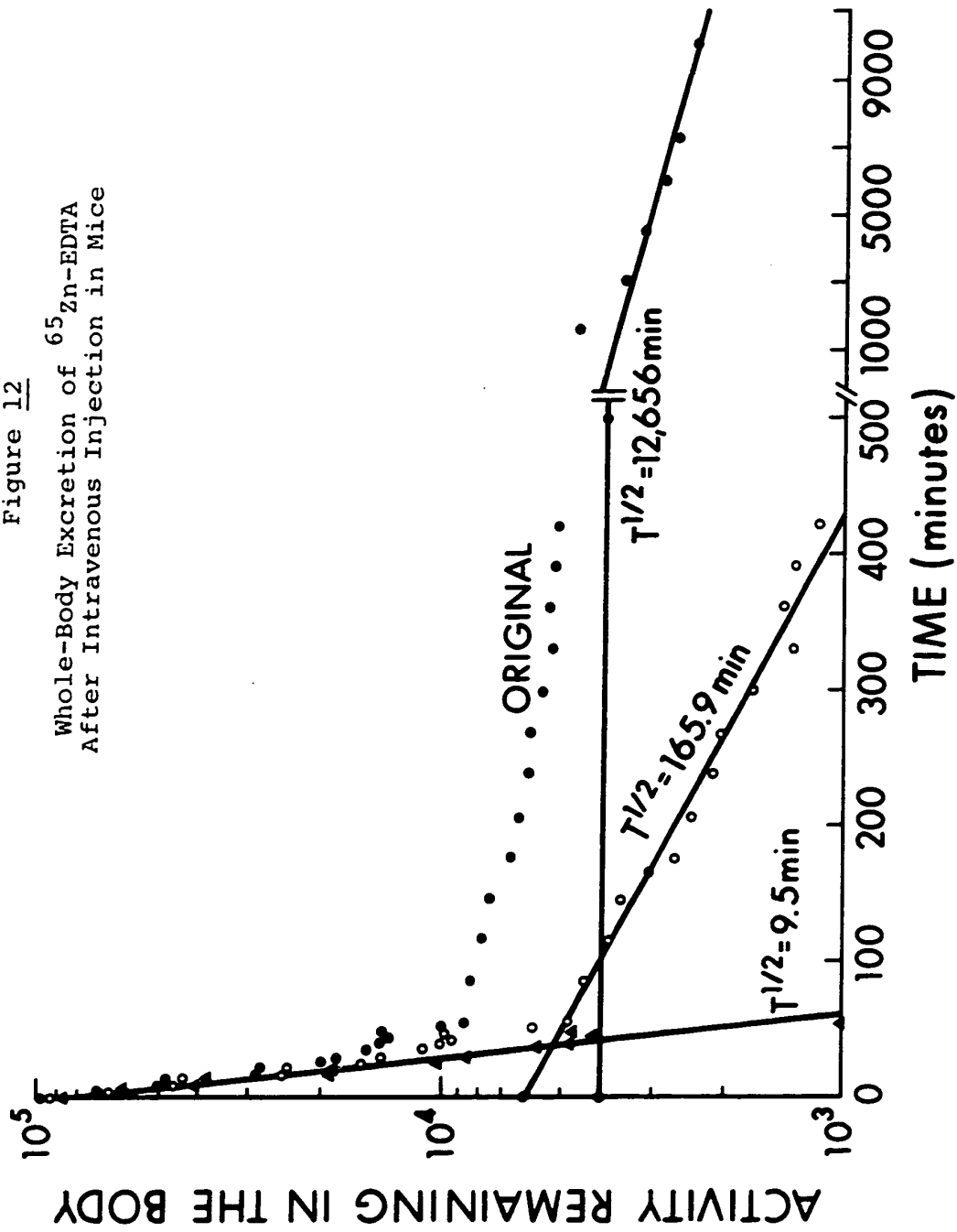
specific activities used were much higher than could be produced in our facility. Furthermore, high specific activities would be necessary because of its high toxicity. The zinc chelate, however, appeared to be relatively non toxic and further investigation was carried out using this compound.

C. EXCRETION AND BIOLOGICAL TURNOVER OF  $^{65}\text{Zn}$ -EDTA IN MICE

It was necessary to determine how fast Zn-EDTA is eliminated from the body and by what route. Therefore, mice were injected intravenously with 0.8 g/Kg of  $^{65}\text{Zn}$ -EDTA and the amount of activity remaining in the body was determined by whole-body counting. The urine and feces were collected for two days to determine the amount excreted by each route.

From the results obtained during whole-body counting, a decay spectrum was plotted for each mouse (figure 12). Each spectrum was then analyzed by employing curve peeling as described previously, and the various compartments were separated starting with the longest lived component. The latter was analyzed for a bound fraction according to the method of Dick and Lea (285). From this analysis, no bound fraction was apparent and the curve followed a series of exponential functions. As the first six minutes of whole-body counting gave essentially constant activity, this segment was eliminated from the spectrum. Therefore, zero time on the spectrum actually represents 6 minutes post-injection. The results obtained from each analysis were then pooled.

Figure 12  
Whole-Body Excretion of  $^{65}\text{Zn-EDTA}$   
After Intravenous Injection in Mice



The half-life and initial activity present in each component was determined (Table 4).

Table 4  
Whole-Body Excretion of  $^{65}\text{Zn}$ -EDTA in Mice

Mouse	Compartment					
	1		2		3	
	$T_b$ 1/2 <sup>a</sup>	Original %	$T_b$ 1/2 <sup>a</sup>	Original %	$T_b$ 1/2 <sup>a</sup>	Original %
1	10.5	91.5	186.8	14.1	13683	3.3
2	7.6	69.3	188.0	19.3	11267	3.3
3	13.0	78.1	160.0	22.4	14248	3.3
4	11.9	100.3	200.0	9.6	11859	4.0
5	9.8	93.0	210.8	10.9	12034	4.2
6	9.5	86.5	165.9	6.0	12656	4.0
<sup>b</sup>	10.4	86.4	185.1	13.8	12624	3.7
$\bar{X}$	$\pm 0.8$	$\pm 4.6$	$\pm 7.8$	$\pm 2.5$	$\pm 446$	$\pm 0.1$

- a. Biological half-life expressed in minutes
- b. Results expressed as mean  $\pm$  standard error of the mean

It is evident from these results that three compartments exist; a short lived compartment with a biological half-life of  $10.4 \pm 0.8$  minutes constituting about 86 per cent of the injected dose; a medium compartment with a biological half-life of  $185.1 \pm 7.8$  minutes, constituting about 14 per cent of the injected dose; and a long-lived compartment with a biological half-life of  $12,624 \pm 466$  minutes constituting about 4 per cent of the injected dose.

A series of mice were then injected with 0.8 g/Kg of

$^{65}\text{Zn}$ -EDTA and the urine and feces were quantitatively collected for a period of two days. The results of this study are shown in Table 5.

Table 5  
Excretion of  $^{65}\text{Zn}$ -EDTA in Urine and Feces of Mice<sup>a</sup>

Mouse	Urine	Feces
1	98.72	1.79
2	96.51	2.43
3	94.37	1.91
4	89.84	4.61
5	88.98	5.54
6	93.78	2.38
$\bar{X}^b$	93.70 $\pm$ 1.54	3.11 $\pm$ 0.64

a. Expressed as per cent of injected dose

b. Results expressed as mean  $\pm$  standard error of the mean

It can be seen that during this two-day period about 96.8 per cent of the injected dose had been eliminated. Of this, 93.7 per cent was excreted in the urine and 3.1 per cent was excreted in the feces.

A further study was performed to determine the chemical nature of the radioactive compound excreted. In this experiment, three mice were injected with the chelate solution (about 59  $\mu\text{Ci/ml}$ ), and the urine and feces collected for the periods 0-6 hours and 24 hours - 1 week post-injection, respectively. These two periods were felt to represent the

short and medium-lived compartments on one hand, and the long-lived compartment on the other hand.

Ten microliters of the urine samples obtained during the first six hours were spotted on chromAR<sup>®</sup> 500 sheets, and the chromatogram developed using a solvent system consisting of ethyl alcohol:water (1:1). Standards of  $^{65}\text{Zn-EDTA}$  and  $^{65}\text{ZnCl}_2$ , dissolved in urine, were also spotted on the same sheet. After development, an autoradiogram was performed using medical non screen X-ray film. The autoradiogram of the excreted urine sample exhibited one radioactive spot only, and the latter corresponded in its Rf value to  $^{65}\text{Zn-EDTA}$  ( $R_f = 0.78$ ).

On the other hand, analysis of the urine samples obtained during the period 24 hours - 1 week post-injection, showed no radioactivity present. This indicates that all of the urinary excretion occurred within 24 hours of injection.

Chemical analysis of radioactivity in feces was unsuccessful. Since ZnEDTA is easily hydrolyzable by acids, careful handling of the samples was necessary in order to avoid the appearance of any artifacts. Attempts of extraction with ethyl alcohol, chloroform, petroleum-ether, as well as percolation with water in a soxhlet apparatus for 24 hours, were unsuccessful in obtaining the radioactivity from the feces. A 24-hour incubation of  $^{65}\text{Zn-EDTA}$  solution and control feces showed that only 50% of the radioactivity could be recovered from the supernatant. It thus appears that the excreted zinc was firmly attached to certain molecules present in fecal matter.

From the results of these investigations, it would seem that  $^{65}\text{Zn}$ -EDTA was excreted mainly in the urine. This is in agreement with other investigators using EDTA chelates (165, 166, 146). Our study shows that 85 per cent of the injected dose was excreted in the first hour. This is slightly higher than the 60 per cent figure mentioned by Stand et al. (94). These same authors reported that 10 per cent of the injected dose was excreted in the feces, a value much higher than the 3 per cent excretion found by us. This could be attributed to the fact that they used a 1:2 metal-to-chelate ratio for their study and the excess chelating agent could have seriously altered the metabolism of ZnEDTA. About 96 per cent of the injected dose was excreted in 24 hours. This is in agreement with the results found by Foreman et al. (165). Foreman (173) also examined the excretion of  $^{65}\text{Zn}$ -EDTA using a whole-body counter. However, he only counted the mice for a period of 2 days and was able to detect only 2 compartments. Had he counted for a longer period, the long component might have become evident. He found that the fast component comprised 81 per cent of the injected dose which compares with the 86 per cent found in the present investigation. The 19 per cent found for the second component is slightly larger than the 14 per cent found by us. However, as mentioned previously, this could actually be composed of two components and therefore yielded a higher value.

The fast component was probably due to direct elimination of the chelate by glomerular filtration with no plasma

binding. The intermediate component may have been due to weak binding of the chelate to tissues or plasma proteins, but this fraction was also excreted quite rapidly via the kidney. Both these fractions consisted of undissociated  $^{65}\text{Zn-EDTA}$ . The slow fraction, however, was not as certain. It could be that chelated zinc was exchanged with endogenous zinc as found by others (185, 302). It is also possible that other metals or chelates in the body, due to their relative abundance or higher stability constants, replaced the zinc from the EDTA complex (174, 173). In either case, it would appear that the slow fraction consisted of unchelated zinc as the calculated half-life of  $12,624 \pm 466$  minutes for this component in the body compared with the 12180 minute half-life found by Gilbert and Taylor (89), after the injection of  $^{65}\text{Zn}$  labelled plasma. The possibility also exists that the long-lived compartment was due to chelated zinc in the body as evidenced by the presence of radioactivity in fecal material. However, this is unlikely as EDTA chelates do not pass the gastro-intestinal tract in the normal animal (164, 71).

