GENERAL METABOLISM OF COBALT STUDIED IN CHICKENS AND DOGS

By

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A THESIS

Submitted to the School of Graduate Studies of Michigan

State College of Agriculture and Applied Science

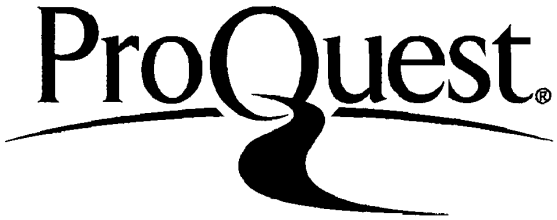
in partial fulfillment of the requirements

of the degree of

DOCTOR OF PHILOSOPHY

Department of Physiology and Pharmacology

1952



ACKNOWLEDGMENTS

The author wishes to express his appreciation to Dr. Lester F. Wolterink for his encouragement, guidance and advice during the course of these experiments and in the preparation of this manuscript. Acknowledgment is also due to Dr. B. V. Alfredson for his encouragement and for his aid in providing facilities in the department; and to Drs. W. D. Collings, E. P. Reineke, J. Meites and C. F. Huffman for their interest and suggestions.

Many thanks are also due to Mr. W. Friedberg, Mrs. E. F. Monkus and Mr. R. L. Cornwall for their assistance in the preparation of the experimental animals; and to Mr. J. Monroe for caring for the animals used in these experiments.

Finally, the author wishes to express his gratitude to the Division of Biology and Medicine, U. S. Atomic Energy Commission, for the funds under which this work was done.

THESIS ABSTRACT

Although specific cobalt deficiency diseases have been described in ruminants from many parts of the world, little is known with respect to the passage of cobalt through the living system. Experiments were set up to study the general metabolism of cobalt particularly in non-ruminants (chickens and dogs). Particular emphasis was placed on the role of the intestinal tract, of the renal tubules, and of the liver. A radioactive isotope of cobalt, Co^{60}, was used.

Cobalt 60, as $Co^{60}SO_4$, is both readily absorbed from the intestinal tract and "secreted" or "continuously diffused" into the intestinal tract through its wall in both the chicken and the dog.

In the chicken, most of the Co^{60} found in the caecum enters from the gut. In the dog, the caecum plays an insignificant role.

In the chicken, Co^{60} is greatly concentrated in the caecal contents, in the caecal wall, and in the large and small intestines regardless of the route of administration. A very small amount of Co^{60} is found in the bladder bile of the chicken. In the dog, the highest concentrations of Co^{60} are found in the liver, in the intestinal wall and in the contents of the first part of the jejunum, in the kidney, in the gall bladder bile and in the bladder urine. The spleen contains a very low amount of Co^{60}.

Cobalt 60 passes into the extracellular fluid soon after intravenous injection of its inorganic form in the dog. Blood cells contain an insignificant amount of Co^{60}.

In the dog, the plasma Co^{60} quickly filters through the glomeruli following a single intravenous injection. The maximal values for the rate of urinary excretion are reached within one-half to three hours. On the other

hand, the rate of biliary Co^{60} excretion reaches a maximum about five to seven hours after injection.

In dogs receiving a constant infusion, relatively constant plasma Co^{60} concentrations are maintained. The rate of urinary Co^{60} excretion in these dogs remains constant but at a level dependent upon the rate of infusion. However, the rate of biliary excretion increases gradually and reaches a plateau at about four to seven hours.

The renal clearance of Co^{60} within three hours after intravenous injection averages 27 ml. per minute per square meter of surface area. About three-fourths of the filtration load is reabsorbed by the tubules. In acute experiments of long duration (up to thirteen hours), renal clearances of Co^{60} decline due to an increase in the tubular reabsorption.

In the dog, the half-time for the removal of Co^{60} from the blood is 20% faster when injected as the cysteine-Co^{60} complex than when injected as $Co^{60}SO_4$. The transfer of Co^{60} from the peritoneal cavity to the blood is 3.3 times faster when injected as the cysteine-Co^{60} complex than when injected as $Co^{60}SO_4$.

A form (or forms) of Co^{60} other than its inorganic form is found in the bile and in the urine. The Co^{60} in these samples is reabsorbed from the gut of young chicks at a considerably faster rate than is inorganic Co^{60}.

CONTENTS

INTRODUCTION

The metabolism of cobalt, present in trace amounts in the tissues of animals and plants and in the cells of microorganisms, has attracted much attention in the last two decades. Interest was first aroused by the findings of Filmer and Underwood (1935), Marston (1935) and Lines (1935), that "enzootic marasmus" and "coast disease", of common occurrence in areas of the West Australia and the South Australia, were due to cobalt deficiency. At the present time, cobalt is known to serve no functional purpose in the animal body other than as an integral part of the accessory food factor, vitamin B_{12}, and as an agent to induce erythrocytosis. Our present knowledge of the metabolism of inorganic cobalt is illustrated diagrammatically in Figure 1.

As indicated in the diagram cobalt is probably lost from the body by three routes: in the feces, in the urine and in the bile. Fecal loss constitutes a large fraction of the dose when administered orally or when introduced directly into the stomach. Intravenously injected cobalt, on the other hand, is chiefly excreted through the urine. Biliary loss is at least partially included in the fecal cobalt. It represents that fraction of the cobalt which is carried through the liver and returned to the intestinal tract in the bile. Whether some of this can be reabsorbed subsequently does not appear to have been investigated.

In view of the lack of knowledge concerning the passage of cobalt through the living system, it becomes highly desirable to investigate:

1. what proportion of administered inorganic cobalt is tied up in the intestinal tract when it is given either orally or intravenously;

2. the relative amounts of cobalt excreted through the intestinal tract and through the kidneys following the two different routes of administration;

3. partitioning of cobalt between the fluid compartments of the body;

4. the rates of biliary and urinary excretion of cobalt in relation to the blood cobalt level;

5. the physiological role of the tubules of the kidney in handling this trace element;

6. the different turnover rates for inorganic cobalt and amino acid cobalt complexes in the animal body;

7. the reabsorbability of the forms of cobalt found in bile and in urine.

The chicken is a favorable experimental animal in which the first two problems may be investigated, because large numbers are readily available and because the surgical techniques are convenient. Since the caeca of birds are believed to be the main site for the synthesis of various accessory food factors which are the by-products of the metabolism of their bacterial population, emphasis was also placed on these structures. Determinations were made of the amount of cobalt "fixed" in the entire caeca, or in the caecal wall versus the caecal contents, both when these organs were free and when they were tied off at their junction with the intestine.

The dog was chosen as the experimental animal to be employed in the studies of the next four problems, due to its large body size and its relatively high rate of urine and bile flow. In order to compare the metabolic role of cobalt in the intestines with that in the chicken, inorganic cobalt was also injected intravenously or introduced directly into a loop of small

intestine of the dog. Various segments of the intestinal tract and tissues were analyzed for cobalt distribution.

For the last of the problems listed, the reabsorbability of cobalt in the urine and bile samples collected from the experimental dog was studied by determining the rate of their intestinal absorption in three-day-old White Leghorn chicks. Preliminary attempts to identify the cobalt compounds present in these samples were made using paper partition chromatographic and radioautographic techniques.

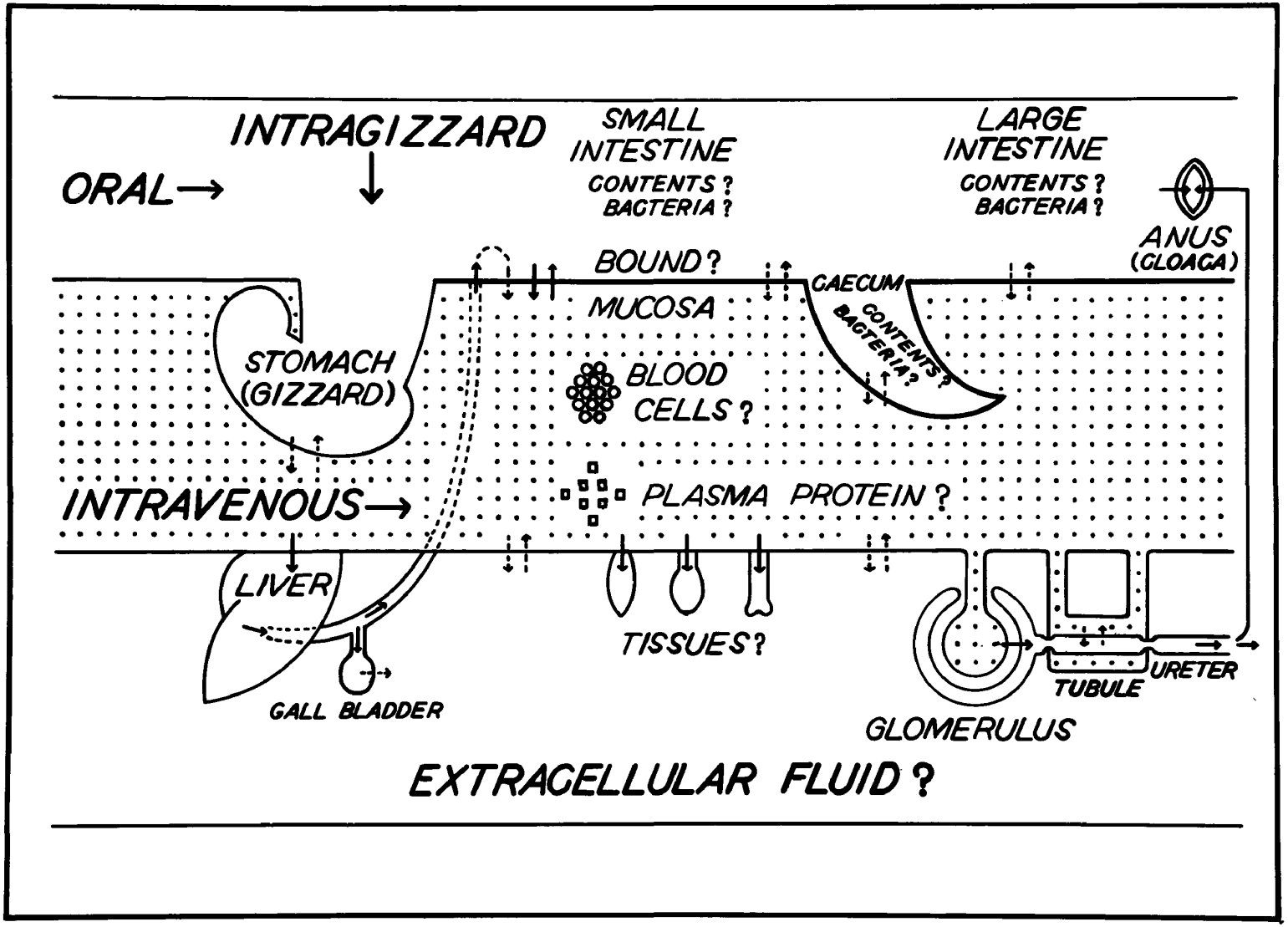


FIGURE 1. DIAGRAM OF COBALT CIRCULATION IN THE BODY.

REVIEW OF LITERATURE

The Physiological Function of Cobalt

History and Importance of Cobalt in the Nutrition of Ruminants:

Certain peculiar diseases among grazing ruminants have been known for many years and all over the world. Animals suffering from these diseases lose appetite and weight, become weak and anemic, and finally die. Each disease has been recognized as being limited to a certain area of the world and prevention and cure can be achieved by transferring animals from one locality to another. All these diseases have different names but similar symptoms in different parts of the world, and now appear to have the same etiology. As summarized by Beeson in the United States (1950) and by Marston in Australia (1952), among these disorders are "bush sickness" and "Norton Main disease" of New Zealand, "enzootic marasmus" of Western Australia, "coast disease" of South Australia, "pining disease" of the Cheviots and of other parts of Scotland, and of Devon, Cornwall, Herefordshire and Worcestershire of England, an unnamed disease in Dartmoor of England, "nakuritis" of Kenya Colony in East Africa, "salt sickness" of Florida, "neck ail" of Massachusetts, "Burton-ail" of New Hampshire, "Grand Traverse disease" of northern Michigan, "ailments" of Wisconsin, and similar diseases in Canada, Hebrides, Ireland, Norway, Denmark, Sweden and Estonia. There is little doubt that these diseases seriously menace the ruminants in other sections of the world where there is a shortage of cobalt in fodder plants.

The development of the studies on these maladies of grazing animals has been summarized by Marston et al. (1938), and discussed extensively by

Wunsch in New Zealand (1937, 1939) where the studies on "bush sickness" began a half century ago.

In view of the regional distribution of these ailments and of the curative effect of shifting animals from "sick" to "healthy" areas, it has been hypothesized that the nature of the soil, and thus of the forage, in the affected regions was involved in producing these nutritional disturbances among ruminants. The first publication in this series appeared in 1911, and was cited and summarized in 1924 by Aston (1924), who concluded that the disease was due to iron deficiency, and could be cured and prevented by administration of ferric ammonium citrate. Although he later claimed to have confirmed his conclusion (1932), further studies made in the North Island of New Zealand (Grimmett and Shorland, 1934), and in South Island (Rigg and Askew, 1934; 1936) produced no evidence to support the curative treatment of iron salts.

The discovery of cobalt as the deficiency element was made independently by two groups of investigators. Filmer (1933) reported that "enzootic marasmus" in West Australia and similar diseases are due to a shortage of some mineral essential for iron metabolism and associated in nature with the latter. Later, he and Underwood obtained an iron-free extract of limonite curative for "enzootic marasmus" (1934), and identified the curative agent as cobalt (1935). In the meantime, initiated by the fact that animals with "coast disease" in South Australia suffer from anemia and that cobalt produces polycythemia in rats (Waltner and Waltner, 1929), Marston (1935) and Lines (1935) succeeded in the treatment of this disease with cobalt. Their reports in great detail were reviewed later (Marston et al., 1938).

Following the reports of successful use of cobalt in Australia, cobalt salts were demonstrated to be effective in curing and preventing similar diseases in the South Island of New Zealand (Askew and Dixon, 1936) in the North Island (Wall, 1937), in Florida of the United States (Neal and Ahmann, 1937), in Dartmoor of England (Patterson, 1937; 1938), in western Canada (Bowstead and Sackville, 1939), in the Cheviot Hills (Corner and Smith, 1938) and in Cornwall and Devon of Scotland (Patterson, 1946).

The earlier concepts in regard to cobalt metabolism and its relation to ruminant nutrition have been reviewed by three groups of investigators (Huffman and Duncan, 1944; McCance and Widdowson, 1944; Russell, 1944). The present status of knowledge of this trace element in the nutrition of animals and plants has been summarized by Marston (1952). The role of vitamin B_{12}, an important biological compound of cobalt, in animal nutrition has been reviewed by Smith (1950-1951); and its clinical aspects by Ungley (1951).