#### D. TISSUE DISTRIBUTION IN MICE

Tissue distribution studies were done for all organs listed in Section III, part F. The results of this distribution are given in Table 6 for organs of minor uptake. Table 7 and figures 13 and 14 represent the data obtained for organs of major uptake. The bladder muscle, gallbladder,

Table 6  
Total Uptake of  $^{65}\text{Zn}$ -EDTA by Spleen,  
Heart, Testicles and Stomach of Mice<sup>a</sup>

Time	Spleen <sup>b</sup>	Heart <sup>b</sup>	Testicles <sup>b</sup>	Stomach <sup>b</sup>
2 min	0.14±0.02	0.15±0.01	0.18±0.02	0.35±0.03
6 min	0.10±0.02	0.12±0.01	0.15±0.005	0.20±0.01
12 min	0.08±0.01	0.10±0.01	0.12±0.02	0.12±0.01
18 min	0.07±0.01	0.08±0.01	0.12±0.02	0.14±0.02
24 min	0.07±0.01	0.06±0.01 <sup>c</sup>	0.10±0.02	0.09±0.01
30 min	0.02±0.004	0.02±0.004	0.03±0.01	0.04±0.003
40 min	0.03±0.01	0.03±0.004	0.03±0.004	0.05±0.02
50 min	0.01±0.004	0.01±0.003	0.02±0.005	0.02±0.002
1 hr	0.02±0.002	0.02±0.003	0.03±0.01	0.03±0.01
3 hr	0.01±0.003	0.01±0.003	0.03±0.01 <sup>c</sup>	0.03±0.004
5 hr	0.02±0.01	0.02±0.01	0.01±0.002	0.04±0.01
2 days	0.02±0.005	0.01±0.004	0.01±0.002	0.02±0.002
4 days	0.02±0.002	0.01±0.002	0.02±0.002	0.02±0.003
6 days	0.02±0.005	0.02±0.01	0.01±0.002	0.02±0.004
14 days	0.02±0.004	0.02±0.005	0.01±0.002	0.01±0.001

- a. Expressed as per cent of injected  $^{65}\text{Zn}$ -EDTA per total organ.
- b. Mean values from six mice ± standard error of the mean except where indicated.
- c. Mean values from five mice ± standard error of the mean.



Table 7

Total Uptake of  $^{65}\text{Zn}$ -EDTA by Liver, Kidney, Muscle, Bone, Lung, Pancreas and Blood of Mice<sup>a</sup>

Time	Liver <sup>e</sup>	Kidney <sup>e</sup>	Muscle <sup>b</sup>	Bone <sup>c</sup>	Lung <sup>e</sup>	Pancreas <sup>e</sup>	Blood <sup>d</sup>
2 min	1.92±0.09	5.59±0.44	16.40±0.65	3.78±0.12	0.86±0.07	0.33±0.03	12.77±0.77
6 min	1.29±0.08	4.95±0.22	11.66±0.68	2.87±0.14	0.66±0.08	0.27±0.01	9.48±0.33
12 min	0.87±0.07	3.62±0.38	8.02±0.88	1.78±0.14	0.41±0.05	0.16±0.01	6.59±0.27
18 min	0.72±0.04	3.11±0.80	6.20±0.37	1.73±0.10	0.37±0.04	0.19±0.01	4.40±0.44
24 min	0.73±0.08	2.31±0.23	4.75±0.32	1.34±0.12	0.21±0.03	0.16±0.01	3.00±0.41
30 min	0.43±0.02	1.39±0.03	1.67±0.18	0.89±0.08	0.08±0.01	0.08±0.01	1.37±0.08
40 min	0.39±0.02	1.22±0.20	1.38±0.33	0.79±0.11	0.08±0.01	0.11±0.01	0.83±0.07
50 min	0.40±0.02	0.87±0.10	0.62±0.10	0.62±0.06	0.06±0.01	0.11±0.01	0.55±0.05
1 hr	0.38±0.01	0.80±0.09	1.13±0.24	0.66±0.14	0.05±0.01	0.11±0.01	0.54±0.02
3 hr	0.48±0.03	0.38±0.05	0.29±0.04	0.74±0.05	0.04±0.01	0.18±0.02	0.21±0.03
5 hr	0.58±0.01	0.20±0.01	0.48±0.10	0.60±0.04	0.03±0.01	0.13±0.02	0.13±0.01
2 days	0.37±0.03	0.06±0.004	0.33±0.05	0.44±0.07	0.02±0.01	0.06±0.01	0.06±0.01
4 days	0.24±0.02	0.04±0.004	0.28±0.05	0.54±0.05	0.02±0.002	0.03±0.01	0.03±0.01
6 days	0.17±0.01	0.03±0.01	0.56±0.05	0.62±0.06	0.02±0.01	0.03±0.01	0.04±0.01
14 days	0.12±0.01	0.02±0.003	0.38±0.02	0.36±0.06	0.02±0.004	0.01±0.003	0.03±0.01

a. Expressed as per cent of injected  $^{65}\text{Zn}$ -EDTA per total organ.

b. Total muscle was calculated on basis of 45 per cent of body weight (94).

c. Total bone was calculated on basis of 6 per cent of body weight (94).

d. Total blood was calculated on basis of 77.8 ml/Kg body weight (303).

e. Mean values from six mice ± standard error of the mean.

Figure 13

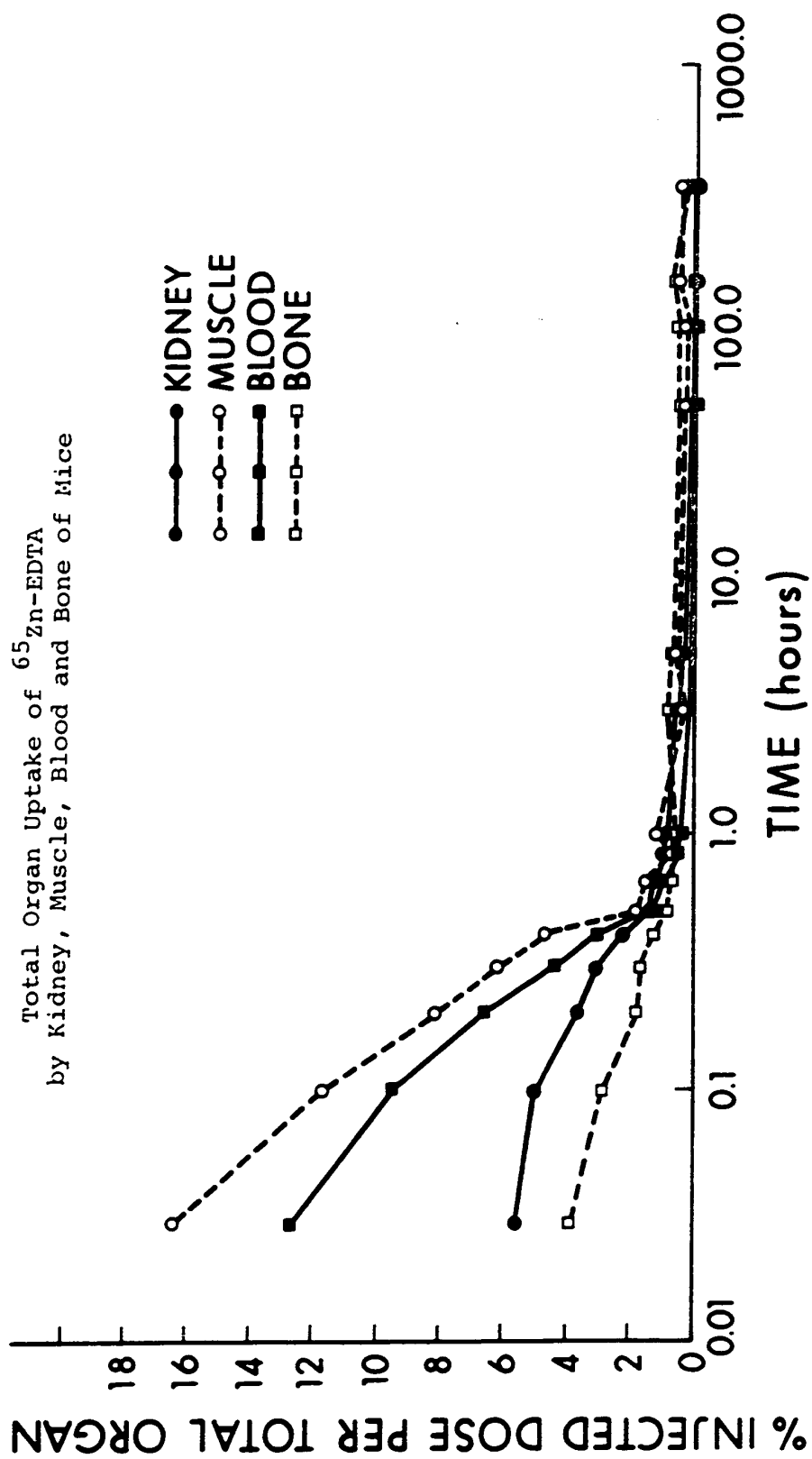
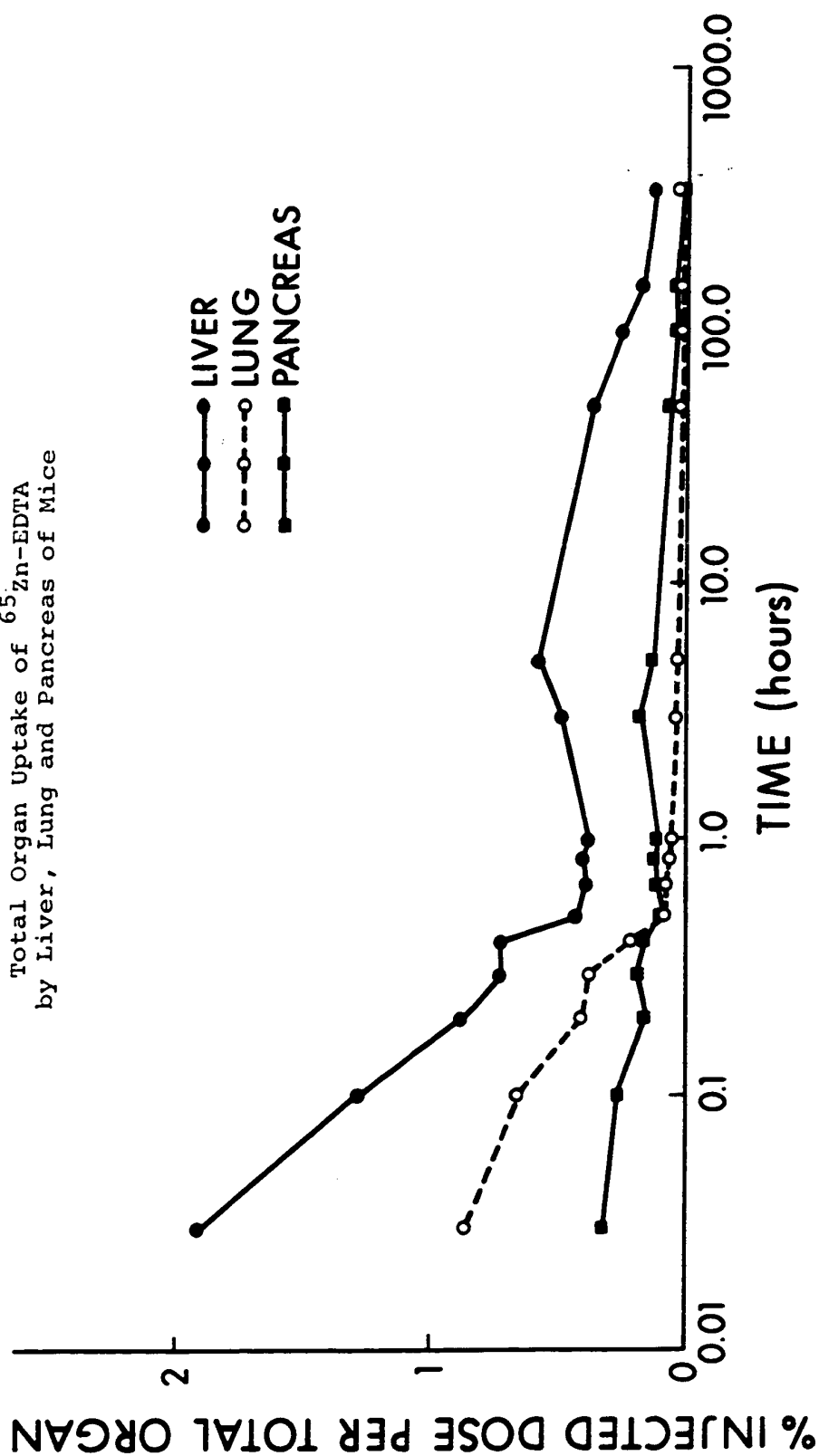


Figure 14  
Total Organ Uptake of  $^{65}\text{Zn-EDTA}$   
by Liver, Lung and Pancreas of Mice



brain, eyes and adrenals were also analyzed but their uptake was less than 0.1 per cent of the injected dose and therefore their results were not reported. It should also be noted that the tail was analyzed at the site of injection and was sometimes found to contain a maximum of 1 per cent of the injected dose. As seen in Table 6, the total organ uptake for the spleen, heart, testicles and stomach was extremely low and it was apparent that these organs did not concentrate  $^{65}\text{Zn-EDTA}$ . From Table 7 and figures 13 and 14, it could be seen that  $^{65}\text{Zn-EDTA}$  accumulation was greatest in the muscle, then blood, kidney, bone, liver, lung and finally pancreas, consecutively. All of these organs exhibited a rapid decrease in activity during the first 50 minutes. The liver showed a slight increase in activity at 3 hours post-injection and this increase continued until 5 hours post-injection. After this period, a steady decrease in activity was observed. The decrease in activity for the initial period showed a half-life of 15.6 minutes. The kidney exhibited a rapid decay which continued throughout the duration of the experiment. Compartmental analysis of its decay revealed three compartments; a long compartment of 11,517 minutes, a medium compartment of 117 minutes and a short compartment of 12.4 minutes. These compare reasonably well with the compartments determined during whole body analysis. After the initial rapid decrease of activity in the muscle, there was an increase at 1 hour. This was then followed by another decrease at 3 hours after

which the activity remained fairly constant throughout the duration of the experiment. Analysis of the initial portion of the curve for muscle uptake revealed a half-life of 10.5 minutes which is also comparable to the short-lived compartment in the whole-body study. The bone also showed an apparent increase at 1 hour after which the activity remained fairly constant. A half-life of 16.6 minutes was found for the initial segment of this curve. The lung showed a constant decrease in activity until 2 days post-injection after which the activity remained constant. In the case of pancreas, there was a decrease in activity for the first 30 minutes followed by an increase at 40 minutes which continued until 3 hours post-injection. After this, the activity decreased until the end of the experiment. The blood also exhibited a decrease in activity which continued throughout the investigation. Three compartments were determined having half-lives of 36,489 minutes, 85.2 minutes and 9.2 minutes. Although the long and medium compartments do not compare with those determined by whole-body analysis, the short-lived compartment does. It is possible that the medium and long-lived compartments were inaccurate due to the low levels of activity analyzed.