Probable Mechanism of Cobalt Action and Its Relation to Vitamin B_{12}:

The suggestion that cobalt exerts its action, in the ruminant animal, either within the lumen of the alimentary tract or during its passage through the wall is based upon three pieces of evidence. First, cobalt is only essential for the nutrition of ruminants. Other herbivorous animals have not been shown to be affected by low cobalt pastures on which sheep and cattle develop acute deficiency (Marston et al., 1938). Rats have been shown to be unaffected when intake of cobalt is less than 0.6 microgram of cobalt per day (Underwood and Elvehjem, 1938), or less than 0.3 microgram of cobalt per day (Houk et al., 1946). Rats raised on rations which provided between 0.15 and 0.35 microgram of cobalt per day for three generations, grew as normally as those which

received a supplement of 10 micrograms of cobalt daily (Marston, 1949). Rabbits remained healthy when they received less than 0.1 microgram of cobalt per day (Thompson and Ellis, 1947). Thus, non-ruminants require cobalt only in extremely small quantities, ~~if at all~~. Second, cobalt is effective only when administered orally. Parenterally injected cobalt is not beneficial to the animal. This suggests that cobalt functions through some mechanism in the alimentary tract. McCance and Widdowson (1944) were the first to indicate the ineffectiveness of parenteral administration of cobalt and other groups confirmed it (Becker et al., 1949; Becker and Smith, 1949, 1951; Marston and Lee, 1949; Gall et al., 1949; Smith et al., 1950). When injected intravenously, the cobalt concentration in the blood and tissues might reach a level as high as ten times above that usually found in normal sheep without any beneficial effect to the animal (Marston, 1949; Marston and Lee, 1949). Third, the cobalt must be administered frequently. Marston (1949) reported that sheep on cobalt-deficient pastures, given orally a supplement of cobalt of 1 mg. per day thrice weekly, remained in normal health. However, corresponding doses administered at intervals of six weeks could not prevent the fatal malady and larger doses given at intervals of two weeks failed to maintain normal health.

Microorganisms which normally flourish in the alimentary tract have been known to be important in the nutrition of the ruminant (Marston, 1949; Marston and Lee, 1949). Tosic and Mitchell (1948) reported that orally administered cobalt is taken up and concentrated by rumen microorganisms. The nature and the numbers of rumen microorganisms have been observed to be directly influenced by cobalt intake (Gall et al., 1948; 1949; Gall and Huhtanen, 1950). Thus, the function of cobalt in the ruminant might relate to the nature

and activity of the mixed population of microflora. The cellulose-splitting microorganisms of the rumen were found to be not markedly affected in the cobalt-deficient lamb (Becker and Smith, 1949; 1951), as indicated by the fact that the efficiency with which crude fiber is fermented within the digestive tract is unimpaired. A low-cobalt diet might then affect only the bacteria other than the cellulose-splitting microorganisms, or it might influence the bacteria by preventing the synthesis of important food factors which are normally required in the balanced nutrition of the animals.

The essential nature of cobalt for the synthesis of B vitamins by the rumen microorganisms has been suggested (Ray et al., 1949); however, combined treatment with various B vitamins failed to improve the syndrome of cobalt deficiency in lambs (Ray et al., 1949). However, with similar treatment, a favorable result was later claimed by the same group of investigators (Hale et al., 1950b).

Investigators have shown earlier (Filmer, 1933; Marston, 1935) that some factor in liver has therapeutic value in the treatment of the ruminant malady. However, the slight response of cobalt-deficient sheep to continued therapy with liver extract is only temporary (Marston, 1949). It is clear now that the dosage used was too small to meet the requirement of the cobalt-deficient sheep. Subcutaneous injection of 15 U.S.P. units of a purified liver extract daily has been reported to be effective in the treatment of cobalt deficiency in lambs (Becker and Smith, 1951). The absence of response when the same dosage of liver extract was administered orally in the same experiment might be the result of destruction of its activity within the alimentary tract or of inefficient absorption from the tract. It is evident that some factor or group of factors contained in the liver extract possesses curative activity which prevents the onset of the syndrome produced by a cobalt deficiency.

A comparison of the rate of response of cobalt-deficient lambs to oral administration of cobalt and to subcutaneous injections of a purified liver extract have been made (Becker and Smith, 1951). An immediate response to the liver therapy indicates that the liver extract is a direct supply of the accessory food factor or factors required by the deficient lambs. On the other hand, a latent period is essential for the microorganisms normally living in the alimentary tract to synthesize the deficient metabolite or metabolites following the stimulation of cobalt therapy.

With the recognition of vitamin B_{12} (Rickes et al., 1948a; Smith 1948a), which is a cobalt-containing complex (Rickes et al., 1948b; Smith, 1948b), it was suggested that the syndrome of cobalt deficiency originates not from a lack of cobalt per se, but from a shortage of vitamin B_{12}. This belief was immediately strengthened by the important finding that the rumen contents are normally rich in vitamin B_{12} (Gall et al., 1948; Hale et al., 1950a; Lewis et al., 1949), and that the synthesis of this vitamin in the alimentary tract is seriously impaired when the concentration of cobalt in the ingesta falls below the level necessary to secure a balanced nutrition of the animal (Abelson and Darby, 1949; Gall et al., 1948; Hale et al., 1950a). However, attempts failed to correct the deficiency symptoms by supplementation with pure vitamin B_{12}, either per os, or parenterally, in sheep (Marston and Lee, 1949), and in lambs (Becker and Smith, 1949; 1951; Becker et al., 1949). It is now clear that the dosages employed in these experiments were insufficient to meet the nutritional demands of the ruminant. This has been clearly demonstrated by observations with sheep (Marston and Lee, 1951). While no regression of the symptoms was obtained in sheep on cobalt deficient pastures treated for four months with 15 micrograms of vitamin B_{12} per week, injected

intravenously; the sheep responded immediately when the dosage was increased to 300 micrograms of vitamin B_{12} per week.

The most outstanding work concerning the direct relationship between the metabolism of inorganic cobalt and vitamin B_{12} was reported by Davis and Chow (1951) that appropriate amounts of aureomycin or the increased amount of Co^{60} incorporated in the diet of rats caused an increase in Co^{60}-labeled vitamin B_{12} activity. They related this effect of the antibiotic to the intestinal bacterial flora. Monroe et al. (1952b) further demonstrated that considerable biosynthesis of vitamin B_{12} took place in the intestinal tract following oral administration of Co^{60}, and also occurred in the tissues following intravenous injection.

The livers and kidneys of ruminants are a very potent source of vitamin B_{12}. Beef kidney and liver have been found to contain 15 to 20 micrograms of vitamin B_{12} per 100 gm. wet weight (Lewis et al., 1949), and 42 to 47 micrograms per 100 gm. after tryptic or pancreatic digestion (Thompson et al., Since ruminant livers are those chiefly used for the preparation of liver extract (Smith, 1951), there is little doubt that vitamin B_{12} is the curative principle present in liver extract which possesses therapeutic value for the cobalt deficiency symptoms.

It is known that routes of the intermediary metabolism are somewhat different in ruminants and non-ruminants (Marston, 1939; 1948). The shorter chain acids produced by fermentation of carbohydrates in the rumen are the main sources of energy for ruminants. This leads to the belief that ruminants may have different requirements for the accessory food factors, including vitamin B_{12}. The high vitamin B_{12} activity of rumen contents (Hale et al., 1950a; Lewis et al., 1949), and the high concentration of this vitamin in the ruminant liver and kidney (Lewis et al., 1949; Thompson et al., 1950) again

suggests that this concept is true. In view of this, it is hardly surprising that all natural and experimental syndromes of cobalt deficiency have been reported only in ruminants. Other animals may require such small quantities of this particular food factor that they usually can get enough of it from the "basal diet". This may explain the failure to produce experimental cobalt deficiency in rats (Houk et al., 1946; Marston, 1949; Underwood and Elvehjem, 1938), and in rabbits (Thompson and Ellis, 1947).

The possibility has not been entirely excluded, at the present, that other accessory food factors in addition to vitamin B_{12} may, in part, play some role in the syndrome of cobalt deficiency.

Metabolism Studies of Cobalt:

The suggestion that bile serves as a pathway for the elimination of cobalt was first made in 1874 (Mayencon and Bergeret) and again in 1884 (Stuart). But it was not until 1936 (Caujolle) that cobalt was definitely found in the bile after parenteral administration. Greenberg et al. (1943b) were able to collect bile through biliary fistulae and measure its cobalt radioactivity after administration of 0.1 mg. of radioactive isotopes of cobalt to rats. About $3.5 \pm 1.4\%$ of the injected dose was recovered 72 hours after intravenous injection, but only $2.0 \pm 0.2\%$ after oral administration. By using the same technique, Sheline et al. (1946) reported a recovery of 5% of the injected dose from the bile of five dogs at the end of 72 hours after intravenous injection of 10 to 26 micrograms of radioactive cobalt. Significant amounts of cobalt were not found in the pancreatic juice collected from pancreatic fistulae in dogs (Sheline et al., 1946).

Kent and McCane (1941) have reported that little dietary cobalt is absorbed by man and that the kidneys are the organs responsible for the

elimination of the absorbed cobalt. During the past ten years or so, radioactive isotopes of cobalt have been used extensively in the studies of the metabolism of this tracer in the animal body. Urine has been found to be the chief pathway for the true excretion of cobalt in rats (Copp and Greenberg, 1941; Greenberg et al., 1943b; Comar et al., 1946a); and in cattle (Comar and Davis, 1947a; 1947b; Comar et al., 1946a; 1946b). These authors reported that 65% of the injected cobalt in cattle and from 65 to 90% in rats is eventually eliminated in the urine. A large majority of it is excreted during the first hours after intravenous injection. Seven to 30% in cattle and 5% in rats is removed in the feces. However, after oral administration, from 40 to 80% of the dose is eliminated in the feces and from 10 to 20% in the urine in rats. In cattle, when cobalt is given orally or introduced directly into the rumen, between 65 and 80% can be accounted for in the feces and only 0.5% in the urine.

It has been reported that less than 5% of the cobalt is retained in rats four days after intravenous injection (Copp and Greenberg, 1941) and that 5% remains in cattle ten days after injection (Comar et al., 1946a). At the end of 72 hours, the liver retains 2.5 $\pm$ 0.6% of the dose when given orally and 3.5 $\pm$ 0.7% when injected intravenously in rats (Greenberg et al., 1943b). After oral administration or intravenous injection, the distribution of radioactive cobalt in tissues has been studied in mice (Lawrence, 1947); in rats (Copp and Greenberg, 1941; Cuthbertson et al., 1950; Ulrich and Copp, 1951); in rabbits (Comar and Davis, 1947b); in pigs (Braude et al., 1949; Comar and Davis, 1947b); and in cattle (Comar and Davis, 1947a; 1947b; Comar et al., 1946a; 1946b). Injected cobalt is generally distributed throughout the tissues, high concentrations being found in the glandular organs,

especially the adrenals, kidneys, thyroid, liver, thymus, intestinal lymph glands, pancreas, spleen and reproductive organs. Low concentrations of cobalt are found in muscle, bone, cartilage, white bone marrow (long bones), tongue, fat, eyes and brain. No cobalt has been recovered from the pituitary. A consistently higher concentration in the lymph glands might suggest the importance of the lymphatic system in the transport of cobalt (Comar and Davis, 1947b). Small amounts can be detected in the contents of the abomasum (Comar and Davis, 1947a); none in that of the rumen (Comar et al., 1946b).

Orally administered cobalt is poorly absorbed in mammals, and in general, the distribution in the tissues parallels that of injected cobalt. A young calf absorbs a greater percentage of orally administered cobalt than does the older animal (Comar and Davis, 1947b).

In comparing the tissue distribution of Co^{60} and vitamin B_{12} labeled with Co^{60} in chicks, Monroe et al. (1952a) reported that vitamin B_{12} is retained by the tissues to a greater extent than is Co^{60} following intraperitoneal injection. The distribution of Co^{60} in tissues is in general agreement with the reports from other animals except that a very low concentration is found in liver.

Monroe et al. (1952b) have recently reported evidence for vitamin B_{12} biosynthesis in the tissues of sheep. They claimed that more than ten times as much Co^{60} is converted to vitamin B_{12} after oral administration as after intravenous injection, and that there is apparently considerably biosynthesis of vitamin B_{12} in tissues after intravenous injection. Their evidence suggests that the adrenals and spleen are possibly the chief tissues for this internal synthesis. They found three distinct fractions containing labeled cobalt, namely a vitamin B_{12}-like, an inorganic fraction and a fraction in

which cobalt is in some "bound" form, in various tissues. In the urine, the "bound" form is apparently lacking. About 70 to 90% of the total Co^{60} in the feces, and 30 to 50% in the small intestine are in the vitamin B_{12}-like and bound forms. About 30% of the total blood Co^{60} is in vitamin B_{12}-like and bound forms seven days after oral administration of inorganic Co^{60}, as compared with 75% after intravenous injection. During the seven-day period, 84.4% of the oral cobalt dose was recovered in the feces and 10.8% in the urine. After intravenous injection, there was 8.0% of the dose excreted in the feces and 77.7% in the urine.

The cobalt concentration in blood drops in a matter of minutes (Comar and Davis, 1947b) and its total recovery in the blood falls to 1% or less after 10 to 15 days (Comar et al., 1946b) when intravenously injected into cattle. However, none can be detected in the blood when cobalt is introduced directly into the rumen (Comar et al., 1946b). Very small amounts or no cobalt at all is found in the milk or in the saliva (Comar et al., 1946b). Extremely small but definite amounts of cobalt are transmitted across the placenta for storage in the liver of the fetus when cobalt is injected or fed to pregnant cows (Comar and Davis, 1947a). In regard to the internal metabolism of cobalt, no indication of species differences between rabbits, swine and cattle has been found (Comar and Davis, 1947b). It has been reported that blood cells account for 20 to 30% of the whole blood Co^{60} in two pigs 16 weeks old which received 2.138 mg. of radiocobalt chloride in the diet during a period of 43 days (Braude et al., 1949). In weanling rats after 90 days on a diet containing 0.006 (2 microcuries) ppm. inorganic radioactive cobalt, the blood cells contained 40 to 50% of the whole blood Co^{60}. This was further increased to 70 to 80% in the weanling rats after 40 days on diet containing

0.21 microcuries of Co^{60} in the form of radioactive yeast extract daily (Cuthbertson et al., 1950).