It is apparent that zinc injected as the EDTA chelate was rapidly eliminated from the body and no organ appeared to accumulate the chelate for significant periods of time. This has also been reported by Foreman et al. (1965). After

a period of about 1 hour, very little activity remained in the organs analyzed and the activity remaining was eliminated very slowly. It is felt that this prolonged retention was due to unchelated zinc which had either undergone isotopic exchange or had been replaced as mentioned previously.  $^{65}\text{ZnEDTA}$ , however, was rapidly excreted which would agree with Foreman and Trujillo (166) who recovered almost all of their  $\text{CaNa}_2\text{EDTA-}^{14}\text{C}$  in the urine by 24 hours. About 96 per cent of  $^{65}\text{Zn-EDTA}$  was eliminated after 24 hours. Brahm-anandam et al. (174) have also found the majority of injected  $^{65}\text{Zn-EDTA}$  to be located in the blood, muscle, bone, kidneys and lungs and that excretion was very rapid. The latter authors reported a significant amount of activity was found in the skin but this was not analyzed during the present study. They also found an increase in activity in the muscle between 10 and 15 minutes post-injection which was not observed in the present investigation. However, their experiments were performed on rats which might account for the difference.

When the concentrations of the labelled chelate were expressed relative to the blood (Table 8, figures 15 and 16), the only organ that appeared to rapidly concentrate the chelate was the kidney. In the liver, the ratio of organ-to-blood was below 1 for 40 minutes post-injection. It then showed a continuous rise until 2 days post-injection after which it began to decline. The kidney showed a constant ratio for the first 12 minutes after which it increased

Table 8  
Organ/Blood Distribution of  $^{65}\text{Zn}$ -EDTA in Mice a

Time	Liver Bloodb	Kidney Bloodb	Lung Bloodb	Muscle Bloodb	Bone Bloodb	Pancreas Bloodb
2 min	0.20±0.01	2.31±0.18	0.51±0.03	0.23±0.02	0.39±0.02	0.29±0.01
6 min	0.19±0.01	2.53±0.13	0.66±0.05	0.22±0.01	0.41±0.02	0.30±0.01
12 min	0.24±0.02	2.87±0.24	0.86±0.06	0.22±0.01	0.43±0.04	0.36±0.02
18 min	0.26±0.01	3.25±0.40	0.90±0.09	0.24±0.02	0.48±0.02	0.53±0.04
24 min	0.43±0.05	3.97±0.45	0.95±0.05	0.29±0.03	0.62±0.07	0.84±0.09
30 min	0.39±0.03	5.50±0.38	0.70±0.05	0.22±0.03	0.96±0.09	0.87±0.10
40 min	0.68±0.05	7.46±1.07	1.04±0.09	0.30±0.03	1.22±0.14	1.52±0.14
50 min	1.00±0.07	8.12±0.86	1.18±0.10	0.19±0.03	1.34±0.10	2.51±0.29
1 hr	1.10±0.06	6.95±0.68	1.26±0.09	0.33±0.06	2.00±0.22	3.06±0.20
3 hr	3.18±0.26	10.92±0.89	2.06±0.22	0.29±0.05	4.78±0.50	9.48±0.82
5 hr	6.52±0.57	8.28±0.46	3.09±0.35	0.66±0.11	5.57±0.42	9.70±0.93
2 days	17.01±0.98	7.11±1.30	3.57±0.66	0.90±0.21	7.40±1.25	9.24±1.06
4 days	11.00±2.00	6.25±1.08	9.00±1.56	1.33±0.25	16.92±2.17	8.17±0.65
6 days	6.36±0.49	4.75±0.92	5.30±0.78	2.86±0.40	21.64±0.92	8.22±0.56
14 days	4.83±0.86	2.67±0.36	3.58±0.37	2.00±0.32	10.67±1.96	3.50±0.36

a. Expressed as ratio of per cent injected dose per g of organ to per cent injected dose per ml of blood.

b. Mean values from six mice ± standard error of the mean.

Figure 15  
Organ/Blood Distribution of  $^{65}\text{Zn-EDTA}$   
in Kidney, Lung and Muscle of Mice

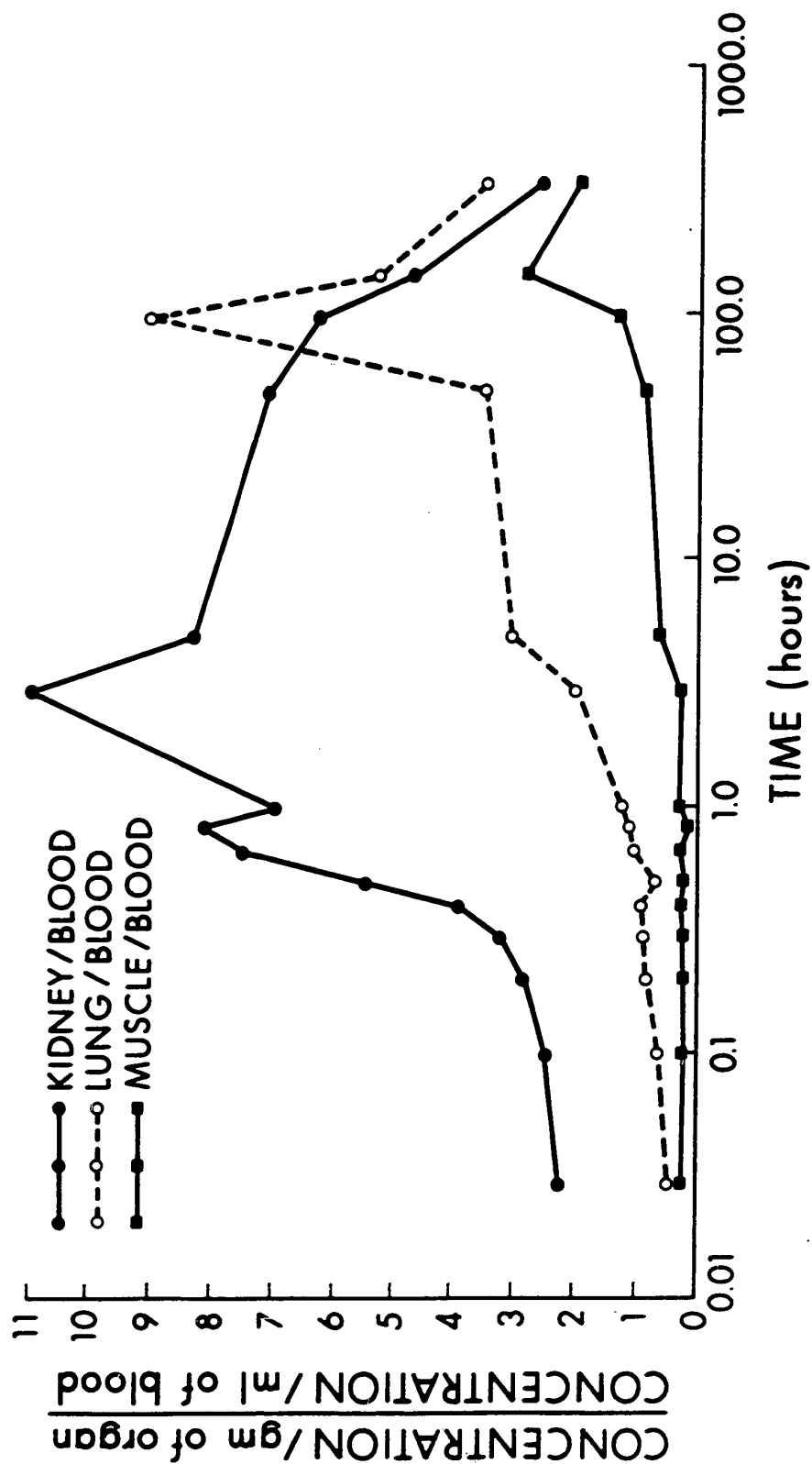
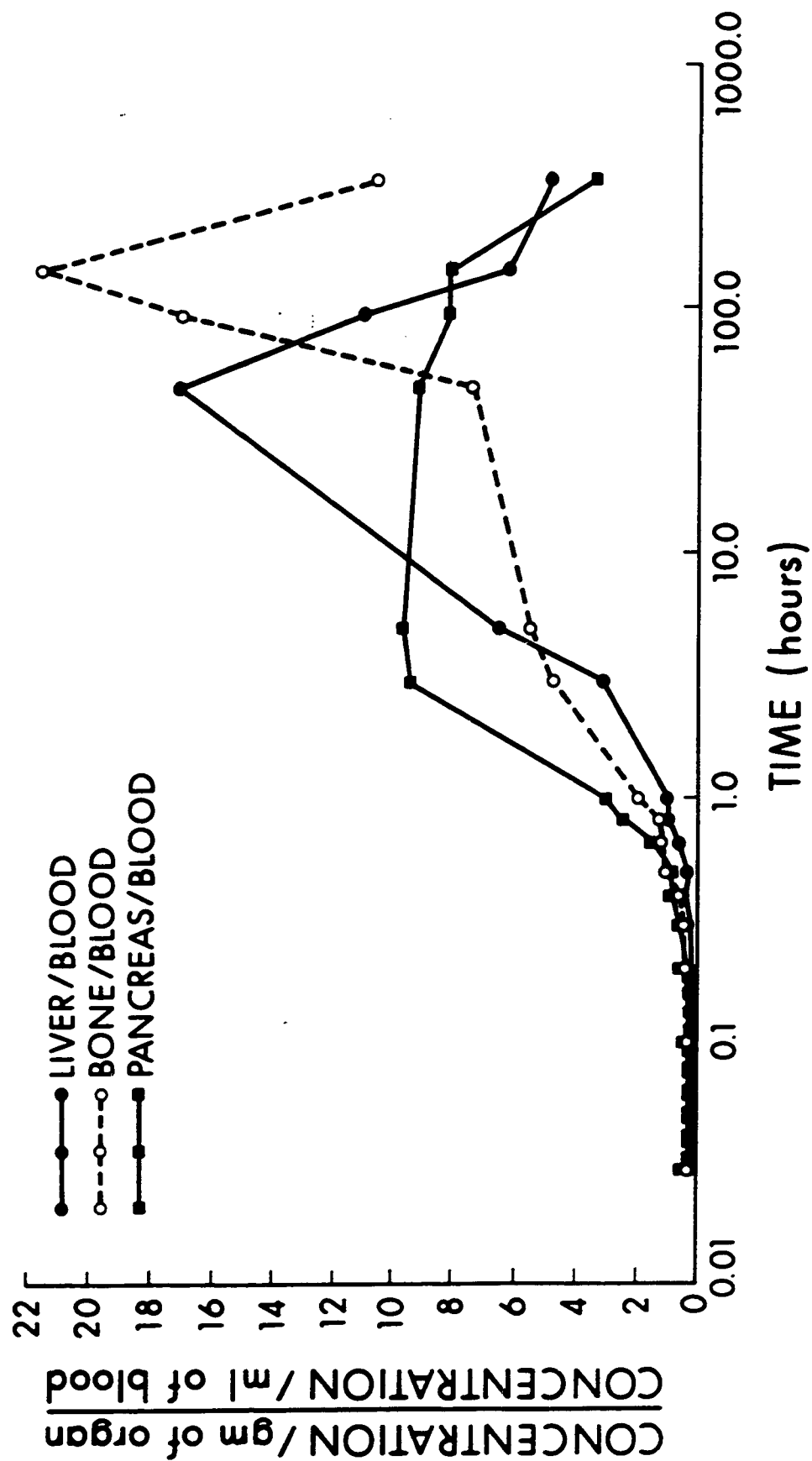




Figure 16  
Organ/Blood Distribution of  $^{65}\text{Zn-EDTA}$   
in Liver, Bone and Pancreas of Mice



until 3 hours. It then declined until the end of the experiment. The lung ratio remained fairly constant for the first hour after which it increased until 4 days post-injection. It then declined until the end of the experiment. The muscle remained essentially constant for the 3 hours post-injection. The ratio then began to increase slowly until day 6 after which it started to decline. The bone had a ratio of less than one for 30 minutes after injection. This ratio then increased until day 6 post-injection after which it started to decline. The ratio for the pancreas was also less than one for the first 30 minutes. It then increased until 5 hours post-injection after which it slowly decreased until day 14.