The Physiology of the Caecum in the Fowl

That digestion of protein and starch takes place in the caeca has been known since the beginning of the century through a direct study of the digestion employing a caecal fistula (Maumus and Launoy, 1901; Maumus, 1902). Crude fiber digestion has been reported to be a particular function of the caeca in chickens, as indicated by the lower digestibility of fiber in caecectomized chickens than in normal birds (Radeff). This was substantiated by the finding that the caecal content of crude fiber is always lower than the intestine content (Roseler, 1929). It has been suggested that bacterial activity in the caecum is responsible for the digestion of crude fiber (Mangold, 1928). Further work indicates that bacterial decomposition in the caecum converts the crude fiber into glucose and organic acids (Blount, 1937). The report that the total population of microorganisms in the caeca is larger than in any other part of the alimentary tract in chickens (Johanson et al., 1948) also suggests that a mixed population of microorganisms plays an important role in caecal digestion in the bird. It is believed that such food materials as crude fiber and certain proteins resistant to peptic and intestinal digestion are decomposed by mixed activities of microorganisms and by enzymes in the caeca of chickens. Kaupp (1919) was the first to report a neutral or slightly acid reaction in the caecum of domestic fowls. A decrease of pH in the caeca and large intestine (Olson and Mann, 1935; Farner, 1942) might be attributed to the presence of organic acids resulting from bacterial decomposition of starch and proteins. Evidence has not been

found to support the view that any significant amount of fat digestion takes place in the caeca of granivorous fowl.

Browne (1922), on the basis of the microscopic anatomy of the caeca, suggested that absorption may readily take place during the passage of intestinal contents into and out of the caeca. By giving colored fluid orally as well as by injecting it directly into the cloaca, evidence was obtained that caeca in chickens may serve as fluid reservoirs. Since crude fiber digestion in the caeca has been reported, glucose absorption has been suggested (Blount, 1937; Mangold, 1928; Radeff). Non-protein nitrogenous substances appear to be absorbed from the caecal contents (Mangold, 1928). The presence of less crude protein but of more pure protein in the caecal contents than in the intestinal contents may indicate an absorption of amides (Roseler, 1929). The synthesis of "B" vitamins by coliform microorganisms has been claimed to take place in the caeca of chickens (Johanson et al., 1948). Their absorption, however, is not certain. Mineral absorption has been postulated (M'Gowan, 1930). There is little doubt that water is absorbed in caeca. Thus, caecectomized chickens pass more moist feces (Mangold, 1928; Radeff), and the percentage of water in the intestinal contents is higher than that in the caecal contents (Keith et al., 1927), and chickens with ligated caeca suffer from diarrhea (Browne, 1932).

After the present experiments on the Co^{60} metabolism in chickens had been completed, Pandurang (1952) of this laboratory, by injecting Co^{60} solution directly into the lumen of the tied caecum in chickens, demonstrated that the caecum absorbs both Co^{60} and water, and that Co^{60} is in some bound form in the caecal contents.

The caecum is apparently not essential to fowl. The removal of both caeca in the chicken has been reported to cause no harm to health (Blount, 1937; Radeff), nor has any reduction in egg production been observed (Mayhew, 1934). Similar results were observed following ligation of both caeca in turkeys (Schlotthauer et al., 1934). Fertility remains unimpaired (Schlotthauer et al., 1934) and digestibility of the rations remains unchanged (Hunter et al., 1930) in turkeys which have undergone the same operation. Therefore, the caecum is not absolutely required by fowl under normal conditions. However, on minimal or deficient rations, it may assume greater importance. The caecal microorganisms might then come into the picture by either synthesizing certain essential nutrients available for their host, or competing with their host for limited essential nutrients.

Browne (1922) was the first to suggest that some intrinsic mechanism within the caecal tubes controls the caeca, causing them to fill up and later to empty. The distension stimulus resulting from the filling of the caeca causes evacuation. In live chickens with a caecal fistula, when the caecum was flushed with water, the contents were found to be ejected forcibly (Pandurang, 1952). It is generally stated that contraction brought about by distension stimulus leads to emptying and that "suction" (due to a negative pressure created by active dilatation) results in filling (Browne, 1922; Mangold, 1928). It has been observed that the caeca empty independently of each other and of the rest of the intestines (Mangold, 1928), and that one caecum evacuates one day and the other the next day (Roseler, 1929). A complete passage of food through the alimentary tract of chickens rarely requires more than twelve hours; however, a complete replacement of caecal contents requires about five days and one caecal discharge occurs for every

seven to eleven intestinal discharges, depending upon the nature of the feed (Roseler, 1929). In testing the rate of passage of various inert materials through the alimentary tract of a variety of animals, including human beings, Hoelzel (1930) found that most of the heavy materials are retained in the gizzard in the pigeon and chicken and in the duodenum and ileum in the chicken as well. No test materials were found in the caeca of chickens, although food materials were present.

The nervous control of caecal activity is not understood. Observations have been made on young house wrens in which "reflexes" were found associated with feeding and defecation (Reed and Reed, 1925). Immediately upon swallowing food, a complicated "reflex" is observed, which leads the young wren to defecate in a very unusual position in which the parent bird can easily collect the excreta as voided, and carry it away.

Caeca are usually paired in fowls. However, four general types of caeca have been reported (Maumus, 1902): 1. well developed caeca in vegetarians; 2. rudimentary caeca in carnivores; 3. only one caecum; 4. no caecum at all.

Renal and Hepatic Clearances

Renal Clearance:

It was Moller, McIntosh and Van Slyke (1929) who first used the term "CLEARANCE" in connection with the excretion of urea. They defined it as the volume of blood which contains the amount of urea excreted each minute by the kidneys. This is not necessarily a real volume cleared completely but a "virtual" volume. No attempt was made to explain this clearance in terms of any particular process in the kidney. In 1931, Jolliffe and Smith

extended this term to the excretion of creatinine; and since then it has been generally used to describe the excretion of other substances.

In his recent book, "The Kidney", Smith (1951) has pointed out that inulin clearance is, in all species, a direct measurement of the glomerular filtration rate. In the dog, creatinine and inulin clearances have been found to be identical under all conditions (Richards et al., 1936; Shannon, 1935; 1936; Van Slyke et al., 1935). Diodrast clearance has been used to measure the "effective renal plasma flow" (Smith et al., 1938). Because of the observations that at low plasma levels sodium para-aminohippurate (PAH) clearance is identical with that of diodrast, that PAH does not penetrate the red blood cell in vivo, that it is not toxic, that it is less bound by plasma proteins than is diodrast, and that the chemical determination is simple, PAH has been extensively used in place of diodrast for the determination of plasma flow through the kidney (Chasis et al., 1945; Goldring and Chasis, 1944).

Houck (1948) has presented a statistical analysis of filtration rate and effective renal plasma flow in 75 normal, trained, female dogs (516 to 774 individual 10-minute urine collection periods in 258 simultaneous clearance observations). The mean filtration rate per sq. m. of body surface area is 84.4 ml. per minute, with a standard error of 2.2; the range of observation is from 43 to 133 ml. per minute. Corresponding figures for the effective renal plasma flow are: mean 266 ml. per minute, standard error 7.6, range 139 to 430 ml. per minute. A mean filtration fraction of 0.317 in these dogs indicates that about 32% of the plasma passing through the glomeruli of the kidney is filtered.

For experimental investigations certain anesthetic agents are required to perform surgical operations in acute experiments or to facilitate procedures such as those required in the study of renal problems. Among the convenient anesthetic agents, sodium pentobarbital has been widely used. Mylon et al. (1943) pointed out that certain side effects of the anesthesia may complicate the experimental conditions. The first systematic investigation of the influence of sodium pentobarbital on renal hemodynamics was made by Corcoran and Page (1943). They reported that the renal function of dogs was not impaired during a period of two hours' anesthesia induced by 30 mg. per kg. body weight of sodium pentobarbital injected intraperitoneally. Sodium pentobarbital, 30 mg. per kg. given intravenously and additional small amounts to maintain uniform anesthesia for a period of five hours, has been found to induce a negligible effect on the sodium reabsorption mechanism (Selkurt and Glauser, 1951). Prolonged barbital or pentobarbital anesthesia of 5 to 6 hours' duration suitable for surgical purposes has no apparent effect on the glomerular filtration rate, although a decrease in the overall effective plasma flow and T_m for PAH may be observed (Glauser and Selkurt, 1952).

In a study of renal problems, urine flow is of primary importance and the problem of diuresis is always encountered. Administration of water to rabbits induces marked increases in renal plasma flow, glomerular filtration rate and urine flow (Forster, 1947). However, under controlled experimental conditions, a 52-fold variation in urine flow (0.014 - 0.725 ml./kg./min.) has been found to have no effect on glomerular filtration rates in rabbits (Forster, 1952).

It was Kirk (1936) who first reported a very low value for total amino acid clearance, ranging from 1 to 8 ml. per minute in man. Doty (1941) early found that a complete reabsorption of tyrosine and histidine took place in the dog when the filtration load was increased moderately. Later, Pitts (1943) found that tubular reabsorption of glycine in dogs amounted to more than 98% of the filtration load at normal plasma amino acid levels (below 10 mg. nitrogen per 100 ml.). The reabsorption of DL-alanine in the dog remains at a high level of 64 to 81% with high loads of 40 mg. nitrogen per 100 ml. (Goettsch et al., 1944). Such essential amino acids as leucine, isoleucine, tryptophane, valine (Beyer et al., 1946), threonine, phenylalanine (Russo et al., 1947), histidine and methionine (Wright et al., 1947) are so effectively reabsorbed by the tubules in the dog that their T_m values could not be reached with the dosages without causing severe effects on the subjects. In the human, only a minute fraction of any of the ingested amino acids is excreted when subjects are placed on a diet containing 1 gm. of protein per kg. body weight per day (Harvey and Horwitt, 1949; Kirsner et al., 1949; Sheffner et al., 1948).

"Hepatic Clearance":

Lewis (1948) first applied the concept of "hepatic clearance" to the study of liver function in the similar manner that "clearance" has been applied to kidney function. He suggested the following formula:

$$\frac{P - P'}{P \times P'} = \frac{C}{V}$$

where P is the initial plasma concentration, P' is the final concentration greater than zero, C is the volume of plasma cleared during this time interval, V is the total volume of the fluid compartment containing the dissolved

material, and C/V is a fractional clearance which represents the fraction of the fluid volume (or solvent compartment) cleared during the clearance period.

The utilization of this fractional clearance formula is based upon three assumptions: (1) relative constancy in fluid volume under different conditions, (2) removal of test substance is accomplished solely by the liver, and (3) the time-concentration is a simple logarithmic curve. With this technique, a clearance may be calculated from the changing plasma concentrations.

A more direct method for determining the clearance of the plasma by any organ or tissue is to compute the amount of the plasma which contains that amount of the test material accumulated or excreted by the organ or the tissue per unit time. Organ or tissue clearances of certain test materials have been studied for some years. The general theory has been summarized byStrajman and Pace (1951). The term "disappearance clearance" indicates the rate at which a test substance is removed from the tissue. The removal of Na^{24} from different tissues has been extensively studied in this connection. The term "accumulation clearance" indicates the rate of accumulation of a test substance in the tissue. The accumulation rate of iodine in the thyroid has been also extensively studied. In the present studies, the hepatic clearance of Co^{60} is computed from direct measurement of the quantities recovered in the bile.

METABOLISM OF COBALT 60 IN CHICKENS

The purpose of the experiments with chickens is to compare the fraction of cobalt which is recoverable from the intestinal tract following oral administration with the fraction recovered following intravenous injection. Data will be presented showing differences between recoveries from the various segments of the tract. An indirect estimate of the fecal loss and the urinary fraction in the bird will also be presented.

A. Method

Two mixed populations of birds culled from the poultry flock of Michigan State College and averaging one kg. in body weight, were used. The necessary surgery was performed on the first day, and on the second day, 20 micrograms of Co^{60} solution* per kg. body weight was administered. Twenty-four hours later, the birds were killed and analyzed for radioactivity.

Trial 1 consisted of 64 birds. The junction of the caeca and the large investine in each bird was pulled to the surface of the body through a ventro-lateral abdominal incision. In the first group of 16 birds, both caeca were left intact; in a second group of 16 birds, both caeca were ligated at the junction with the large intestine (see Introduction, p. 2);

*Co^{60}, as $Co^{60}SO_4$, obtained from Tracerlab, Boston, Mass. The stock solution containing 400 micrograms of cobalt per ml. of 0.1 N HCl solution was diluted to about 70 micrograms per ml. (pH 2) with physiological saline. The 20 micrograms injected contained 10 microcuries of Co^{60}. Physical decay corrections were made at each time of counting.

in a third group of 16 birds, the right caecum was ligated; and in the last group of 16 birds, the left caecum was similarly ligated. The incision was then closed with three or four stitches. Half of the birds in each group received the Co^{60} injection through the wing vein. In the other half, the Co^{60} was injected directly into the lumen of the gizzard.

After decapitation, the intestinal tract from the proventriculus down to the cloaca was removed, leaving behind the pancreas and mesenteries. Neither the cloaca nor the esophagus and crop were included. Each caecum was then removed and its contents mechanically separated from the caecal wall. The samples obtained were spread on the bottom of separate 20 ml. beakers and counted while moist above a thin mica end-window G-M tube. The standard, made by adding a known quantity of the Co^{60} injection solution to 2 ml. of water, was likewise counted in a 20 ml. beaker. The remainder of the intestinal tract was placed in a 250 ml. beaker and similarly counted. Due to the differences in the total volume of the intestinal tracts, a number of standards were prepared by adding a known quantity of Co^{60} to different volumes of water. The precise standard employed was that having the same volume as the sample of intestine being measured.

Trial 2 consisted of 32 birds. The large intestine of each bird was ligated below the caecal opening into the intestine. In the first group of 16 birds, both caeca were left intact; and in a second group of 16 birds, the left caecum was ligated. The incision was then sutured. Cobalt 60 was injected into the wing vein of half of the birds and into the lumen of the gizzard of the other half as in Trial 1. Twenty-four hours later, the birds were killed.

After dissection, the small intestine and its standards were counted in a similar manner to that employed with the birds in Trial 1. The caecal wall, the caecal contents and the whole large intestine, however, were placed in separate porcelain crucible covers, and ashed at 500 to 600 degrees Centigrade in a muffle furnace for five hours. These samples were then counted under a thin mica end-window G-M tube. Standards for these samples were prepared in triplicate by pipetting a known quantity of Co^{60} injection solution onto the covers and drying. A self absorption correction was applied to each sample (Appendix 1).

In both trials, one ml. blood samples were obtained in porcelain crucibles by cutting the wing vein immediately before the killing. Two-tenths ml. of bladder bile was also taken from each bird and placed in separate porcelain covers. The total volume of bladder bile in each bird was recorded. All blood and bile samples were dried at room temperature for 24 to 36 hours and counted under a thin mica end-window G-M tube. Standards were prepared by adding known quantities of Co^{60} to blood and bile taken from non-injected birds.

All data were treated statistically. The groups' means and their standard errors (Snedecor, 1946) are summarized in the appropriate tables. Aberrant data were rejected (after initial statistical evaluation) according to the Chauvenet criterion (Calvin, 1949) to give the population from which the final statistics were computed.

B. Results

The recoveries of Co^{60} from the digestive tract in both trials, computed as percentages of the injected dose, are summarized in Tables 1 to 4 and Figures 2 to 6.