From these results, it would appear that the kidney is the only organ which rapidly accumulates the radioactive  $^{65}\text{Zn}$  compound. The other organs had ratios of less than one during the period when most of the chelate was eliminated from the body. This may indicate that their activity was mainly due to blood in the organ. After this initial phase, all organs examined showed an increase in the ratio of organ-to-blood which could indicate that the organs were concentrating radioactivity. Although the actual activity of the organs was very low after 1 hour (Table 7), their relative concentrations were higher than that of the blood. It is also significant to note that the pancreas, bone, lung and liver which showed the greatest increases in ratio are also organs which are reported to concentrate injected

$\text{ZnCl}_2$  (94, 86, 304, 88). This would further suggest that some of the zinc-65 had been exchanged or had been replaced from the chelate. Initially, this uptake was not apparent due to the high blood levels. However, there could have been a release of zinc from the protein complexes, reabsorption by the renal tubules, and redistribution in the various organs (94).

The relative uptake of  $^{65}\text{Zn}$ -EDTA in various organs as a function of time has also been calculated (Table 9, figures 17 and 18). It can be seen that the greatest relative uptake was in the kidneys followed by blood, lung, bone, pancreas, muscle and liver. Again, it is apparent that the chelate was rapidly eliminated from all the organs. The liver concentration declined rapidly for the first 40 minutes. The concentration then remained fairly constant until 3 hours post-injection. At 5 hours post-injection, it rose slightly and then continued to drop until day 14. The kidney exhibited a continuous decrease in activity throughout the entire experimental period. In the case of muscle, there was a continuous decrease in activity until 50 minutes post-injection. A slight increase then occurred at 1 hour after which the activity remained fairly constant throughout the investigation period. There was a continuous decrease in concentration in the bone for 50 minutes post-injection. At 1 hour, there was a slight increase which remained fairly constant until 4 days post-injection. At 4 days, there was another increase, reaching a peak at 6 days.

Table 9  
Relative Organ Uptake of  $^{65}\text{Zn-EDTA}$  in Mice<sup>a</sup>

Time	Liver <sup>b</sup>	Kidney <sup>b</sup>	Muscle <sup>b</sup>	Bone <sup>b</sup>	Lung <sup>b</sup>	Pancreas <sup>b</sup>	Blood <sup>b,c</sup>
2 min	1.56±0.12	18.31±2.08	1.77±0.14	3.05±0.19	4.00±0.21	2.24±0.08	7.84±0.37
6 min	0.78±0.06	10.50±0.74	0.91±0.07	1.69±0.13	2.68±0.18	1.27±0.10	4.58±0.17
12 min	0.55±0.05	6.32±0.56	0.51±0.07	0.88±0.07	1.96±0.23	0.88±0.06	2.26±0.22
18 min	0.46±0.04	5.73±0.66	0.42±0.03	0.86±0.06	1.70±0.22	0.92±0.06	1.80±0.16
24 min	0.45±0.04	4.17±0.38	0.31±0.02	0.65±0.07	1.05±0.12	0.78±0.08	1.12±0.16
30 min	0.28±0.02	3.32±0.20	0.13±0.02	0.54±0.06	0.42±0.02	0.52±0.06	0.61±0.04
40 min	0.24±0.02	2.55±0.31	0.10±0.02	0.44±0.07	0.38±0.07	0.52±0.03	0.35±0.03
50 min	0.23±0.02	1.70±0.13	0.04±0.01	0.32±0.04	0.25±0.02	0.53±0.05	0.22±0.02
1 hr	0.24±0.01	1.67±0.22	0.08±0.02	0.40±0.07	0.27±0.03	0.68±0.06	0.23±0.01
3 hr	0.28±0.02	0.77±0.05	0.02±0.003	0.36±0.02	0.17±0.03	0.78±0.06	0.08±0.01
5 hr	0.34±0.02	0.42±0.02	0.04±0.01	0.29±0.02	0.16±0.02	0.52±0.07	0.05±0.01
2 days	0.23±0.03	0.12±0.01	0.03±0.01	0.23±0.04	0.10±0.02	0.31±0.05	0.02±0.01
4 days	0.14±0.01	0.09±0.01	0.02±0.004	0.34±0.04	0.12±0.01	0.14±0.02	0.02±0.002
6 days	0.19±0.04	0.09±0.02	0.06±0.01	0.47±0.03	0.11±0.02	0.16±0.03	0.02±0.002
14 days	0.08±0.02	0.04±0.01	0.04±0.002	0.23±0.05	0.08±0.01	0.07±0.01	0.02±0.002

a. Expressed as per cent of injected  $^{65}\text{Zn-EDTA}$  per g of organ weight except where indicated.

b. Mean values from six mice ± standard error of the mean.

c. Expressed as per cent of injected  $^{65}\text{Zn-EDTA}$  per ml of blood.

Figure 17  
Relative Uptake of  $^{65}\text{Zn-EDTA}$   
in Kidney, Lung and Blood of Mice

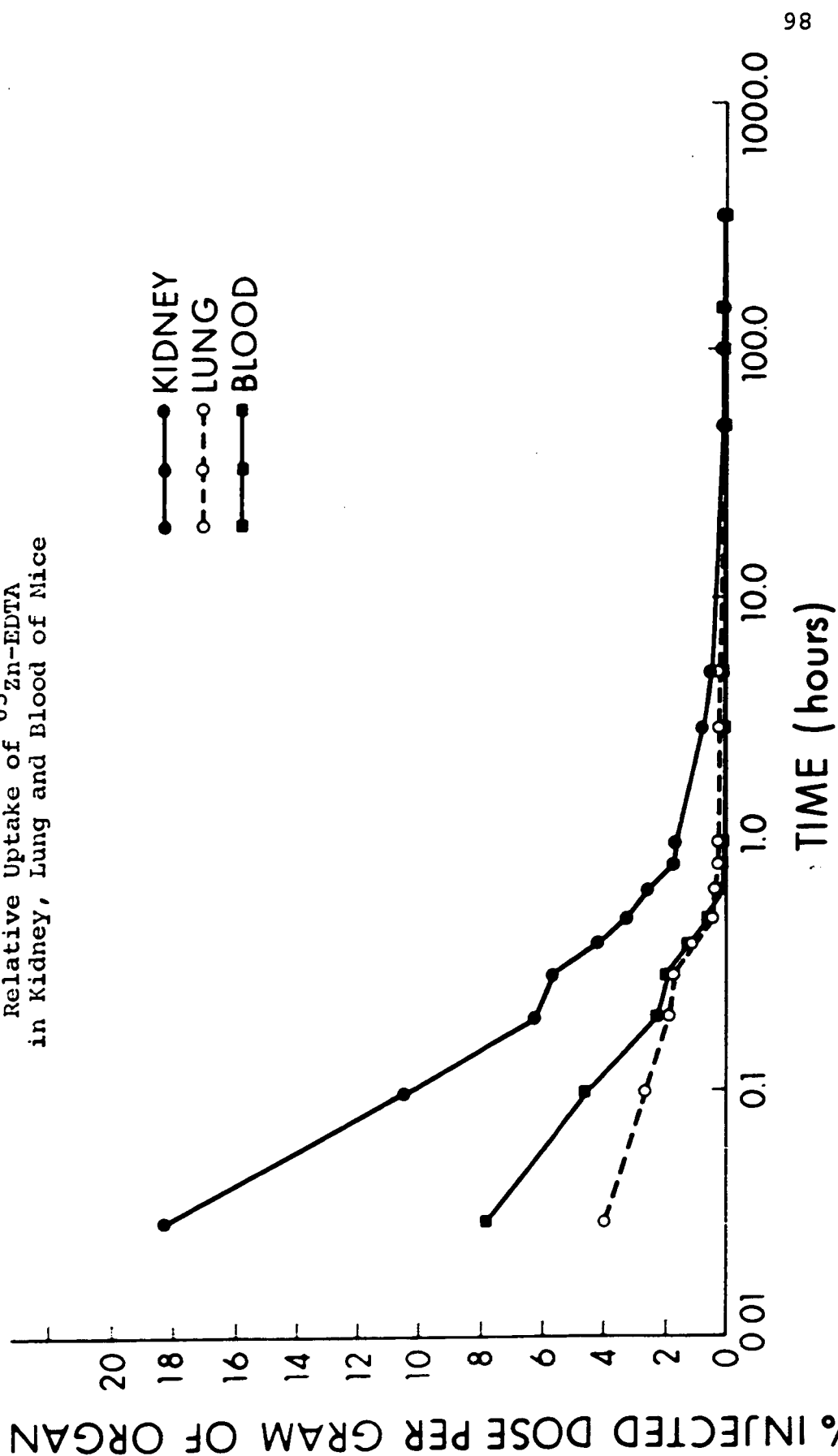
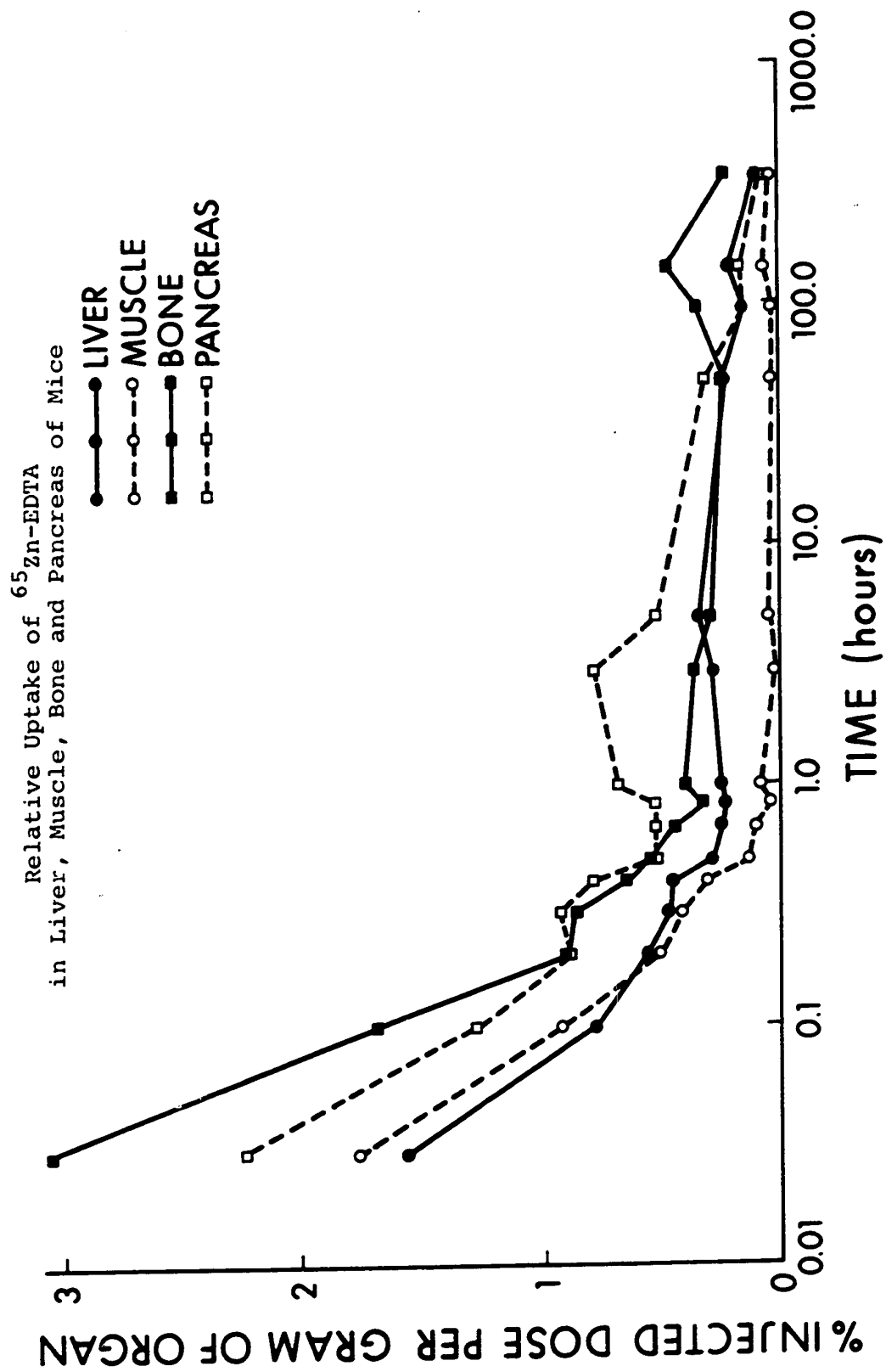


Figure 18



Activity was again decreased at day 14. There was a fairly rapid decrease in concentration in the lung for the first hour post-injection after which the activity remained quite constant. The pancreas exhibited a decrease in activity for the first 30 minutes. The activity then remained constant until 1 hour post-injection, when it again rose. This increase continued until 3 hours post-injection after which a continuous decrease occurred until day 14. The concentration of activity in the blood decreased until 2 days post-injection after which it remained constant.

Stand et al. (94) also found the greatest concentration of activity was in the kidney. However, they found the next greatest concentration to occur in the pancreas, followed by the liver, lung, bone, muscle and blood. When comparing the concentration between 1 and 24 hours post-injection, Stand et al. (94) found the pancreas, liver, bone, muscle, lung and blood to remain unchanged. However, by comparing the results obtained in the present investigation at 1 hour and 2 days, it is noted that the liver and muscle concentrations did not change significantly, but a large decrease in activity was observed in the bone, pancreas and blood. This difference could have been due to the difference in time period, or due to the excess EDTA that was added in the Stand experiment (94), which possibly changed the tissue distribution (174).

Autoradiography was also done on the kidneys at various time intervals after injection of about 50  $\mu$ Ci of  $^{65}\text{Zn}$ -EDTA

(figures 19, 20, 21 and 22). Figure 19 shows that the chelate was concentrated mainly in the cortex at 30 seconds post-injection. At five minutes post-injection (figure 20) it had progressed mainly to the medulla, calyces and renal pelvis. After 1 hour post-injection (figure 21), the activity in the kidney was extremely low.

Figure 22 demonstrates that at 1 minute post-injection, the chelated zinc was passing through the medulla and that the  $^{65}\text{Zn-EDTA}$  was concentrated in the collecting tubules.

From the results of tissue distribution, it is apparent that the kidneys accumulated a significant amount of the injected  $^{65}\text{Zn-EDTA}$  and rapidly eliminated it from the body.

#### E. RENOGRAM STUDIES USING $^{65}\text{Zn-EDTA}$ IN DOGS

Since it was evident that  $^{65}\text{Zn-EDTA}$  was rapidly eliminated from the mouse via the kidneys, and because of the relative amount of activity in the kidney when compared to the other organs, it was suggested that the compound be tested as a kidney scanning agent.

A renogram was performed on a 17 kilogram male dog to determine if the elimination of injected  $^{65}\text{Zn-EDTA}$  was comparable to that of the mouse. About 100  $\mu\text{Ci}$  of  $^{65}\text{Zn-EDTA}$  was injected into the antecubital vein of the dog and a renogram was recorded for a period of 30 minutes (figure 23). Analysis of urine results showed constant fluctuation with an apparent half-life of 17.9 minutes (figure 24). This compares with the results obtained by





Figure 19 Autoradiogram of a mouse kidney 30 seconds after injection of 50 $\mu$ Ci of  $^{65}\text{Zn}$ -EDTA X 8



Figure 20 Autoradiogram of a mouse kidney 5 minutes after injection of 50 $\mu$ Ci of  $^{65}\text{Zn}$ -EDTA X 8



Figure 21 Autoradiogram of a mouse kidney 1 hour after injection of 50 $\mu$ Ci of  $^{65}\text{Zn}$ -EDTA X 8



Figure 22 Autoradiogram of a mouse kidney 1 minute  
after injection of 50 $\mu$ Ci of  $^{65}\text{Zn}$ -EDTA

Magnification X 37

Activity concentrated in collecting tubules

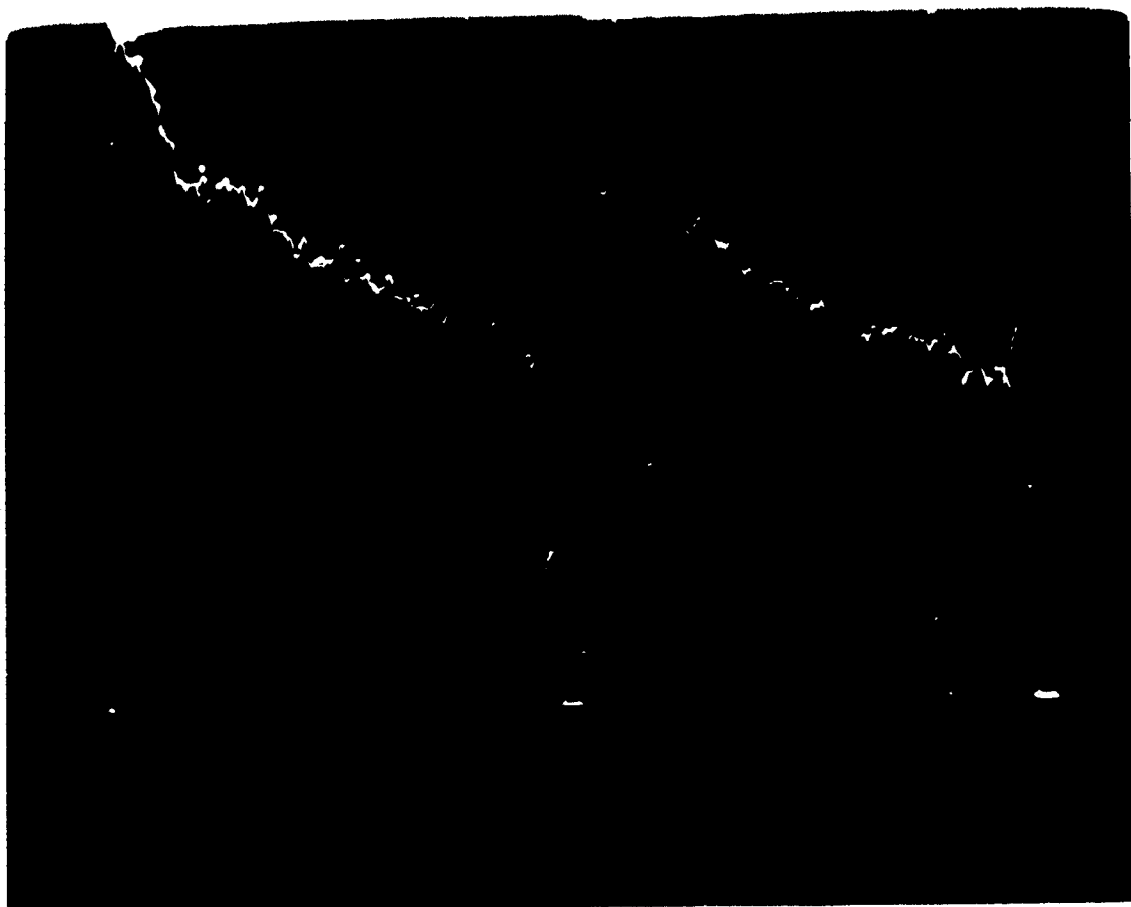


Figure 23 Renogram obtained from injection of  $^{65}\text{Zn}$ -EDTA in a dog

Myers and Diener (305) who found a clearance half-time of 15-20 minutes by frequent bladder washing of rats after injection of  $^{51}\text{Cr}$ -EDTA. Activity in the blood showed an initial rapid decrease having a half-life of 1.7 minutes, followed by a slow fraction with a half-life of 36 minutes (figure 25). It can be seen from figure 25 that the activity rapidly decreased in the blood during the first 10 minutes post-injection after which time it remained fairly constant for the duration of the renogram. Analysis of activity in the urine (figure 24) showed an initial lag time of 11 minutes before any activity was detected. The initial 6 minute delay was probably due to the dead space in the bladder catheter which had a volume of 1.8 ml and urine flow was at a rate of 0.3 ml per minute. It would therefore appear that a two minute delay occurred before detection of activity in the urine. These results essentially agree with those depicted in figure 23 where there was a 100-second delay before peak activity in the right kidney, and an 80 second delay before peak activity in the left kidney. This period is referred to as the "renal transit time" (217, p. 32). The biological half-life of  $^{65}\text{Zn}$ -EDTA in the canine kidney was calculated to be 38 minutes using the "drainage segment" of the renogram. This renal transport was slow when compared to the mouse but other investigators have found a prolonged "drainage segment" when using  $^{51}\text{Cr}$ -EDTA in humans (306).

Figure 24  
Excretion of  $^{65}\text{Zn}$ -EDTA in Dog Urine

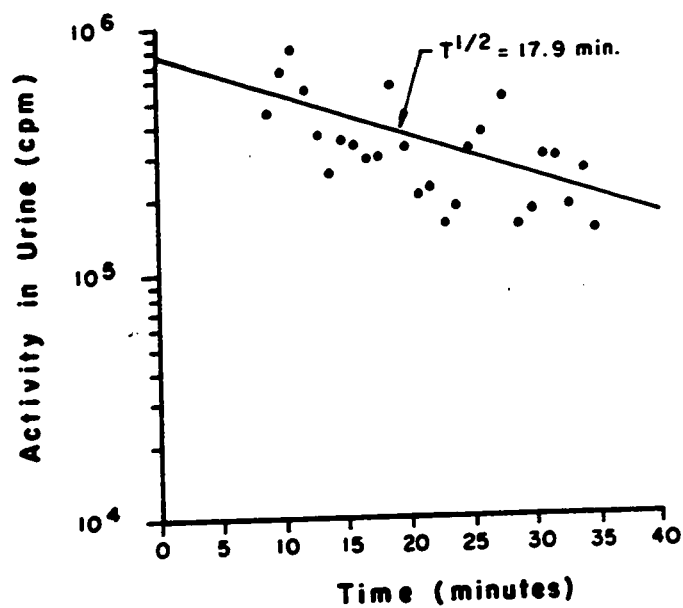
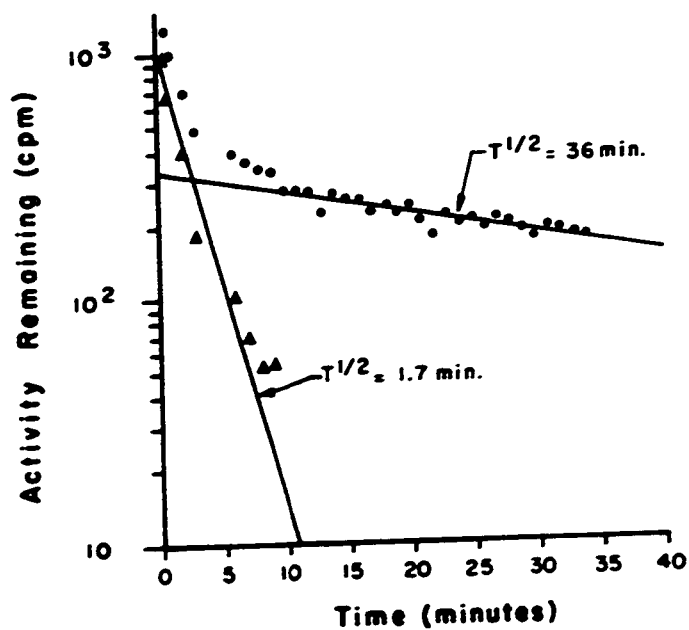


Figure 25  
Elimination Rate of  $^{65}\text{Zn}$ -EDTA from Dog Blood



#### F. SCAN OF THYROID PHANTOM USING $^{63}\text{Zn-EDTA}$

From the results of the investigation with mice and the renogram study in dogs, the possibility of using  $^{63}\text{Zn-EDTA}$  in kidney scanning was further explored. Because of the physical distance between the accelerator and scanning laboratories and since the half-life of  $^{63}\text{Zn}$  is only 38 minutes, it was not possible to obtain a kidney scan within the limits of toxicity as determined previously. Extensive decay had occurred during the transportation period. It was therefore decided to test the technique with larger quantities of  $^{63}\text{ZnEDTA}$  using thyroid phantoms. Two vials of the chelate, each containing 10 g of ZnEDTA, were prepared and bombarded for 1 hour at 1 ma and 120 Kev. After bombardment, two thyroid phantoms were filled with the  $^{63}\text{Zn-EDTA}$  solution and a scan was performed on a Nuclear Chicago Pho/Gamma III camera using a pinhole collimator (figure 26). A second phantom was examined by means of an Ohio nuclear rectilinear scanner using a fine grain high energy focusing collimator (figure 27). Absolute activity at the time of scanning was determined using a  $^{22}\text{Na}$  standard as described previously. Figure 27 was obtained with an initial activity of 10.6  $\mu\text{Ci}$ , and figure 26 was recorded with an initial activity of 20.1  $\mu\text{Ci}$ . From this investigation, it is evident that sufficient activity could be prepared with a Cockroft-Walton accelerator to obtain a scan. If a positron head had been available, the efficiency would have been improved and a better scan could have been obtained.