Trial I, Large Intestine Not Ligated

Intestinal Recovery: As shown in Table 1 and Figures 2 and 3, when the large intestine was not ligated, there were no significant differences in the Co^{60} recoveries from the intestinal tract 24 hours after injection whether the Co^{60} was administered intravenously or injected directly into the lumen of the gizzard. In the sham operation, the total recovery from the intestinal tract averaged 12.1%, out of which an average of 7.2% was from the small and large intestines. The remaining 4.9% was recovered from both the caeca. When both caeca were tied, the total recovery averaged only 7.6%, a figure which is in close agreement with the 7.2% recovered from the small and large intestines alone in the previous group. In this case, only a small amount of the injected dose (less than 1%) was recovered from both the caeca. With only one caecum tied, total recovery was between that recovered from the sham operated group and that recovered from the group with both caeca tied. However, due to large individual variations, there were no statistical differences between any of these groups.

Extracellular Fluid Recovery: The Co^{60} recovery from the extracellular fluid was calculated from the whole blood concentration. It was assumed that the Co^{60} enters the entire extracellular compartment and that it does not penetrate into the blood cells. Both assumptions will be demonstrated to be correct from the data of the dog experiments. If one assumes a hematocrit value of 40% and 25% of the body weight to be extracellular fluid in the chicken, recovery from the total extracellular space averaged 5.4% when all groups of birds were considered. Inspection of Table 1 reveals no significant differences between groups injected intravenously or groups in which the Co^{60} was introduced directly into the lumen of the gizzard.

Caecal Recovery: A consideration of the Co^{60} recoveries from individual caeca showed that the ligation of one caecum does not affect the recovery from the caecum of the opposite side. Accordingly, Table 2 and Figure 4 were tabulated so that the Co^{60} recoveries from all the untied caeca may be compared with recoveries from all the tied caeca. Comparisons may also be made between recoveries following intravenous or intragizzard injections and between recoveries from the left or right caeca. As seen from this table and figure, when the large intestine was not ligated, the Co^{60} recovery from a single caecum or its contents 24 hours after injection was independent of the route of Co^{60} administration. No significant difference could be detected between right and left caeca. However, the recoveries were much less when the caecum was tied than when it was free. The recovery from a single caecum or from its contents averaged 2.3% and 1.8%, respectively, when the caecum was free; as compared with only 0.28% and 0.08%, respectively, when it was tied. The Co^{60} recovery from the caecal wall was also independent of the route of administration and whether the caecum was on the right or left side of the bird. In addition, the presence or absence of a ligature did not affect the Co^{60} found in the caecal wall. The recovery from the caecal wall, averaged for all groups of birds under all conditions, was 0.34%.

Trial II, Large Intestine Ligated

Intestinal Recovery: As presented in Figure 5 and Table 3, when the large intestine was ligated at its junction with the small intestine, the total intestinal recovery (in the sham operation) 24 hours after injection was 25% when Co^{60} was injected intravenously and 63% when it was introduced directly into the lumen of the gizzard. The proportion of the recovery from the gut which was found in the small intestine was about 70% when Co^{60} was

injected intravenously and 90% when it was introduced directly into the lumen of the gizzard. The recovery from both caeca averaged 4.5% of the injected dose and that from large intestine 1.6% of the injected dose regardless of the route of administration.

Extracellular Fluid Recovery: The Co^{60} recovery from the extracellular fluid was computed as before and found to average 4.1% in all groups of birds. This does not differ significantly from the value of 5.4% for those groups of birds with large intestine not ligated.

Caecal Recovery: It may be noted, in Table 4 and Figure 6, that when the large intestine was ligated, the Co^{60} recovery from a single caecum and its contents 24 hours after injection tended to be higher when Co^{60} was injected intravenously than when it was introduced directly into the lumen of the gizzard. In the sham operation, the total recovery from a single caecum and the recovery from its contents averaged 2.6% and 2.3%, respectively, when injected intravenously as compared with 1.9% and 1.5%, respectively, when introduced directly into the gizzard. However, due to the large individual variations, the differences between these recoveries were not statistically significant. Neither the presence or absence of a ligature, nor the route of administration, nor whether the caecum was on the left or right side of the bird significantly affected the Co^{60} recovery. The Co^{60} recovery from the caecal wall in this trial averaged 0.33%, a value no different from that found in the previous trial in which the large intestine was not ligated.

Ratio to Blood: Table 5 and Figure 7 show the relative Co^{60} concentrations in the caecal wall, and in the large intestine plus its contents relative to the whole blood level. The concentration ratio of caecal wall

to whole blood averaged 22 and of the large intestine plus its contents to whole blood averaged 49 in all groups of birds. There were no significant differences between any of these groups.

Comparative Data from Both Trials: The ratios of the Co^{60} concentrations in the caecal wall to whole blood and in the bile to blood in both trials are presented in Tables 6 and 7, and in Figures 8 and 9.

With the large intestine not ligated, the Co^{60} concentration ratio of caecal contents to whole blood in the untied caecum was 53 to 69 when Co^{60} was injected intravenously and 90 to 106 when it was introduced directly into the lumen of the gizzard (Table 6 and Figure 8). In the tied caecum, these ratios were 0.3 to 3.8. When the large intestine was ligated, on the other hand, all concentration ratios were above 100 (104 to 147) with one exception. In that one case, the ratio was only 68 (when the caecum was tied and when Co^{60} was introduced directly into the gizzard).

The Co^{60} concentration ratios of bladder bile to whole blood, as presented in Table 7 and Figure 9, were less than 1 in those groups of birds with the large intestine not ligated and between 1.5 and 3.7 in those groups in which it was ligated. Ratios in all groups receiving intravenous injection tended to be higher than those in the groups receiving intragizzard injection. However, these differences were not significant.

As shown in Table 8, there were no significant differences in the wet weights of either the caecal contents (group mean per caecum 1.13 to 2.05 gm.), or the caecal wall (group mean per caecum 1.22 to 1.74) gm.), or the large intestine plus its contents (group mean per bird 3.20 to 3.30 gm.), or in the total volume of the bladder bile (group mean 0.60 to 1.19 ml. per bird) between any of the different groups of birds in both trials.

TABLE 1

Co^{60} Recoveries (Per Cent of Injected Dose) from Small and Large Intestines, Both Caeca and Extracellular Fluid Twenty-four Hours after Injection. The large intestine was not ligated. The percentage recovery in extracellular fluid was computed from whole blood activity as indicated in the text.

Treatment	Route of Injection	Small & Large Intestines	Both Caeca	Total Intestinal Recovery	Extracellular Fluid
Sham	Intravenous	8.2 ± 1.0*	4.4 ± 3.7	12.5 ± 3.8	4.4 ± 2.0
	Intragizzard	6.2 ± 2.5	5.4 ± 2.1	11.7 ± 3.3	5.3 ± 4.7
Both Caeca Tied	Intravenous	7.1 ± 2.7	0.8 ± 0.3	8.0 ± 2.7	7.9 ± 2.9
	Intragizzard	6.9 ± 2.8	0.3 ± 0.2	7.2 ± 2.8	4.1 ± 1.2
Right Caecum Tied	Intravenous	7.7 ± 1.7	2.3 ± 1.0	10.0 ± 2.0	4.3 ± 1.3
	Intragizzard	5.7 ± 1.6	2.8 ± 1.4	8.6 ± 2.1	5.9 ± 1.2
Left Caecum Tied	Intravenous	5.4 ± 2.7	3.4 ± 2.5	8.9 ± 3.7	5.1 ± 1.8
	Intragizzard	6.3 ± 2.6	1.4 ± 1.2	7.6 ± 2.9	6.5 ± 2.8

* Mean ± standard error.

TABLE 2

Co^{60} Recoveries (Per Cent of Injected Dose) from Caecal Wall and Caecal Contents Twenty-four Hours After Injection. The large intestine was not ligated.

Treatment	Route of Injection	Caecum	Caecal Wall	Caecal Contents	Total
Caecum Untied	Intravenous	Right	0.49 ± 0.58*	1.69 ± 1.06	2.18 ± 1.16
		Left	0.64 ± 0.48	1.20 ± 1.05	1.84 ± 1.11
	Intragizzard	Right	0.35 ± 0.71	2.22 ± 1.08	2.57 ± 1.23
		Left	0.42 ± 0.53	2.07 ± 1.25	2.49 ± 1.13
Caecum Tied	Intravenous	Right	0.27 ± 0.17	0.10 ± 0.05	0.37 ± 0.18
		Left	0.29 ± 0.18	0.17 ± 0.11	0.46 ± 0.21
	Intragizzard	Right	0.10 ± 0.09	0.01 ± 0.01	0.11 ± 0.09
		Left	0.14 ± 0.13	0.03 ± 0.02	0.17 ± 0.13

* Mean ± standard error.

TABLE 3

Co^{60} Recoveries (Per Cent of Injected Dose) from Small Intestine, Both Caeca, Large Intestine Plus Its Contents and Extracellular Fluid Twenty-four Hours After Injection. The large intestine was tied off at its junction with the small intestine. The percentage recovery in extracellular fluid was computed from whole blood activity as indicated in the text.

Treatment	Route of Injection	Small Intestine	Both Caeca	Large Intestine	Total Intestinal Recovery	Extracellular Fluid
Sham	Intravenous	17.9 ± 6.0*	5.3 ± 1.9	2.0 ± 0.9	25.2 ± 8.4	4.6 ± 2.7
	Intragizzard	57.6 ± 8.6	3.6 ± 1.9	1.3 ± 0.9	62.5 ± 8.9	4.0 ± 1.7
Left Caecum Tied	Intravenous	20.8 ± 4.8	4.9 ± 2.4	1.4 ± 0.8	27.1 ± 5.4	3.7 ± 1.3
	Intragizzard	59.9 ± 6.9	2.2 ± 0.5	1.5 ± 0.9	63.6 ± 7.0	4.2 ± 0.6

* Mean ± standard error.

TABLE 4

Co^{60} Recoveries (Per Cent of Injected Dose) from Caecal Wall and Caecal Contents Twenty-four Hours After Injection. The large intestine was tied off at its junction with the small intestine.

Treatment	Route of Injection	Caecum	Caecal Wall	Caecal Contents	Total
Sham	Intravenous	Right	0.38 ± 0.20*	2.23 ± 1.13	2.61 ± 1.50
		Left	0.30 ± 0.07	2.30 ± 0.30	2.60 ± 1.34
	Intragizzard	Right	0.43 ± 0.18	1.41 ± 0.71	1.84 ± 0.80
		Left	0.38 ± 0.27	1.66 ± 1.33	2.04 ± 1.56
Left Caecum Tied	Intravenous	Right	0.27 ± 0.11	2.00 ± 1.41	2.27 ± 1.51
		Left	0.25 ± 0.10	2.12 ± 0.98	2.37 ± 1.03
	Intragizzard	Right	0.34 ± 0.17	1.01 ± 0.34	1.35 ± 0.41
		Left	0.26 ± 0.10	0.69 ± 0.22	0.95 ± 0.27

* Mean ± standard error.

TABLE 5

Co^{60} Concentration Ratios of Caecal Wall and Large Intestine plus Its Contents to Whole Blood* Twenty-four Hours After Co^{60} Injection. The large intestine was tied off at its junction with the small intestine.

Treatment	Route of Injection	Concentration Ratio		
		Caecal Wall / Blood		Large Intestine plus Its Contents / Blood
		Left	Right	
Sham	Intravenous	19	21	58
	Intragizzard	23	26	44
Left Caecum Tied	Intravenous	19	18	44
	Intragizzard	24	26	51
Mean		21	23	49

* Ratios to plasma would be 0.6 of the recorded values if a hematocrit of 40% is assumed and no cobalt enters the cells.

TABLE 6

Co^{60} Concentration Ratio of Caecal Contents to Whole Blood* Twenty-four Hours After Co^{60} Injection

Treatment		Routes of Injection	Concentration Ratio Caecal Contents: Blood	
			Left Caecum	Right Caecum
Large Intestine Intact	Sham	Intravenous	69	60
		Intragizzard	103	106
	Both Caeca Tied	Intravenous	3.8	3.0
		Intragizzard	0.3	0.5
	Right Caecum Tied	Intravenous	53	1.8
		Intragizzard	101	0.9
	Left Caecum Tied	Intravenous	2.4	69
		Intragizzard	2.0	90
Large Intestine Tied	Sham	Intravenous	119	117
		Intragizzard	136	147
	Left Caecum Tied	Intravenous	120	104
		Intragizzard	68	127

* Ratios to plasma would be 0.6 of the recorded values if a hematocrit of 40% is assumed and no cobalt enters the cells.

TABLE 7

Co^{60} Concentration Ratio of Bile to Whole Blood* Twenty-four Hours After Co^{60} Injection

Treatment		Route of Injection	Concentration Ratio Bile: Blood
Large Intestine Intact	Sham	Intravenous	0.92
		Intragizzard	0.28
	Both Caeca Tied	Intravenous	0.58
		Intragizzard	0.14
	Right Caecum Tied	Intravenous	0.19
		Intragizzard	0.15
	Left Caecum Tied	Intravenous	0.63
		Intragizzard	0.32
Large Intestine Tied	Sham	Intravenous	3.7
		Intragizzard	2.3
	Left Caecum Tied	Intravenous	3.4
		Intragizzard	1.5

* Ratios to plasma would be 0.6 of the recorded values if a hematocrit of 40% is assumed and no cobalt enters the cells.

TABLE 8

Bile Volume, Wet Weight of Caecal Contents, Caecal Wall and Large Intestine plus Its Contents in Chickens

Treatment		Route of Injection	Bladder Bile Volume cc.	Wet Weight, gm.				
				Caecal Contents		Caecal Wall		Large Intestine plus Its Contents
				Left	Right	Left	Right	
Large Intestine Tied	Sham	Intravenous	1.2 ± 0.5	1.7 ± 0.4	1.9 ± 0.4	1.6 ± 0.3	1.7 ± 0.4	3.3 ± 0.6
		Intragizzard	1.2 ± 0.5	1.5 ± 0.5	1.8 ± 0.7	1.5 ± 0.6	1.4 ± 0.2	3.2 ± 0.7
	Left Caecum Tied	Intravenous	1.1 ± 0.3	1.7 ± 0.7	1.7 ± 0.7	1.2 ± 0.3	1.3 ± 0.3	3.2 ± 0.9
		Intragizzard	1.2 ± 0.4	1.4 ± 0.7	1.9 ± 0.8	1.2 ± 0.5	1.5 ± 0.6	3.2 ± 0.9
Large Intestine Intact	Sham	Intravenous	0.7 ± 0.3	1.5 ± 0.8	1.7 ± 0.8			
		Intragizzard	0.6 ± 0.3	1.5 ± 0.6	1.1 ± 0.7			
	Both Caeca Tied	Intravenous	1.0 ± 0.7	1.8 ± 0.7	1.7 ± 0.8			
		Intragizzard	0.6 ± 0.4	1.3 ± 0.7	1.3 ± 0.4			
	Right Caecum Tied	Intravenous	0.9 ± 0.5	2.0 ± 0.7	1.7 ± 0.6			
		Intragizzard	0.6 ± 0.3	1.2 ± 0.7	1.7 ± 0.4			
	Left Caecum Tied	Intravenous	0.7 ± 0.2	2.0 ± 0.7	2.1 ± 1.2			
		Intragizzard	0.6 ± 0.3	1.7 ± 0.6	1.7 ± 0.7			

TABLE 9

Metabolic Balance of Co^{60} Following Intravenous and Intragizzard Injections. All data are presented as percentage of injected dose.

	Intravenous		Intragizzard	
	Large Intestine not Ligated	Large Intestine Ligated	Large Intestine not Ligated	Large Intestine Ligated
Injected	100	100	100	100
Recovered from Whole Intestinal Tract	12	25	12	63
From Small Intestine		18	7	58
From Large Intestine	7	2		1.3
From Both Caeca	5	5	5	3.6
Recovered from Extracellular Fluid	5	4.6	5	4
Fecal Loss (by calculation)	13	0	51	0
Urinary Loss and Tissue Retention (by calculation)	70	70	32	33

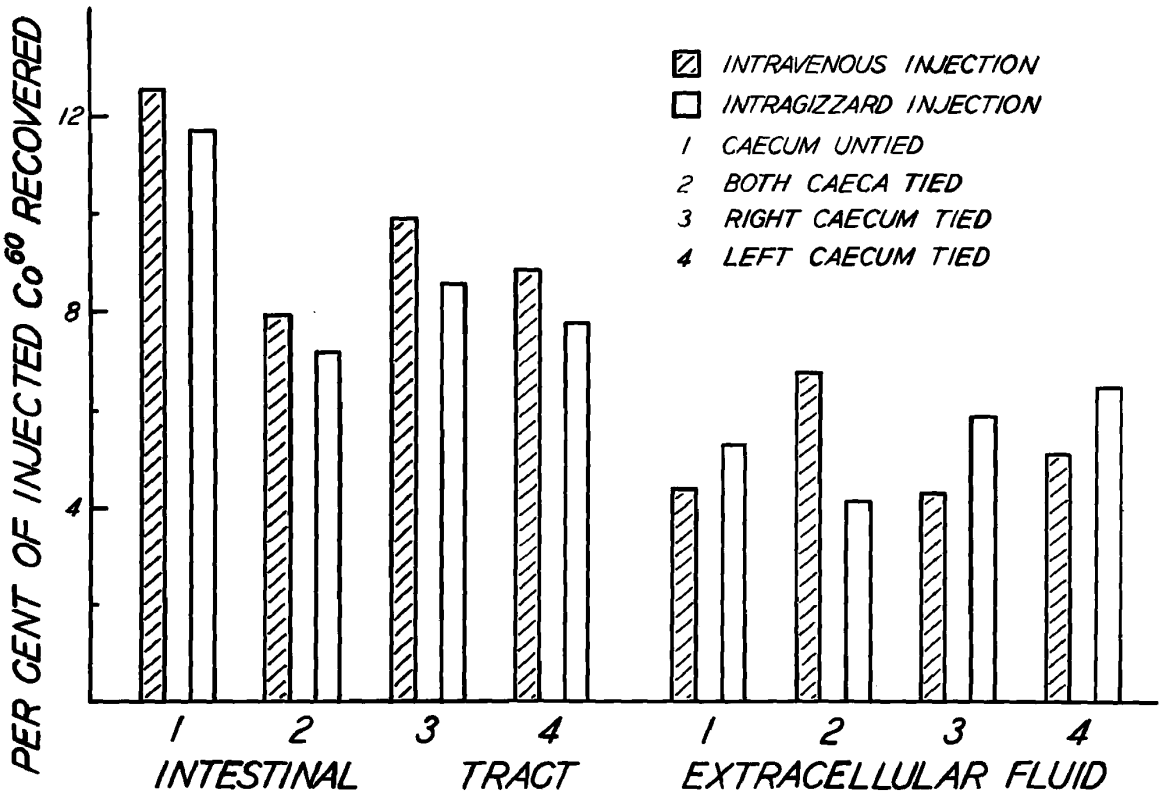


Figure 2. Co^{60} Recoveries (Per cent of Injected Dose) from Intestinal Tract and Extracellular Fluid Twenty-four Hours After Injection. The large intestine was not ligated. The percentage recovery in extracellular fluid was computed from whole blood activity as indicated in the text.

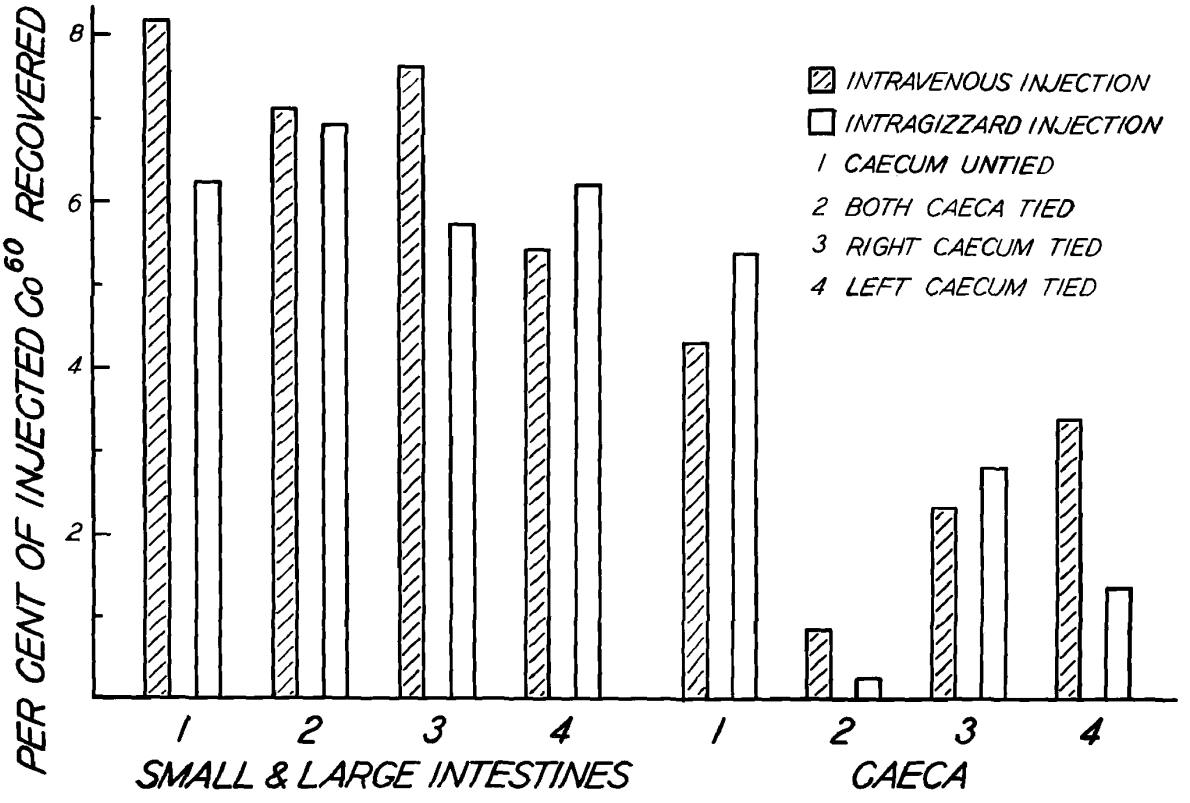


Figure 3. Co^{60} Recoveries (Per Cent of Injected Dose) from Small and Large Intestines and Both Caeca Twenty-four Hours After Injection. The large intestine was not ligated.

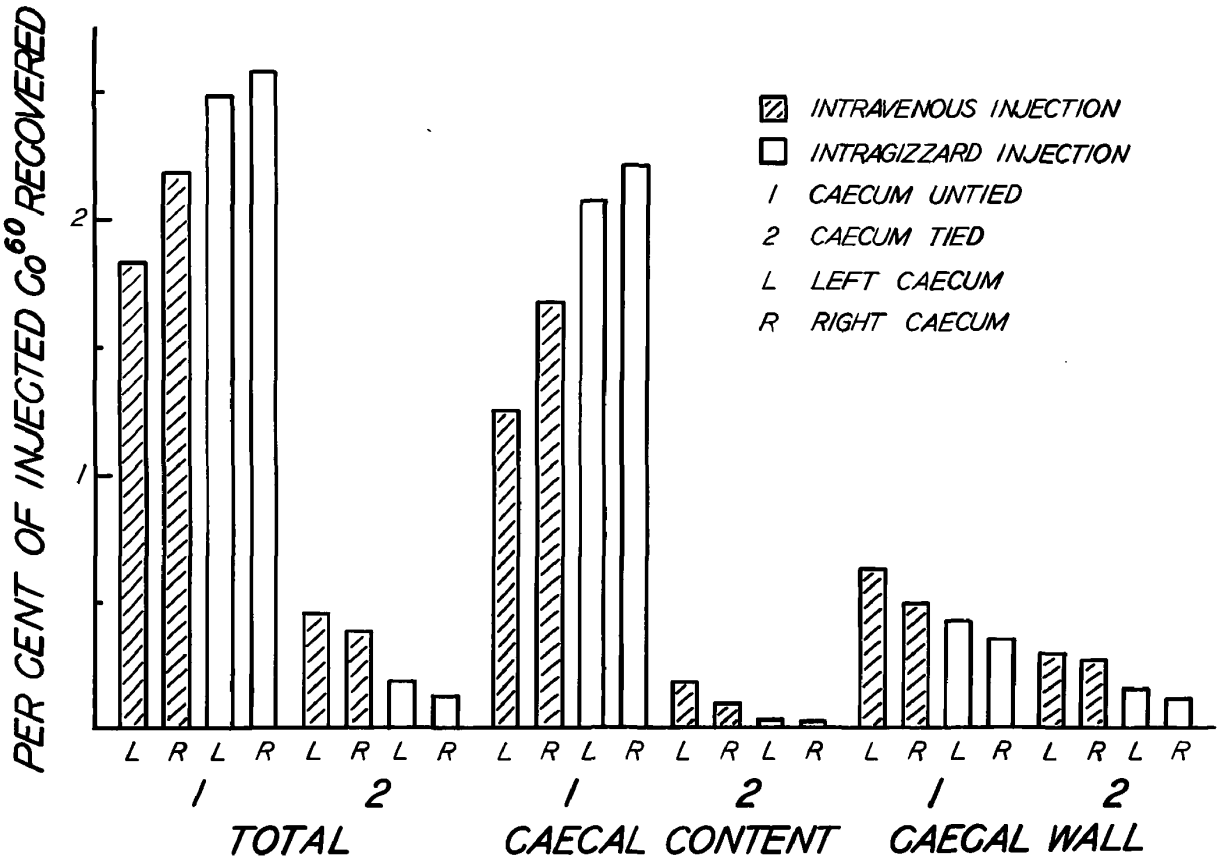


Figure 4. Co^{60} Recoveries (Per Cent of Injected Dose) from Caecal Wall and Caecal Contents Twenty-four Hours After Injection. The large intestine was not ligated.

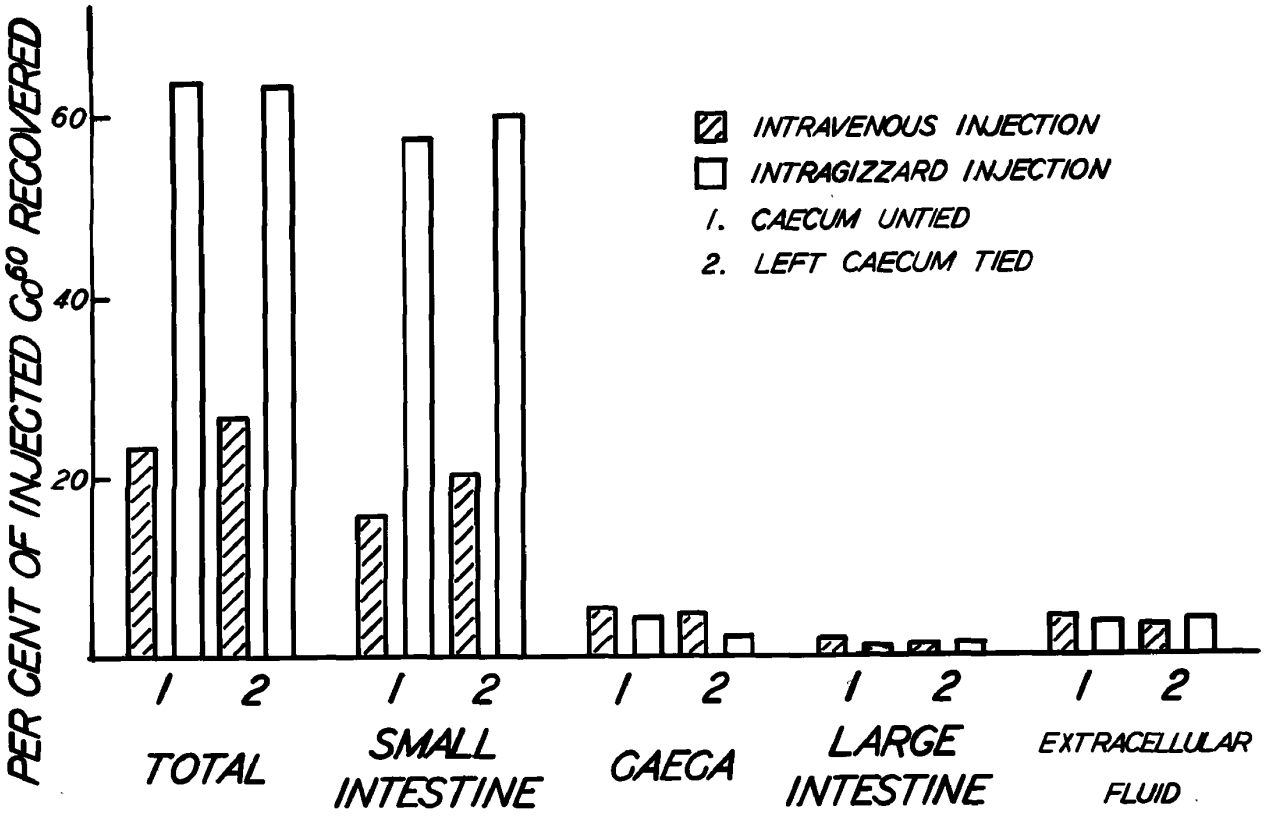


Figure 5. Co^{60} Recoveries (Per Cent of Injected Dose) from Small Intestine, Both Caeca, Large Intestine plus its Contents and Extracellular Fluid Twenty-four Hours After Injection. The large intestine was tied off at its junction with the small intestine. The percentage recovery from extracellular fluid was computed from whole blood activity as indicated in the text.