Figure 26 Scan of thyroid phantom obtained using  $^{63}\text{Zn}$ -EDTA and a Nuclear Chicago Pho/Gamma III camera with a pinhole collimator

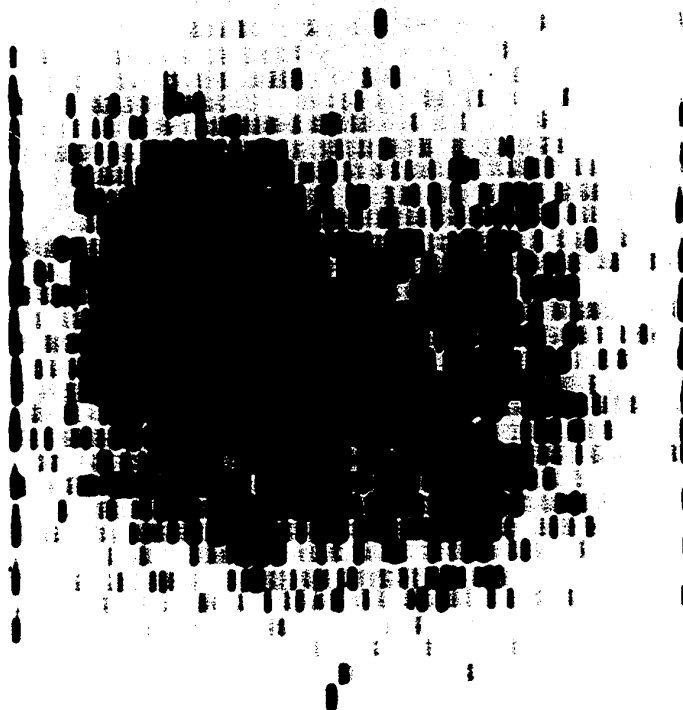


Figure 27 Scan of thyroid phantom obtained using  $^{63}\text{Zn}$ -EDTA and an Ohio Nuclear dual probe rectilinear scanner with a fine grain high energy collimator

### G. DOSIMETRY

Since both  $^{63}\text{Zn-EDTA}$  and  $^{65}\text{Zn-EDTA}$  showed promise as potential radiopharmaceuticals, a partial dosimetry was computed for the two compounds. The kidney, being the major organ of translocation, was principally used as a model. The whole-body dose was also calculated. Because human data are unavailable, parameters obtained from animal experimentation were used for dosimetric purposes. The following assumptions were made:

- a. Ninety-four per cent of the injected dose is eliminated via the kidneys and six per cent via the feces.
- b. A biological half-life of 38 minutes (obtained from the renogram study) was used for elimination by the kidneys.
- c. A biological half-life of 12,624 minutes (obtained from whole-body excretion in mice) was used for elimination by the feces.
- d. The uptake half-life was negligible.
- e. The absorbed dose for human kidneys was calculated assuming a mass of 288 g and for the whole-body assuming a mass of 70 Kg (307).
- f. The bladder contribution to the absorbed dose was neglected.

The absorbed doses were calculated using equation (1) proposed by Loevinger and Berman (308) and the general outline of Overton (309) was followed for calculations.



$$\bar{D}_{(v \leftrightarrow v)} = \tilde{C}_v \Sigma \Delta_i \phi_i (v \leftrightarrow v) \text{ rad} \quad (1)$$

where:

$\bar{D}_{(v \leftrightarrow v)}$  = the dose to the volume from a uniform distribution of radioactivity throughout the same volume.

$\Delta_i$  = equilibrium absorbed dose constant for radiation of type  $i=1,2,3,4,-----$  with a fractional frequency  $n_i$  per disintegration, and a mean energy,  $\bar{E}_i$  in Mev.  
 $= 2.13 n_i \bar{E}_i \left( \frac{\text{g-rads}}{\mu\text{Ci-hr}} \right)$

$\phi_i (v \leftrightarrow v)$  = the absorbed dose fraction in a volume,  $v$ .  
 Values for  $\phi_i$  were obtained from Snyder et al. (307).

$\tilde{C}_v$  = cumulated uniform concentration of activity in volume  $v$ , in  $\mu\text{Ci-hr/g}$ .  
 $= \frac{\tilde{A}_v}{M_v}$

$\tilde{A}_v$  = the cumulative activity ( $\mu\text{Ci-hr}$ ).

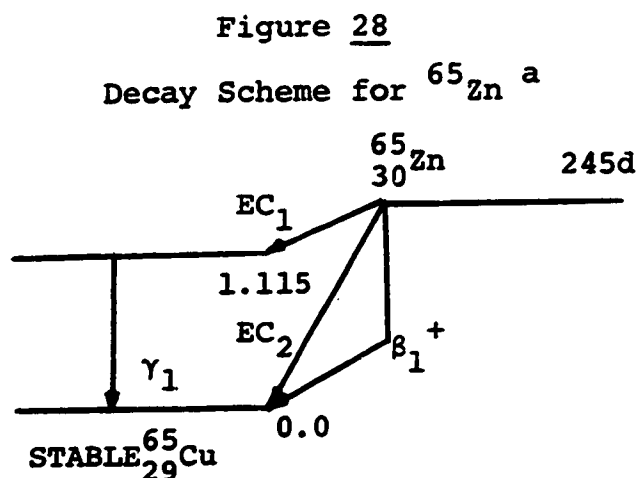
$M_v$  = mass of volume under consideration.

For zinc-65, values of  $\Delta_i$  were obtained from the decay scheme and results outlined by Dillman (310). The  $\Delta_i$  and  $\phi_i$  values for kidneys and whole-body are shown in Tables 10 and 11. The values for  $\Delta_i$  for zinc-63 were calculated using the values of  $n_i$  and  $\bar{E}_i$  obtained from Lederer (9), and the results are shown in Tables 12 and 13 with the corresponding

$\phi_i$  values.

1. Theoretical Absorbed Dose from  $^{65}\text{Zn}$ -EDTA

Zinc-65 decays according to the scheme outlined in figure 28.



a. From Dillman (310)

Table 10

Values for  $\Delta_i$  and  $\phi_i$  for Various Emissions from  $^{65}\text{Zn}$  of Uniform Distribution in the Kidneys

$\bar{E}_i$ (Mev)	$n_i$	$\Delta_i \left( \frac{\text{g-rad}}{\mu\text{Ci-hr}} \right)$	$\phi_i$ kidney	$\phi_i$ whole-body
0.1414	0.0170	0.0051	1.0	1.0
0.5110	0.0340	0.0400	0.073	0.408
1.1150	0.4899	1.1657	0.067	0.385
1.1050	0.0080	0.0002	0.067	0.385
0.0080	0.2125	0.0036	0.932	0.996
0.0080	0.1061	0.0018	0.932	0.996
0.0089	0.0370	0.0007	0.932	0.996
0.0071	0.4103	0.0062	0.932	0.996
0.0079	0.1087	0.0018	0.932	0.996
0.0088	0.0041	0.0001	0.932	0.996
0.0008	1.34	0.0023	1.0	1.0
0.0001	2.85	0.0006	1.0	1.0

$$\Sigma \Delta_i = 1.2281$$

$$\Sigma \Delta_i \phi_i = 0.1023$$

$$\Sigma \Delta_i \phi_i = 0.4874$$

Zinc-65 has a physical half-life of 245 days. Therefore, the effective half-life in the body, assuming a biological half-life of 38 minutes, is:

$$\begin{aligned}\frac{1}{T_{\text{eff}}} &= \frac{1}{T_{\text{phys}}} + \frac{1}{T_{\text{biol}}} \\ &= 0.63 \text{ hr}\end{aligned}$$

$$\lambda_{\text{eff}} = \frac{0.693}{0.63} = 1.1 \text{ hr}^{-1}$$

$$\tilde{C}_v = \frac{\tilde{A}_v}{M_v} \int_0^{\infty} e^{-\lambda t} dt$$

$$\text{where } \lambda = \lambda_{\text{eff}}$$

The integral evaluates as

$$\tilde{C}_v = \frac{\tilde{A}_v}{M_v} \left[ \frac{1}{\lambda_{\text{eff}}} \right]$$

We have determined that about 94 per cent of the injected Zn-EDTA is excreted in the urine. Therefore, for an injected dose of 10  $\mu\text{Ci}$ , 9.4  $\mu\text{Ci}$  would pass through the kidneys.

$$\begin{aligned}\tilde{C}_v &= \frac{9.4}{288} \cdot \frac{1}{1.1} \\ &= 0.0297 \text{ } \mu\text{Ci-hr/g}\end{aligned}$$

Then, the total absorbed dose for the kidneys using equation (1) would be,

$$\begin{aligned}\bar{D}_{(v \leftrightarrow v)} &= 0.0297 \times 0.1023 \left\{ \frac{\mu\text{Ci-hr}}{\text{g}} \times \frac{\text{g-rad}}{\mu\text{Ci-hr}} \right\} \\ &= 0.0030 \text{ rads/10 } \mu\text{Ci of } ^{65}\text{Zn}\end{aligned}$$

Similar calculation for absorbed dose in the whole-body using the appropriate  $\phi_i$  values from Table 10 gives a

$$\bar{D}_{(r \leftarrow v)} = 0.00006 \text{ rads/10 } \mu\text{Ci of } {}^{65}\text{Zn}$$

where  $\bar{D}_{(r \leftarrow v)}$  = the dose to organ  $r$  from a uniform distribution of radioactivity throughout volume  $v$ .

It is also apparent that a portion of the injected Zn-EDTA is not eliminated in the urine and this must also be considered.

Table 11

Values for  $\Delta_i$  and  $\phi_i$  for Various Emissions from  ${}^{65}\text{Zn}$  of Uniform Distribution in the Whole-Body

$\bar{E}_i$ (Mev)	$n_i$	$\Delta_i \left( \frac{\text{g-rad}}{\mu\text{Ci-hr}} \right)$	$\phi_i$ kidney	$\phi_i$ whole-body
0.1414	0.0170	0.0051	1	1
0.5110	0.0340	0.0400	0.00142	0.340
1.1150	0.4899	1.1657	0.00161	0.321
1.1050	0.0080	0.0002	0.00161	0.321
0.0080	0.2125	0.0036	0.00410	0.959
0.0080	0.1061	0.0018	0.00410	0.959
0.0089	0.0370	0.0007	0.00410	0.959
0.0071	0.4103	0.0062	0.00410	0.959
0.0079	0.1087	0.0018	0.00410	0.959
0.0088	0.0041	0.0001	0.00410	0.959
0.0008	1.34	0.0023	0.00410	1
0.0001	2.85	0.0006	0.00410	1

$$\Sigma \Delta_i = 1.2281 \quad \Sigma \Delta_i \phi_i = 0.0071 \quad \Sigma \Delta_i \phi_i = 0.4095$$

Zinc-65 has a physical half-life of 245 days. Therefore, the effective half-life in the body, assuming a biological

half-life of 12,624 minutes, is:

$$\begin{aligned}\frac{1}{T_{\text{eff}}} &= \frac{1}{T_{\text{phys}}} + \frac{1}{T_{\text{biol}}} \\ &= 203.1 \text{ hr}\end{aligned}$$

$$\lambda_{\text{eff}} = \frac{0.693}{203.1} = 0.0034 \text{ hr}^{-1}$$

We have determined that about 6 per cent of the injected Zn-EDTA is excreted at this rate. Therefore, for an injected dose of 10  $\mu\text{Ci}$ , this would amount to 0.6  $\mu\text{Ci}$ . Therefore, for the kidneys,

$$\begin{aligned}\tilde{C}_v &= \frac{0.6}{288} \cdot \frac{1}{0.0034} \\ &= 0.6126 \text{ } \mu\text{Ci-hr/g}\end{aligned}$$

$$\begin{aligned}\text{then } \bar{D}_{(v \leftrightarrow v)} &= 0.6126 \times 0.0071 \left\{ \frac{\mu\text{Ci-hr}}{\text{g}} \times \frac{\text{g-rad}}{\mu\text{Ci-hr}} \right\} \\ &= 0.0043 \text{ rads/10 } \mu\text{Ci of } ^{65}\text{Zn}\end{aligned}$$

Similar calculation for absorbed dose in the whole-body using the appropriate  $\phi_i$  values from Table 11 gives a

$$\bar{D}_{(v \leftrightarrow v)} = 0.0010 \text{ rads/10 } \mu\text{Ci of } ^{65}\text{Zn}$$

The total relative whole-body dose is therefore 0.00006 rads + 0.0010 rads = 0.00106 rads/10  $\mu\text{Ci}$  of  $^{65}\text{Zn-EDTA}$ .

The total relative kidney dose is the sum of the whole-body dose and the specific organ dose. Therefore  $D_{\text{total}} = 0.0030 \text{ rads} + 0.0043 \text{ rads} + 0.00006 \text{ rads} + 0.0010 \text{ rads} = 0.00836 \text{ rads/10 } \mu\text{Ci of } ^{65}\text{Zn-EDTA}$ .

## 2. Theoretical Absorbed Dose from $^{63}\text{Zn-EDTA}$

The decay scheme as outlined by Lederer (9) was used to determine  $n_i$  and  $\bar{E}_i$  values (see page 30). These values were then used to calculate  $\Delta_i$ . Values for  $\bar{E}_i$ ,  $n_i$ ,  $\Delta_i$  and  $\phi_i$  are given in Table 12.

Table 12

Values for  $\Delta_i$  and  $\phi_i$  for Various Emissions from  $^{63}\text{Zn}$  of Uniform Distribution in the Kidneys

Type of Radiation	$\bar{E}_i$ (Mev)	$n_i$	$\Delta_i \left( \frac{\text{g-rad}}{\mu\text{Ci-hr}} \right)$	$\phi_i$ Kidney	$\phi_i$ Whole-body
Positron $\beta^+$	(1) 2.36	0.80	4.0214	1	1
	(2) 1.71	0.07	0.2550	1	1
	(3) 1.41	0.05	0.1502	1	1
	(4) 1.02	0.005	0.0109	1	1
Gamma $\gamma$	(1) 0.669	0.01	0.0142	0.073	0.408
	(2) 0.962	0.01	0.0205	0.067	0.385
	(3) 1.42	0.004	0.0121	0.060	0.359
	(4) 1.55	0.0011	} neglect in calculations		
	(5) 1.83	0.0002			
	(6) 2.04	0.0013			
	(7) 2.34	0.0007			
	(8) 2.55	0.0006			
	(9) 2.77	0.0004			
	(10) 3.10	0.0002			
Annihilation	0.51	1.85	2.0096	0.073	0.408

note:  $\gamma_1^{e/\gamma} = 0.00052$  and  $\gamma_2^{e/\gamma} = 0.00023$  were neglected in calculations.

Kidney  $\Sigma \Delta_i \phi_i = 4.5873$

Whole-body  $\Sigma \Delta_i \phi_i = 5.2754$

Zinc-63 has a physical half-life of 0.63 hours. Therefore,

the effective half-life in the body, assuming a biological half-life of 38 minutes, is

$$\begin{aligned}\frac{1}{T_{\text{eff}}} &= \frac{1}{T_{\text{phys}}} + \frac{1}{T_{\text{biol}}} \\ &= 0.315 \text{ hr}\end{aligned}$$

$$\lambda_{\text{eff}} = \frac{0.693}{0.315} = 2.2 \text{ hr}^{-1}$$

Again, assuming that 9.4  $\mu\text{Ci}$  of radioactivity pass through the kidneys, the cumulative activity would be

$$\begin{aligned}\tilde{C}_v &= \frac{9.4}{288} \cdot \frac{1}{2.2} \\ &= 0.0148 \mu\text{Ci-hr/g}\end{aligned}$$

the total absorbed dose for the kidneys would be

$$\begin{aligned}\bar{D}_{(v \leftrightarrow v)} &= 0.0148 \times 4.5873 \left\{ \frac{\mu\text{Ci-hr}}{\text{g}} \times \frac{\text{g-rad}}{\mu\text{Ci-hr}} \right\} \\ &= 0.0680 \text{ rads/10 } \mu\text{Ci of } {}^{63}\text{Zn}\end{aligned}$$

Similar calculations for absorbed dose in the whole-body using the appropriate  $\phi_i$  values from Table 12 give a

$$\bar{D}_{(r \leftarrow v)} = 0.0003 \text{ rads/10 } \mu\text{Ci } {}^{63}\text{Zn}$$

The amount of activity that is not eliminated in the urine must again be considered and  $\phi_i$  values for uniform distribution in the whole-body are summarized in Table 13.

Zinc-63 has a physical half-life of 0.63 hours. Therefore, the effective half-life in the body, assuming a biological half-life of 12,624 minutes, is

Table 13

Values for  $\Delta_i$  and  $\phi_i$  for Various Emissions from  $^{63}\text{Zn}$  of Uniform Distribution in the Whole-Body

$\Delta_i \left( \frac{\text{g-rad}}{\mu\text{Ci-hr}} \right)$	$\phi_i$ kidney	$\phi_i$ whole-body
4.0214	1	1
0.2550	1	1
0.1502	1	1
0.0109	1	1
0.0142	0.00142	0.340
0.0205	0.00161	0.321
0.0121	0.00152	0.302
2.0096	0.00142	0.340
$\Sigma \Delta_i = 6.4939$	$\Sigma \Delta_i \phi_i = 4.44042$	$\Sigma \Delta_i \phi_i = 5.13583$

$$\frac{1}{T_{\text{eff}}} = \frac{1}{T_{\text{phys}}} + \frac{1}{T_{\text{biol}}}$$

$$= 0.63 \text{ hr}$$

$$\lambda_{\text{eff}} = \frac{0.693}{0.63} = 1.1 \text{ hr}^{-1}$$

If we again assume that 6 per cent of the injected Zn-EDTA is excreted at this rate, this would involve 0.6  $\mu\text{Ci}$  for an injected dose of 10  $\mu\text{Ci}$ .

Therefore, for the kidneys,

$$\tilde{C}_v = \frac{0.6}{288} \cdot \frac{1}{1.1}$$

$$= 0.0019 \mu\text{Ci-hr/g}$$



$$\begin{aligned}\bar{D}_{(v \leftrightarrow v)} &= 0.0019 \times 4.4404 \left\{ \frac{\mu\text{Ci-hr}}{\text{g}} \times \frac{\text{g-rad}}{\mu\text{Ci-hr}} \right\} \\ &= 0.0084 \text{ rads/10 } \mu\text{Ci of } {}^{63}\text{Zn}.\end{aligned}$$

Similar calculation for absorbed dose in the whole-body using the appropriate  $\phi_i$  values from Table 13 gives a

$$\bar{D}_{(v \leftrightarrow v)} = 0.00004 \text{ rads/10 } \mu\text{Ci of } {}^{63}\text{Zn}.$$

The total relative whole-body dose is therefore  $0.0003 \text{ rads} + 0.00004 \text{ rads} = 0.00034 \text{ rads/10 } \mu\text{Ci of } {}^{63}\text{Zn-EDTA}$ .

The total relative kidney dose is the sum of the whole-body dose and the specific organ dose. Therefore  $D_{\text{total}} = 0.0680 + 0.0084 + 0.0003 + 0.00004 = 0.07674 \text{ rads/10 } \mu\text{Ci of } {}^{63}\text{Zn-EDTA}$ .

Table 14 summarizes the results obtained for the theoretically calculated dosimetry of  ${}^{65}\text{Zn}$  and  ${}^{63}\text{Zn}$ .

Table 14

Calculated Absorbed Dose of Zinc-65 and Zinc-63  
in the Kidney and Whole-Body

Radioactive Compounds	Absorbed dose (rads/10 $\mu\text{Ci}$ )	
	Kidney	Whole-Body
${}^{65}\text{Zn-EDTA}$	0.00836	0.00106
${}^{63}\text{Zn-EDTA}$	0.07674	0.00034

It can be seen from Table 14 that the relative absorbed dose in the kidney after the use of zinc-63 is greater than that for zinc-65. However, the relative absorbed dose for

whole-body is less for zinc-63 than for zinc-65 due to the prolonged retention time. In all cases dosages delivered are well below the maximum permissible weekly dose for the kidney of 300 mRad, or whole-body dose of 100 mRad recommended by the ICRP (311). Sodee (312) has calculated an absorbed dose of 14 rads for the kidney and 2.2 rads for whole-body after injection of 250  $\mu$ Ci of  $^{75}\text{Se}$ -selenomethionine. The absorbed doses reported in the present investigation are well below these levels. Results reported for whole-body doses from  $^{131}\text{I}$ -hippuran and  $^{125}\text{I}$ -hippuran, however, are below those found in the present investigation (313). Chantraine (313) reported that the renal dose from adult administration of 10  $\mu$ Ci of  $^{131}\text{I}$ -hippuran and  $^{125}\text{I}$ -hippuran was 0.01 mRad, and 0.002 mRad respectively. From the absorbed dose values it would appear that  $^{65}\text{Zn}$ -EDTA or  $^{63}\text{Zn}$ -EDTA would both be safe for use in nuclear medicine. However, zinc-65 is not well suited for scanning using present instrumentation due to the high energy of its gamma ray whereas zinc-63 can be detected with a positron camera. It should be noted that the absorbed dose for the two isotopes of zinc were based on rapid elimination from the body. If the elimination of the chelate was hindered due to kidney impairment, the absorbed dose from the zinc-65 would be greatly increased due to its long physical half-life whereas the absorbed dose from the zinc-63 would not be affected as significantly. Therefore, zinc-63 would be the isotope of choice from a safety standpoint. Although the bladder con-

tribution to total-body dose is important it has been neglected in the present discussion. This contribution could be calculated based on the material excreted from the kidneys and after consideration of the various retention times. However it was felt that a complete dosimetry study was beyond the scope of this investigation.

#### H. THEORETICAL CONSIDERATIONS

The activity of an isotope that can be produced by activation analysis using a Cockroft-Walton accelerator depends on many factors. These are as follows: flux and energy of neutrons that pass through the sample, cross section of the reaction, number of target atoms in the sample, irradiation time, and half-life of radiation product. All these factors have been considered in the derivation of formula 1 (given in appendix),

$$\frac{dB}{dt} = \left[ \frac{\lambda}{\lambda - \theta} \right] \left[ \frac{Ym\sigma}{4\pi x^2} \right] \left( e^{-\theta t_b} - e^{-\lambda t_b} \right) \quad (1)$$

where  $\frac{dB}{dt}$  = number of activated atoms per unit time

$\lambda$  = decay constant of activated isotope

$\theta$  = decay constant of target

$Y$  = initial neutrons/sec from target

$m$  = number of target atoms in sample

$\sigma$  = cross section of target atom

$x$  = distance of sample from target

$t_b$  = time of bombardment.

As the tritium target depletes during bombardment, due

to sputtering, there is an optimum time during which one can bombard a sample to achieve maximum activity. This optimum time for bombardment can be determined using formula 2 (given in appendix),

$$t = \frac{\ln (\lambda/\theta)}{\lambda - \theta} \quad (2)$$

where  $t$  = optimum time of bombardment

$\lambda$  = decay constant of activated isotope

$\theta$  = decay constant of target.

For the conditions of our bombardment (120 Kev and 1 ma) the half-life of the target was found to be 100 minutes. Therefore, the optimum time for bombardment to produce zinc-63, which has a half-life of 38 minutes, was found to be 85.8 minutes.

Thus, the bombardment of a sample containing a mixture of 1.2975 g of  $\text{ZnCl}_2$  and 3.5940 g of  $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$  (that is, 50 mg more than the amount required to chelate the  $\text{ZnCl}_2$ ), for a period of 85 minutes, would produce 10.0  $\mu\text{Ci}$  of zinc-63. This was calculated for an experimentally determined target-to-sample distance of 2 cm and for an initial yield of neutrons of  $7.02 \times 10^{10}$  neutrons/sec. The other values used in the calculation were for  $\lambda = 3.04 \times 10^{-4} \text{ sec}^{-1}$ ,  $\theta = 1.16 \times 10^{-4} \text{ sec}^{-1}$ ,  $\sigma = 1.67 \times 10^{-25}$ , and  $t_b = 5.1 \times 10^3 \text{ sec}$ . It should be noted that the quantity of  $\text{Na}_2\text{EDTA}$  (used in the mixture) contained  $1.1589 \times 10^{22}$  atoms of  $^{14}\text{N}$ . During a bombardment period of 85 minutes, only 1.3  $\mu\text{Ci}$  of  $^{13}\text{N}$  would have been produced due to the small cross-section for

14 Mev neutrons (206). Dosimetry for this quantity of isotope was determined using the decay scheme reported by Dillman (310) and  $\phi_i$  values from Snyder et al. (307). The absorbed dose in the kidney was found to be 0.0002 rads/1.3  $\mu\text{Ci } ^{13}\text{N}$  and 0.000005 rads/1.3  $\mu\text{Ci } ^{13}\text{N}$  for whole-body. These values can be considered insignificant.