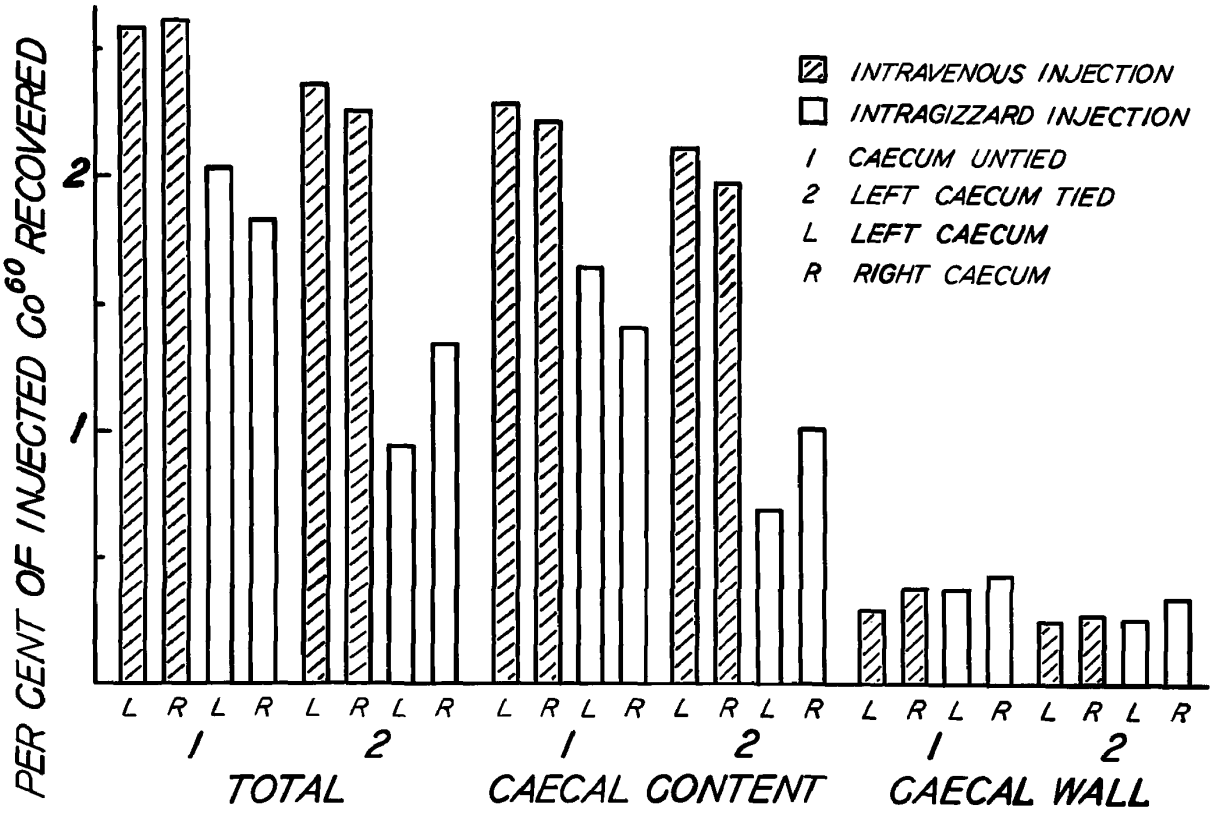


Figure 6. Co^{60} Recoveries (Per Cent of Injected Dose) from Caecal Wall and Caecal Contents Twenty-four Hours After Injection. The large intestine was tied off at its junction with the small intestine.

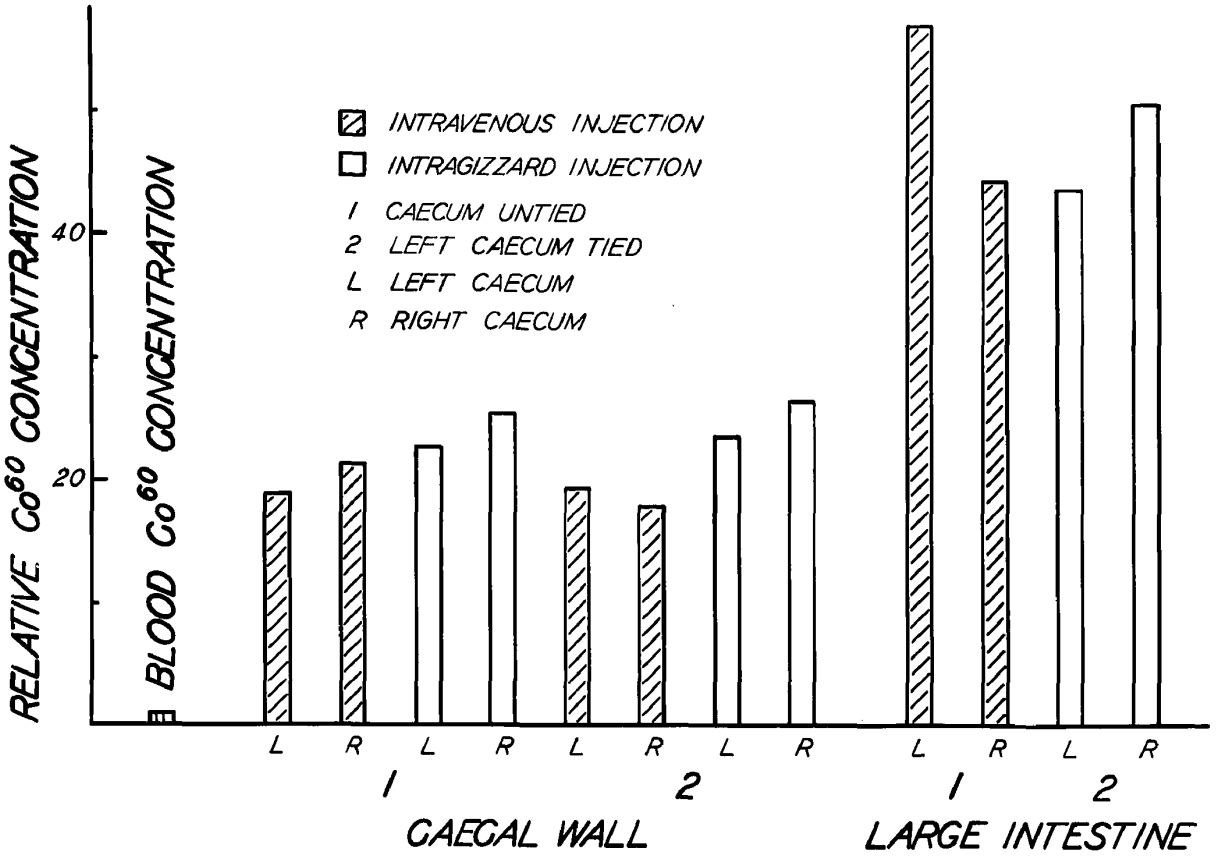


Figure 7. Relative Co^{60} Concentrations in the Caecal Wall and the Large Intestine plus Its Contents to Whole Blood Twenty-four Hours After Co^{60} Injection. The large intestine was tied off at its junction with the small intestine.

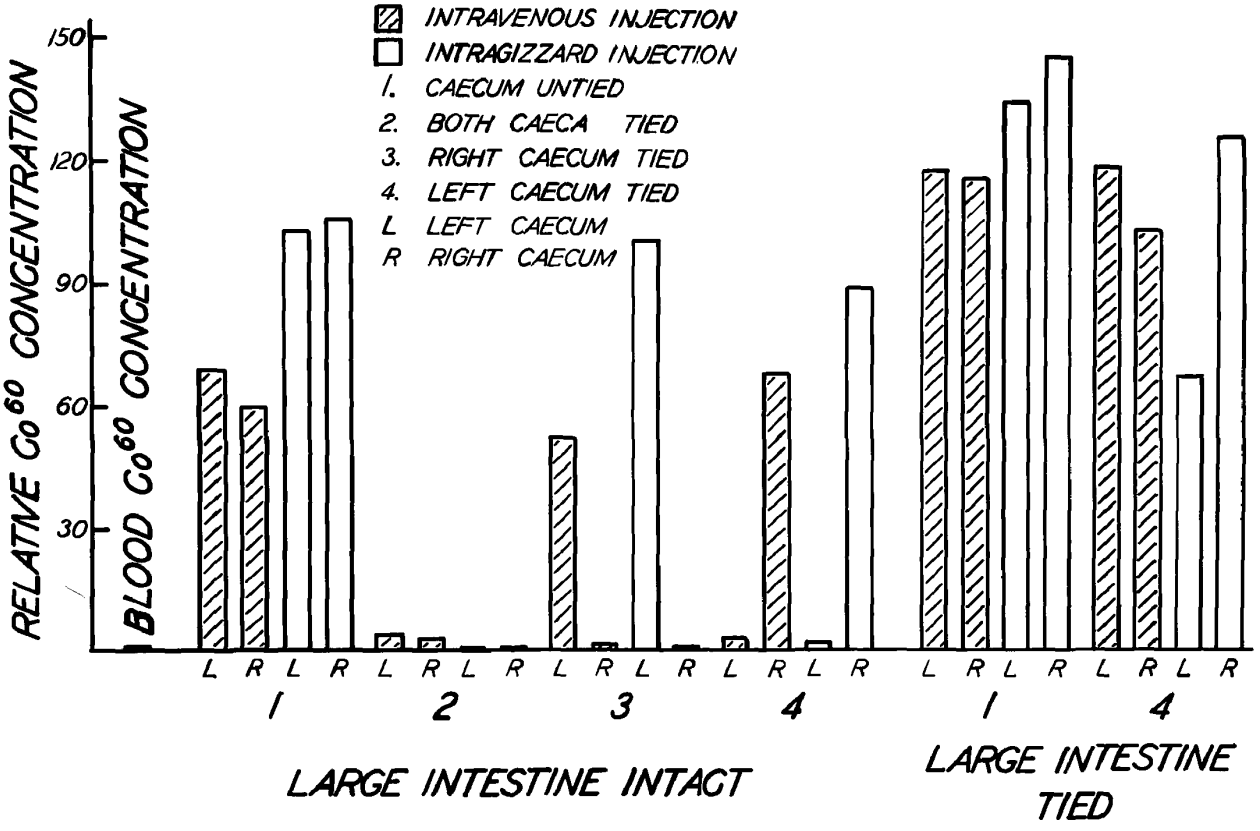


Figure 8. Relative Co^{60} Concentrations in the Caecal Contents to Whole Blood Twenty-four Hours After Co^{60} Injection.

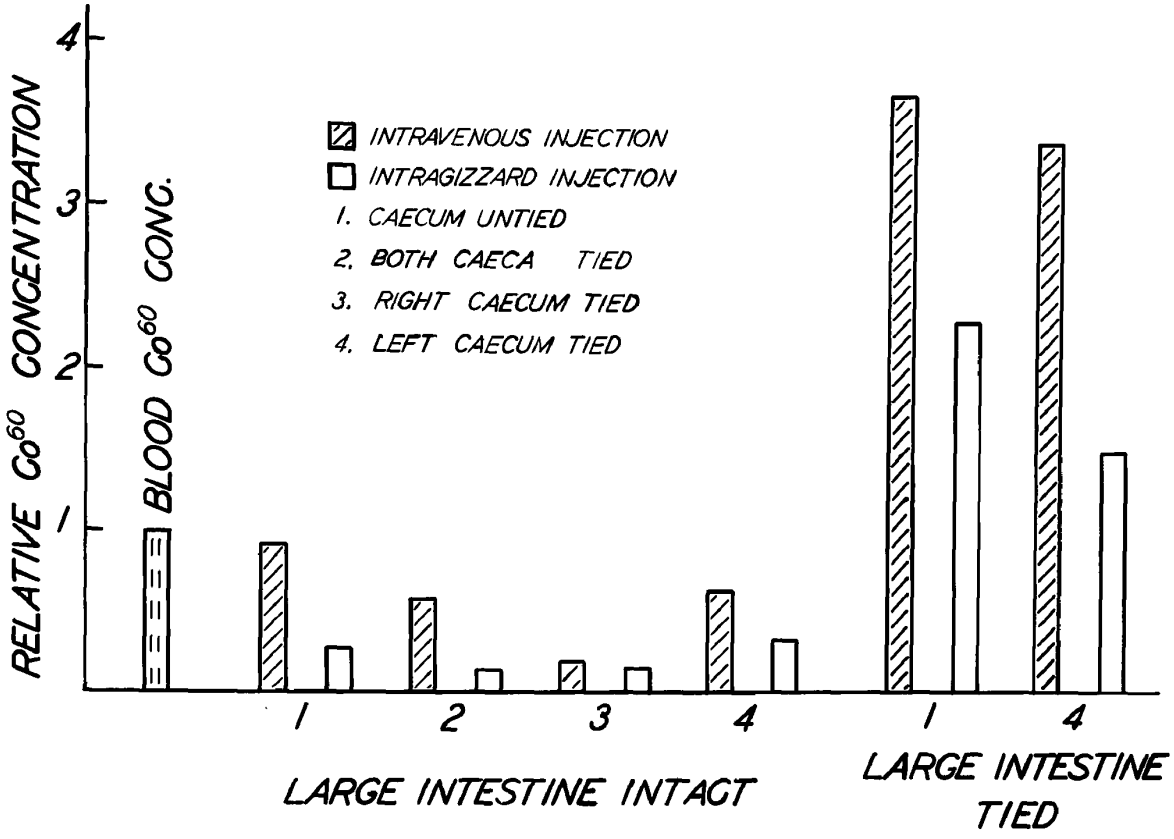


Figure 9. Relative Co^{60} Concentrations in the Bile to Whole Blood Twenty-four Hours After Co^{60} Injection.

C. Discussion

When the large intestine was not ligated, recovery of Co^{60} from the intestinal tract or its appearance in the extracellular fluid 24 hours after injection is not affected by routes of administration used in these experiments (Table 1 and Figure 2). These results would be anticipated if the organism approached an equilibrium in which Co^{60} is partitioned in a uniform and standard manner between body fluids, tissues and intestinal contents. Evidence for such a standard partitioning may also be drawn from the data obtained by Monroe *et al.* (1952a) within 24 hours after intraperitoneal Co^{60} injection. Thus, it appears that the time scale for exchange with the various Co^{60} compounds of the bird is such that "uniform labeling" is attained in about a day's time. The route of administration is of no consequence for events after 24 hours.

This is in contrast to the findings in the ruminants summarized by Comar (1948) and reaffirmed in a subsequent report by Monroe *et al.* (1952b). In the ruminants, the time required to reach the "uniform labeling", in the above sense, was much longer than 24 hours. Since the ruminants have an extremely large volume of bacterial flora in the rumen, a large amount of the orally administered Co^{60} might be absorbed by the bacteria rather than by their host. Consequently, the tissue content of Co^{60} following oral administration would be expected to be less than that following intravenous injection.

In addition, the rate of flow of materials through the intestinal tract of the ruminants is so slow that considerable time must necessarily elapse before an appreciable fraction of Co^{60} in the tract can leave the animal. Since the transport rate of materials through the intestinal tract is slow,

it might be thought that there would be a greater opportunity for the absorption of the Co^{60} in ruminants. The fact that low absorption was found in sheep following oral administration (Monroe et al., 1952b) is a direct evidence for large scale Co^{60} binding by the intestinal contents in this species.