Although it appears possible to produce zinc-63 to be used in kidney scanning, it would be better if a higher specific activity could be prepared. Therefore, machines with higher flux, or reactions with higher cross sections, should be used and further investigation in this area should be considered.

## **V. SUMMARY AND CONCLUSIONS**

1. Zinc-63-EDTA was obtained by bombarding Zn-EDTA directly with fast neutrons for 1 hour at 120 Kev and a beam current of 1 ma. No noticeable changes occurred in the zinc-chelate when examined by I.R., N.M.R. and C.H.N.

2. Minor amounts of nitrogen-13 were also produced during bombardment of the Zn-EDTA. Since this isotope is a pure positron emitter, no interference was noted.

3. The  $LD_{50}$  of  $ZnCl_2$ , when administered intravenously to mice, was found to be 9.3(8.2 to 10.6) mg/Kg.

4. The  $LD_{50}$  of  $ZnNa_2EDTA$  containing 50 mg excess of  $Na_2EDTA$  when administered intravenously to mice was found to be 1.32(1.02 to 1.70) g/Kg.

5. The  $LD_{50}$  of  $ZnNa_2EDTA$  containing 50 mg excess of  $CaNa_2EDTA$  when administered intravenously to mice was found to be 2.3(1.91 to 2.77) g/Kg.

6. The excretion and biological turnover of  $^{65}Zn$ -EDTA when administered intravenously to mice consisted of three compartments. The long fraction had a biological half-life of  $12624 \pm 446$  minutes and consisted of  $3.7 \pm 0.1$  per cent of the administered chelate, the medium fraction had a biological half-life of  $185.1 \pm 7.8$  minutes and consisted of  $13.8 \pm 2.5$  per cent of the administered chelate, and the fast fraction had a biological half-life of  $10.4 \pm 0.8$  minutes and consisted of  $86.4 \pm 4.6$  per cent of the administered chelate. It was felt that the slow fraction consisted of free  $^{65}Zn$  from the EDTA chelate which resulted either by isotopic exchange or displacement by other metals or chelates. The medium

compartment might be due to the formation of weak complexes in the body, and the fast fraction was probably due to direct filtration of Zn-EDTA by the kidney.

7. Two days post-injection,  $93.7 \pm 1.54$  per cent of the injected  $^{65}\text{Zn}$  was excreted in the urine and  $3.11 \pm 0.64$  per cent was excreted in the feces. Chromatography revealed that the major portion, which was excreted in the urine, remained undissociated in the body.

8. Tissue distribution studies in mice revealed that no organ concentrated the injected  $^{65}\text{Zn-EDTA}$  for any prolonged period of time. Seventeen different organs were analyzed but only the muscle, blood, kidney, bone, liver, lung and pancreas attained significant amounts of activity. When results were expressed as per cent of injected dose per total organ, the greatest accumulation of activity was in the muscle, blood, kidney, bone, liver, lung and pancreas respectively. Analysis of the initial rate of decrease in activity of these organs corresponded to the fast fraction of whole-body analysis.

9. When data were expressed as a ratio of per cent activity per unit weight of organ relative to per cent activity per unit volume of blood, the only organ which appeared to accumulate activity was the kidney. All other organs had a ratio of less than one for about the first hour post-injection, after which the ratio increased indicating an accumulation of activity. The pancreas, bone, lung and liver which had the greatest increase in ratio are also known to



be organs which concentrate  $\text{ZnCl}_2$ . This further suggested that the long fraction noted in whole-body analysis was due to zinc-65 dissociated from the EDTA chelate.

10. Expression of results as per cent of injected dose per unit weight of organ also revealed that no organ concentrated the injected  $^{65}\text{Zn-EDTA}$  for prolonged periods and the kidney was the target organ for  $^{65}\text{Zn-EDTA}$  in the mouse.

11. Dog renograms resulting after the injection of  $^{65}\text{Zn-EDTA}$  revealed that the compound was not cleared as rapidly from the kidney as was the case in the mouse. Analysis of the drainage segment gave a half-life of 38 minutes. Blood analysis showed a rapid clearance compartment of 1.7 minutes and a slow compartment with a half-life of 36 minutes. The activity excreted in the urine decreased with a half-life of 17.9 minutes.

12. A scan of a thyroid phantom was obtained using a Pho/Gamma III camera and an Ohio-Nuclear dual probe rectilinear scanner and it was seen that enough activity could be obtained to produce a reasonable scan. These scans were obtained without the use of a positron camera which would improve the efficiency even more.

13. The theoretical radiation dose was calculated for the kidney and whole-body after injection of  $^{65}\text{Zn-EDTA}$  and  $^{63}\text{Zn-EDTA}$ . Zinc-65 gave a relative absorbed dose of 8.36 mrad/10  $\mu\text{Ci}$  for the kidney and 1.06 mrad/10  $\mu\text{Ci}$  for the whole-body. Zinc-63, on the other hand, gave a relative absorbed dose of 76.74 mrad/10  $\mu\text{Ci}$  for the kidney and

0.34 mrad/10  $\mu$ Ci for the whole-body. These absorbed doses are well below those recommended by the I.C.R.P. for the corresponding organs.

At the beginning of this investigation it was stated that many requirements had to be fulfilled before a radio-pharmaceutical could be considered useful in nuclear medicine. This investigation has attempted to answer most of these. The zinc-chelate was shown to remain intact after neutron bombardment, it was relatively non-toxic, was rapidly excreted from the body, it was nearly all excreted as the undissociated complex and it was taken up mostly by the kidney. The relative absorbed dose to the kidney and whole-body was relatively low, and enough activity could be prepared to obtain a scan. It therefore would seem that  $^{63}\text{Zn}$ -EDTA may be potentially useful in nuclear medicine. However, it should be mentioned that relatively large amounts of Zn-EDTA are required to obtain sufficient activity and it would be better if less chelate could be injected. Therefore, machines with higher neutron fluxes or elements with higher cross sections should be searched for so that higher specific activities could be obtained and less compound could be injected.

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## VII. APPENDIX

1. Formula for determination of activity produced by bombardment of elements with neutrons.

If 1 neutron intercepts a sample, the probability of an interaction =  $\frac{m\sigma}{A}$

where m = total number of target atoms in the sample

$\sigma$  = cross section of the target atom

A = area of sample bombarded

If N neutrons go through a sample, the probability of an interaction =  $\frac{Nm\sigma}{A}$

If we have m atoms in a sample of area A being bombarded by N' neutrons per second and a cross section of  $\sigma$ , the net number of activated target atoms per second is equal to the number of radioactive atoms produced minus the number of radioactive atoms decayed.

$$\text{Therefore, } \frac{dB}{dt} = \frac{N'm\sigma}{A} - \lambda B$$

where B = number of activated atoms produced

$\lambda$  = decay constant of activated atom

However, in a Cockroft-Walton accelerator, N' varies with time and also with distance of the sample from the accelerator tritium target.

$$N' = \frac{YA}{4\pi x^2} \cdot e^{-\theta t}$$

where Y = total yield at start of bombardment (neutrons/sec)

x = distance of sample from target

$\theta$  = decay constant of tritium target

A = area of sample

Therefore, number of activated atoms/sec

$$\frac{dB}{dt} = \frac{YAm\sigma}{4\pi x^2 A} \cdot e^{-\theta t} - \lambda B$$

$$\text{let } K = \frac{Ym\sigma}{4\pi x^2}$$

$$\text{therefore } \frac{dB}{dt} + \lambda B = Ke^{-\theta t}$$

$$\frac{d}{dt}(Be^{\lambda t}) = Ke^{(\lambda-\theta)t}$$

$$\text{then } Be^{\lambda t} = \frac{Ke^{(\lambda-\theta)t}}{\lambda - \theta} + c$$

where  $c$  = constant of integration.

Inserting initial conditions when  $t = 0$ ,  $B = 0$

$$\text{then } Be^{\lambda t} = \left( \frac{K}{\lambda - \theta} \right) \left( e^{(\lambda-\theta)t} - 1 \right)$$

$$B = \left( \frac{K}{\lambda - \theta} \right) \left( e^{-\theta t} - e^{-\lambda t} \right)$$

Therefore, number of activated atoms

$$B = \left( \frac{Ym\sigma}{4\pi x^2} \right) \left( \frac{1}{\lambda - \theta} \right) \left( e^{-\theta t} - e^{-\lambda t} \right)$$

Number of disintegrations per second at end of bom-

$$\text{bardment} = \lambda B = \left( \frac{Ym\sigma}{4\pi x^2} \right) \left( \frac{\lambda}{\lambda - \theta} \right) \left( e^{-\theta t} - e^{-\lambda t} \right) \quad \text{formula (1)}$$

where  $t$  = time of bombardment.



2. Formula for determination of optimum bombardment time for target atom using a Cockroft-Walton Accelerator.

From formula (1) we can consider  $\left(\frac{Ym\sigma}{4\pi x^2}\right)\left(\frac{\lambda}{\lambda-\theta}\right)$  as a constant (Q).

$$\text{Therefore, } P = \text{DPM} = Q(e^{-\theta t} - e^{-\lambda t})$$

$$\frac{dP}{dt} = Q(-\theta e^{-\theta t} + \lambda e^{-\lambda t})$$

$$\text{for a maximum, } \frac{dP}{dt} = 0$$

$$\text{Therefore, } \theta e^{-\theta t} = \lambda e^{-\lambda t}$$

$$\ln \theta - \theta t = \ln \lambda - \lambda t$$

$$(\lambda - \theta)t = \ln \lambda - \ln \theta$$

$$t = \frac{\ln(\lambda/\theta)}{\lambda - \theta}$$

formula (2)

where  $\lambda$  = decay constant of activated atom

$\theta$  = decay constant of tritium target

3. Program for curve peeling of exponential function using the method of weighted least squares.

C-FOCAL, 69CE

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01.01 E
01.10 F N=1,3;D 1.9
01.20 S M=1;S N=3;D 3
01.30 S B(2)=B(1);S C(2)=C(1);S G(2)=G(1)
01.40 S N=N+1;D 1.9;S M=1;D 3;D 5
01.50 T Z,B(1)," "%4.02,100*B(1)/B(1)," "%6.03,V," "%2,H," ";A A
01.60 I (X)1.7;G 1.3
01.70 S N=-X;S M=1;D 3;S B(2)=-.593147/B(1)
01.80 S Z=C(1);D 8;T !! " INTERCEPT "Z,Z,!" T-HALF "Z,B(2)
01.81 T " SIGMA "%4.02,100*B(1)/B(1);S P=0/w
01.84 A !,LA;I (LA)1.88;A " "LB," "LT
01.85 S Z=B(1)*LA+C(1);D 8;S Q=Z
01.86 S Z=FSQT(S*2+(G(1)*(P-LA))2);D 8
01.87 T " "Z,LB-G," "Z,FSQT(LT2+Z2);S 1.04
01.88 C
01.90 T !Z,N," ";A T(N),A(N),D(N)

03.05 S W=0;S Q=0;S Y=0;S P=0;S S=0;S Q=0
03.10 F K=1,N;S Z=A(K);D 9;D 3.3;D 3.4
03.20 G 3.5
03.30 S U=(A(K)/D(K))2;S Y=Y+J*Z;S Q=Q+J*(Z2);S X=X+J
03.40 S Q=Q+U*T(K);S P=P+Z*J*T(K);S S=S+(T(K)2)*J
03.50 S LT=W*Q-02;S LB=W*P-0*1;S LA=W*Q-Y2
03.60 S C(M)=(1*Q-0*P)/LT;S B(M)=LB/LT
03.70 S G(M)=(LT*LA-LB2)/((N-2)*W*LT);S S=FSQT(G(M)/N)
03.80 S G(M)=FSQT((W*B(M))/LT)

05.10 S V=(B(2)-B(1))/FSQT(G(1)2+G(2)2);S H=2*N-5

08.10 S Z=EXP(Z)
09.10 S Z=FL0G(Z)

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