These data also indicate that Co^{60} passes across the intestinal wall in both directions. The intestinal absorption of inorganic cobalt has been demonstrated by separate experiments in this laboratory. Wolterink and Lee (unpublished data) found that 50% of the administered dose is absorbed within 20 minutes in day-old baby chicks when Co^{60}, as $Co^{60}SO_4$, is injected directly into the lumen of the gizzard. Pandurang (1952) has also been able to show that inorganic Co^{60} is absorbed from the tied caecum of the chicken. The intestinal "excretion" of Co^{60} will be discussed later.

At the end of 24 hours, a total recovery from the entire intestinal tract of 12% when the large intestine was not ligated (Table 1 and Figure 2) and of 25% when it was ligated (Table 3 and Figure 5) was found following intravenous injection. At least 13% of an intravenously injected dose, then, passes out through the feces during the first day. Out of the 75% not recovered from the intestinal tract of these birds, only 5% was found in the extracellular fluid. A large fraction of the remaining 70% must have been excreted in the urine.

On the other hand, in the case of intragizzard injection, 12% of the injected dose was recovered in the entire unligated intestinal tract at the end of 24 hours, whereas 63% was recovered if the large intestine was ligated. Thus, at least 51% of the dose administered into the gizzard appears to be lost in the feces during the first day. In addition to the 4 to 5% found in the extracellular fluid, a large fraction (up to 32 to 33%

of the administered dose) of the 37% not recovered in the intestinal tract must have been excreted in the urine.

In the dog experiments, 35 to 60% of an intravenously injected dose was eliminated in the urine during the first 7 to 13 hours (Table 19). Thus, urine is the chief pathway for the excretion of Co^{60} in both the dog and the chicken.

A summary of the metabolism balance of Co^{60} following these two routes of administration is tabulated in Table 9.

In this table, the Co^{60} recovery from the extracellular fluid was computed on the assumptions of an average hematocrit value of 40% and an extracellular fluid value of 25% of the body weight. By means of thiocyanate, radioactive Cl^{38}, Na^{24}, or Na^{22}, the extracellular space has been observed to be from 21 to 28% of the body weight in the rabbit (Hahn and Hevesy, 1941; Manery and Haege, 1941), dog (Greenbert et al., 1943a; Winkler et al., 1943), rat (Cuthbertson and Greenberg, 1945); and in the adult human being (Fellers et al., 1949; Kaltreider et al., 1941). A higher average value of 41 to 44% of the body weight was found for infants (Fellers et al., 1949; Flexner et al., 1947). For normal chicks (13 to 35 days of age with a body weight ranging 121 to 411 gm.), a thiocyanate space of 43% of the body weight and a hematocrit value of 30.5% have been reported (Hegsted et al., 1951). Although values for older chickens are not available, in view of the data cited from other species, it seems reasonable to use a value of 25% of the body weight for extracellular fluid and of 40% for the hematocrit. It should be emphasized that the Co^{60} recoveries from the extracellular fluid were statistically the same in all groups of birds and in both trials. This means that Co^{60} retained in the body fluids reached a constant level in 24 hours whatever the route of administration and whatever the route of passage out of the body.

After intravenous injections, an unexpectedly large fraction of the Co^{60} was recovered from the intestinal tract. In the dog experiments (Table 19), hepatic bile was found to carry from 5 to 10% of the total intravenously injected dose in periods of 7 to 13 hours. Significant amounts of cobalt have not been eliminated in the pancreatic juice (Sheline et al., 1945-1946). This suggests that Co^{60} is "excreted" into the small intestine through the bile. However, in chickens the low Co^{60} concentration in bile and the low bile to whole blood concentration ratio indicates that an appreciable fraction of the intestinal Co^{60} recovery may reach the small intestinal lumen directly through the wall.

When the large intestine was not ligated, only a very small amount of Co^{60} was found in the contents of the tied caecum regardless of the route of administration (Table 2 and Figure 4). However, recoveries from the caecal contents in the untied caeca were more than 20 times as high. This indicates that the caecum receives most of its Co^{60} from the gut. Only a small fraction actually enters the caecum across the caecal wall. This is demonstrated further by the fact that the Co^{60} concentration ratios of the caecal contents to whole blood were below 4 in all the tied caeca and above 50 in all the untied caeca (Table 6 and Figure 8).

When the large intestine was ligated, on the other hand, the ligation of caecum did not cause any significant decrease in the caecal Co^{60} recovery (Table 4 and Figure 6). This implies that large amounts of Co^{60} can enter the caecum across its wall under appropriate conditions. These conditions are evidently satisfied when both the large intestine and the caecum are ligated. Whether this large scale Co^{60} passage across the caecal wall is due to an alteration of the caecal function caused by ligation or is due merely to a large

reservoir of Co^{60} maintained in the body for a longer time requires further investigation.

Little information concerning the blood and lymphatic supplies of the caecum in birds is available. The ligation of the caecum was made around its neck and close to the junction with the intestine. Surgical damage to the mesentery around the caecum was avoided as much as possible. The placement of a ligature appeared to have little effect on the blood and lymphatic circulation of the caecum, judging from the observation that Co^{60} recoveries from the caecal wall were about the same in all groups of birds and in both trials (Tables 2, 4, Figures 4 and 6). Thus, there is little evidence for large scale circulatory impairment in the ligated caeca.

In view of the small mass of the large intestine plus its contents (group mean, 3.20 to 3.30 gm. wet weight, Table 8), the 1 to 2% Co^{60} recovery from the large intestine when the latter was ligated (Table 3 and Figure 5) is very significant. The high Co^{60} concentration ratio of 49 for the large intestine plus its contents to blood (Table 5 and Figure 7) suggests that the Co^{60} is actively secreted into the large intestine in the bird. Cobalt 60 might have entered the large intestine by antiperistalsis from the cloaca into which the ureters empty. Although no estimate can be made of the fraction of the continuous flowing urine which is forced back into the large intestine, it seems unlikely that any large amount of the Co^{60} recovered from the large intestine could have come from this source.

The existence of high Co^{60} concentration ratios in the wall and/or in the contents of the intestinal tract to whole blood strongly indicates that

the Co^{60} must be in some "bound" form or forms*. The presence of vitamin B_{12} containing Co^{60} has recently been reported both in the excreta and in tissues including small intestine (Davis and Chow, 1951; Monroe et al., 1952b). In addition, Monroe et al. (1952b) have found what they designated as "bound" Co^{60} in the excreta and in tissues of sheep. Pandurang (1952) of this laboratory has also claimed evidence for the presence of some unknown bound form of Co^{60} in the caecal contents as early as one hour after the inorganic Co^{60} was injected into the lumen of the tied caecum in the chicken. Apparently, a large amount of the Co^{60} is present in tissues and in intestinal contents in some "bound" form or forms.

The highest Co^{60} concentration ratios were found in the caecal contents, indicating that the "non-diffusible" Co^{60} was present in larger quantity in the caecal contents than in the caecal wall. This implies that either the size or the metabolic activity of the intestinal bacterial population may be directly concerned with the "binding" of Co^{60}. Davis and Chow (1951) have reported that the fecal concentration of vitamin B_{12} containing Co^{60} activity can be increased by the incorporation of aureomycin in the diet or by increasing the level of inorganic Co^{60} in the ration of rats. They attribute the effect of the antibiotic to some alteration in the intestinal bacterial flora.

The existence of "non-diffusible" Co^{60} in the intestinal wall as well as in the contents may be the result of "continuous diffusion" of inorganic Co^{60} from the circulation through the wall into the lumen of the intestinal tract. In passing through the intestinal wall, and again after entering the lumen, some fraction of the Co^{60} may be complexed into large "non-diffusible"

*The term "bound" is used to indicate a form of cobalt which does not leave the cell despite a concentration gradient favoring diffusion out. These "bound" form or forms of Co^{60} may be true chemical compounds; or they may be coordination complexes; or they may be simple adsorption complexes.

molecules. The "diffusion" process might continue until the wall and the contents of the intestinal tract can no longer bind the Co^{60} into its "non-diffusible" form or forms and a "saturation" point reached.

As shown in Table 6 and Figure 8, the Co^{60} concentration in the caecal contents varies under different experimental conditions. These concentrations were higher following intragizzard injection than following intravenous injection, and they were highest in those groups of birds with the large intestine ligated. These differences in Co^{60} concentrations in the caecal contents may be due to one or more of the following reasons: 1. The total integrated Co^{60} in the body fluid available for "diffusion" into the caecum during the 24 hours of the experiment might be higher under one set of experimental conditions than under another. 2. The number of bacteria in the caecal contents might vary under different experimental conditions in spite of the fact that the wet weights of the caecal contents are about the same (Table 8). The uptake of Co^{60} per bacterial cell might also vary under different experimental conditions. 3. Certain caecal functions, for example the ability of the caecum to absorb water, might be altered by the presence of ligatures, despite the fact that ligation causes no apparent effect on the blood and lymphatic circulation of the caecum. If water transport is interfered with by mechanical stimulus, it would be reasonable to expect alterations as well in the transport of water soluble materials containing Co^{60}.

Monroe et al. (1952a) found a very low concentration of Co^{60} in the liver of young chicks after intraperitoneal injection of inorganic Co^{60}. In the present experiments, the concentration of Co^{60} in the bladder bile of the chicken 24 hours after oral or intravenous administration was much lower than that found in the hepatic bile of the dog. The Co^{60} concentration ratios

of the bladder bile to whole blood of the chicken were below 4 in all groups of birds. No information can be found with respect to the quantity of bile flow or to the emptying time of gall bladder in the bird. However, in 24 hours, emptying of gall bladder must certainly have taken place. This eliminates the possibility that the Co^{60}-containing 24-hour hepatic bile was diluted by Co^{60}-free bladder bile formed earlier. Since hepatic bile is concentrated within the gall bladder by the removal of water, the concentration ratio in the hepatic bile should be still lower than that observed in the bladder bile. In view both of this low concentration ratio and of the low volume of bladder bile, the liver of chicken apparently does not remove cobalt from the blood to the same extent as does the liver of the dog. Further investigation concerning the function of the liver in the excretion of Co^{60} in the chicken is needed.

METABOLISM OF COBALT 60 IN DOGS

The purposes of the experiments with dogs are: to determine rates for both biliary and urinary excretion of cobalt, to evaluate the function of the renal tubules in handling this trace element and to determine the partition of cobalt between the body fluids. Comparisons will be made between the biological turnover rates for the removal of inorganic cobalt and amino acid cobalt complexes from the peritoneal cavity and the blood. Data will also be presented showing the relative distributions of cobalt in the intestines and tissues following different routes of administration. The reabsorbability of the cobalt excreted in the bile and urine collected from the experimental dog will be studied by determining their intestinal absorption in chicks.

A. Methods

General Remarks:

Twenty-one mongrel dogs, weighing between 5.5 and 21.4 kg., and 80 three-day-old White Leghorn chicks were used in these experiments.

The Co^{60} used for all injections was the same diluted solution (about 70 micrograms per ml. with a pH 2) used in the chicken experiments. Infusion solutions and an injection solution of second dilution (0.813 microgram per ml.) were prepared from the first diluted solution in physiological saline.

All blood, urine, bile and plasma samples used for radioactivity determination were dried in separate porcelain crucibles at room temperature for 24 to 36 hours. Standards were prepared from known quantities of Co^{60} added to blood, urine, bile and plasma collected before injection of Co^{60}.

All standards were made in triplicate. Blood standards were prepared for each dog. The other standards were used throughout these experiments.

All the plasma and urine samples were refrigerated before chemical analysis. The group means and their standard errors were computed as in the chicken experiments.

Surface area was calculated from the Meeh-Rubner weight formula:

$$S.A. = \frac{11.2 \times Wt.^{0.667}}{100}$$

where S. A. is the surface area in square meters and Wt. is the body weight in kg.

Distribution of Co^{60} in Blood:

Thirty-four blood samples were collected from the jugular vein of Dogs No. 15*, 16, 21, 24 and 25 at various intervals after intravenous injection of inorganic Co^{60}. Radioactivity measurements from each sample were made on one ml. of heparinized whole blood, on one ml. of plasma and on 3 ml. of the cadmium hydroxide plasma filtrate which was used for the determinations of creatinine and PAH. A standard for the plasma filtrate was prepared by adding a known quantity of Co^{60} to water. The plasma filtrate samples and the standards, in triplicate, were dried in separate porcelain crucibles at room temperature for 96 hours.

The hematocrit determination on each blood sample was made by spinning the well-mixed heparinized whole blood in a Wintrobe hematocrit tube for half an hour at 2250 RPM within one hour after sampling. A change of the cell

*Dog No. 15 was an unsatisfactory preparation for the study of renal clearance of Co^{60}. However, two blood samples were drawn 1 and 2 hours after the intravenous injection of Co^{60}.

volume by less than 1% was observed after an additional half hour of centrifugation at the same speed, indicating satisfactory packing of the red blood cells. A correction for the plasma trapped between cells was made using a factor of 0.96 (Gregerson and Schiro, 1938). Between the time of sampling and centrifugation, blood samples were refrigerated. From the above procedures, the partitioning of Co^{60} between cells, plasma and protein-free plasma filtrate was computed.

One dog (No. 26) received 20 micrograms of Co^{60} per kg. body weight intravenously. Two blood samples of about 40 ml. each were drawn from the jugular vein at one and ten hours after injection and heparinized. After centrifugation 10 ml. of plasma was pipetted into a dialyzing tubing* about four inches in length. The protein in another 2 ml. of plasma was precipitated with cadmium hydroxide as described in Appendix 2. Ten ml. of the supernatant protein-free filtrate was pipetted into a second dialyzing tubing. After all the plasma filtrate was decanted, 20 ml. of distilled water was added to the protein precipitate. Vigorous shaking yielded a suspension of this precipitate. Ten ml. of the suspension was put in a third dialyzing tubing. All tubes were dialyzed against running tap water for eight to thirty hours. Frequent shaking of the tubing containing plasma or protein precipitate was necessary to prevent packing of the denatured proteins. Radioactivity measurements were made on one ml. of plasma, on 3 ml. of protein-free plasma filtrate and on 3 ml. of precipitate suspension both before and after dialysis. All samples were prepared in triplicate

*Dialyzing tubing, Chicago Apparatus Company, Chicago, Illinois

and dried for 48 to 72 hours. The percentage of Co^{60} diffusible through the cellophane membrane was then calculated.

Rate of Co^{60} Absorption from the Peritoneal Cavity and Its Rate of Elimination from Blood When Injected Intraperitoneally:

Twenty micrograms of Co^{60} per kg. body weight was injected intraperitoneally into each of four dogs weighing between 7 and 10.5 kg. Two dogs received the Co^{60} solution. The other two received a complex of Co^{60} with cysteine. To make the Co^{60}-cysteine complex, the pH of Co^{60} solution was first adjusted to between 6 and 7. A sufficient amount of cysteine* was added at room temperature to give a Co^{60}/cysteine molar concentration ratio of 1:30. This mixture contains ten times as much cysteine as is required for the formation of the Co^{60}-cysteine complex (Michaelis and Yamaguchi, 1929).

About 2 ml. of blood were drawn from the cephalic vein at intervals of 5, 10, 15, 30, 60 minutes, and 2, 3, 4, 5, 6, 7, 8 and 20 hours after injection. Radioactivity was measured on one ml. of whole blood. Disappearance curves from the peritoneal cavity and blood were then plotted on semilog paper following the method of Berlin and Siri (1951).

Reabsorption of Co^{60} from the Urine and Bile Samples:

The hepatic bile and urine samples from Dog No. 7 were used. Pooled samples collected between 0-4, 4-8, and 8-12 hours after the initial Co^{60} injection were employed. The pooled samples were then diluted with physiological saline to the concentration indicated in Table 12 and kept in the refrigerator overnight. Seven groups of three-day old White Leghorn chicks were set up, each group consisting of eleven birds. Three groups of these

*Anhydrous cysteine hydrochloride, C. P., Fisher Scientific Co., Pittsburgh, Pennsylvania.

chicks received a urine injection; three groups, a bile injection; and one group, an injection of an inorganic Co^{60} solution. One ml. of these samples or of the Co^{60} solution was injected directly into the lumen of the gizzard of each chick. All chicks were killed half an hour later. The intestinal tract from above the proventriculus down to the cloaca was removed and placed on individual tared procelain crucible covers. They were then ashed in a muffle furnace at 500 to 600 degrees Centigrade for five hours, weighed, and counted under a thin mica end-window G-M tube. Standards were prepared by adding known quantities of Co^{60} solution to the intestinal tract removed from non-injected chicks and ashed and counted in the same manner. Since the weights of all the ashed samples were about the same (group means, 9.6 to 10.5 mg./cm^2), no self-absorption correction was applied.

These urine and bile samples and, in addition, those from Dog No. 5 (which received the same treatment as Dog No. 7) were also studied by means of paper partition chromatography and radioautography. All samples were spotted on Whatman No. 1 filter paper and developed by ascending technique (Williams and Kirby, 1948) with n-butanol saturated with water for a period of 72 hours at room temperature. Radioautograms were prepared by placing Kodak No Screen X-ray film in direct contact with the resultant paper chromatograms for a period of 72 hours.

Renal Clearance, Tubular Reabsorption and Hepatic Clearance of Co^{60}:

A total of fourteen dogs, weighing 5.5 to 21.4 kg., were employed in this section of the experiment. Food was taken away from each dog for about ten hours prior to the experiment. Half an hour before each experiment, 50 ml. of water per kg. was given by stomach tube. Each dog was anesthetized with 30 mg. of sodium pentobarbital per kg. body weight. In those experiments of

long duration (up to 13 hours), additional doses of 3 to 5 mg. of pentobarbital per kg. were administered as necessary to maintain deep slow respiration.

Six of these fourteen dogs* received an intravenous priming dose of 50 mg. of creatinine** and of 2 mg. of PAH (sodium p-amino hippurate)*** per kg. half an hour prior to the beginning of the clearance periods. At that time, infusion of solution containing 0.2% of creatinine and 0.1% of PAH was begun through the exposed femoral vein at the rate of 200 ml. per hour using a mercury drop method. The urine samples were collected from the bladder of four dogs through an indwelling catheter and by supra-pubic cannulation of both ureters with plastic tubing in the remaining two dogs. In cases where the bladder urine was collected, each period was terminated by a single rinse with a known quantity of physiological saline and an air-wash. Three or eight consecutive urine collection periods of 15 minutes' duration were run in each of the six dogs beginning half an hour after a single intravenous injection of 20 micrograms of Co^{60} per kg. Blood was drawn from the jugular vein opposite to the infusion side and heparinized.

In four other dogs, the cystic duct was ligated and the common bile duct cannulated with 1 mm. I.D. plastic tubing through a ventral abdominal incision. Each ureter was similarly cannulated. The trachea was cannulated with a metal T-cannula and the femoral vein with a glass cannula connected

*One of these six dogs, Dog No. 15, was an unsatisfactory preparation. She died one hour after a single intravenous injection of 20 micrograms of Co^{60} per kg. body weight.

**Creatinine, C. P., Pfanstiehl Chemical Co., Waukegan, Illinois.

***PAH, Sodium para-aminohippurate, 20% solution, Sharp and Dohme, Philadelphia, Pennsylvania.

to an injection burette. Arterial blood was sampled as follows: An 18-gauge needle with its adapter end removed was connected to a 1 mm. I. D. plastic tubing, with a close-fitting wire serving as a plug. The needle was passed obliquely through the wall of the artery (the carotid, except in Dog No. 3, which was sampled from the femoral). After sampling, the wire plug was dipped in heparin and inserted through the plastic tubing to the tip of the needle, which was left in situ. An artery clip was placed on the tubing to prevent leakage. Occasionally a soft clot formed in the needle. This was easily pressed out by clamping both ends of the artery and applying pressure in between. About 2 ml. of blood were taken at each sampling and heparinized. Each of these four dogs received 20 micrograms of Co^{60} per kg. body weight from the injecting burette, followed by 5 ml. of saline immediately before the urine and bile collections.

In the remaining four dogs, similar operations were made to collect urine and hepatic bile through ureteral and biliary cannulae. The trachea was also cannulated. Two of these four dogs (Dogs No. 6 and 7) received a priming intravenous injection of 10 micrograms of Co^{60} per kg. followed by a constant infusion of a solution containing, respectively, 37.56 or 25.23 micrograms percent of Co^{60} in physiological saline. Infusion was made through an exposed femoral vein at the rate of 100 ml. per hour using the mercury drop method. Urine and bile collections were made as in the four dogs which received only a single injection of Co^{60}. In the last two dogs (Dogs No. 24 and 25), an intravenous priming dose of 10 micrograms of Co^{60}, 50 mg. of creatinine and 2 mg. of PAH per kg. body weight was given half an hour prior to the beginning of the clearance observation. The infusion solution consisted of 26.57 or 23.38 micrograms percent of Co^{60}, 0.3% of

creatinine and 0.15% of PAH in physiological saline. The infusion rate was the same as for the two previous dogs. Clearance observations were made at three different stages during the experiment. Each stage consisted of three consecutive urine collection periods of 30 minutes' duration. The first stage was started half an hour after the initial Co^{60} injection; the second stage, six hours; and the third, ten and one-half hours. Bile samples were also collected during the urine collection periods.

Radioactivity measurements in all these fourteen dogs were made on one ml. of whole blood, on 0.05 to 0.2 ml. of diluted urine, on 0.1 to 0.2 ml. of the hepatic bile and also, in some cases, on one ml. of plasma.

Creatinine clearances were used to measure the glomerular filtration rates and PAH clearances to measure the effective renal plasma flows. Creatinine was determined on the cadmium hydroxide plasma filtrates (Fujita and Iwatake, 1931) and on diluted urine by the alkaline picrate method (Folin and Wu, 1919). PAH was determined by the method of Smith et al. (1945). All analyses were made in duplicates. The detail methods of these chemical analyses may be found in Appendix 2.

A plasma concentration value interpolated to coincide with the midpoint of the urine collection period was used to calculate the clearances, T_m for PAH and Co^{60} load during that period. Bile samples were collected in 15 ml. graduated centrifuge tubes and urine in 10, 50 or 100 ml. volumetric flasks depending upon the length of the collection period and the urine flow. All samples were capped to prevent evaporation. To determine the urine volume, each urine collection flask was filled to the mark with distilled water from a 50 ml. burette. The difference between the total volume of the flask and water or saline and water added gave the urine volume. One ml. of each of

the urine samples diluted as described above were again diluted to 100 or 250 ml. in another volumetric flask. Determinations of creatinine and PAH were made on these final dilutions.

Data derived from the above determination were computed by the use of the formulae listed in Appendix 2. These include renal clearance of creatinine in ml. per minute (C_{cr}), renal clearance of PAH in ml. per minute (C_{PAH}), renal clearance of Co^{60} in ml. per minute ($C_{Co}60$), tubular secretory mass of PAH in mg. per minute (T_m), filtration fraction (FF), Co^{60} load in micrograms per minute, Co^{60} excreted in micrograms per minute, Co^{60} reabsorbed in micrograms per minute, and Co^{60} reabsorption in percentage.

Tissue Distribution of Co^{60} in Dogs:

Two dogs of 10.0 and 11.0 kg. were anesthetized with 30 mg. of sodium pentobarbital per kg. body weight. Through a ventro-medial abdominal incision, three small intestinal loops, each four inches in length, were ligated at both ends. The first loop was at the first part of the duodenum with the bile duct emptying into its lumen. The second loop was at the last part of the duodenum and the third loop, at the beginning of the jejunum. One dog received 20 micrograms of Co^{60} per kg. intravenously. The same amount of Co^{60} was injected into the lumen of the second intestinal loop of the second dog. With the abdominal incision sutured up, these two dogs were placed back in the cage and supplied with water only.

Twelve hours after the injection of Co^{60}, these two dogs were anesthetized again. Small pieces of the liver, the spleen, the right kidney, the three loops of the small intestine, the caecum and the first part of the large intestine were then removed. All segments of the intestinal wall, including caecum, were separated from their contents. Each sample, about 0.5 to 2.0 gm.,

was wet-weighed, ashed, ash-weighed and counted in separate porcelain crucible covers. The second loop of small intestine from the dog which received intra-intestinal injection of Co^{60} was digested with a minimum amount of concentrated nitric acid until all solid matter had been dissolved. The excess acid was then boiled off. The solution was cooled and diluted with acetone and a little water to form a clear solution of 250 ml. Triplicate samples, containing 2 ml. of each of this diluted solution were placed on separate porcelain crucible covers, and dried, ashed, ash-weighed and counted in a similar manner. Standards were prepared in triplicate by adding a known quantity of Co^{60} to the crucible covers. A self absorption correction (Appendix 1) was applied to all these samples. Radioactivity measurements on one ml. of blood, plasma, bladder urine and bladder bile of these two dogs were also made.

The various treatments of all these twenty-one dogs are summarized in Table 10.

TABLE 10

Treatments Employed on the Dogs Used in These Experiments

Dog No.	Sex	Body Wt. kg.	Injection Agent	Injection Amount per kg.	Injection Route	Infusion Agent	Infusion Amount per 100 ml.	Infusion per hr.	Bile Collection	Bile Collection	Experimental Purpose
26	M	11.0	$Co^{60}SO_4$	20 ug	IV						To study the distribution of Co^{60} in blood
8	M	7.0	$Co^{60}SO_4$	10 ug	IP						To study the turnover rate of Co^{60} in the body
9	M	9.0									
10	F	9.8	Cysteine-	10 ug	IP						
11	M	10.3	Co^{60} Comp.								
21	M	12.0	$Co^{60}SO_4$,	20 ug,	IV	Creatinine, and	0.2 gm,	100 ml.		Dog 21, by suprapubic cannulation of both ureters; other dogs, by catheterization.	To study the renal clearance of Co^{60} beginning half an hour after a single intravenous injection
12	F	12.5	Creatinine, and	50 mg,		PAH in saline	0.1 gm.				
13	F	5.5	PAH.	2 mg.							
15*	F	10.0									
16	F	16.0									
18	F	9.0									
1	M	21.4	$Co^{60}SO_4$	20 ug	IV				by cannulation of common bile duct	by suprapubic cannulation of both ureters	To study the renal and hepatic excretions of injected Co^{60}
2	M	11.5									
3	M	13.5									
4	F	9.5									
6	M	8.0	$Co^{60}SO_4$	10 ug	IV	$Co^{60}SO_4$ in saline	37.56 ug	100 ml.	by cannulation of common bile duct	by suprapubic cannulation of both ureters	To study the renal and hepatic excretions and tubular function of Co^{60}
7	M	10.0					25.23 ug				
24	F	13.0	$Co^{60}SO_4$, Creatinine, and	10 ug, 50 mg,	IV	$Co^{60}SO_4$, Creatinine, PAH	26.57 or 23.38 ug,	100 ml.			
25	F	13.0	PAH	2 mg.		in saline	0.30 mg, 0.15 mg.				
22	M	10.0	$Co^{60}SO_4$	20 ug	IV						To study the relative tissue distribution of Co^{60}
23	M	10.5	$Co^{60}SO_4$	20 ug	II**						

* Dog No. 15 was an unsatisfactory preparation.

** Intraintestinal injection.

B. Results

Distribution of Co^{60} in Blood:

The distribution of Co^{60} in blood components is summarized in Table 11. The relation of the distribution to the time after the Co^{60} injected into the blood is shown in Figures 10 and 11.

It will be noted that among the thirty-four blood samples tested, the cells contained only an average of $3.1 \pm 1.6\%$ of the total Co^{60} present in the whole blood. There was no statistical difference in the amount of Co^{60} in the blood cells in relation to the time after the Co^{60} injected into the blood throughout the period of twelve hours. However, the amount of Co^{60} present in the cadmium hydroxide protein-free plasma filtrate decreased toward the end of the experimental period. Within two hours after the Co^{60} injection, the Co^{60} present in the protein-free plasma filtrate represented about $15.7 \pm 2.4\%$ of the Co^{60} of the whole blood. The amount decreased to $8.9 \pm 1.8\%$ during the period of 6 to 7.5 hours after Co^{60} injection. Only $4.3 \pm 2.3\%$ was found in the protein-free filtrate near the end of the experiment. The differences between the amounts of Co^{60} present in the protein-free plasma filtrates are statistically significant. By subtracting the amounts of Co^{60} present in blood cells and protein-free plasma filtrate from 100%, the amount of Co^{60} present in the protein precipitate were found to be $78.1 \pm 7.7\%$, $91.1 \pm 3.4\%$ and $97.2 \pm 3.5\%$ at various times after initial injection.

By dialysis, the diffusible Co^{60} present in the various plasma fractions was found to be as follows:

A. In the blood sample obtained at one hour after Co^{60} injection.

	In Plasma	In Plasma Filtrate	In Protein Precipitate
After dialyzing of 8 hours	21%	74%	9%
After dialyzing of 30 hours	31%		

B. In the blood sample obtained at ten hours after Co^{60} injection.

	In Plasma	In Plasma Filtrate	In Protein Precipitate
After dialyzing of 22 hours	2.3%	100%	23%

Rate of Co^{60} Absorption from the Peritoneal Cavity and Its Rate of Elimination from Blood When Injected Intraperitoneally:

The rate of absorption and the rate of elimination of Co^{60}, in terms of half-time, when injected intraperitoneally are presented in Table 12. These data were obtained graphically from a semilog plot of the blood levels for the first four hours after injection according to the method of Berlin and Siri (1951).

The half-times for the removal of Co^{60} from the blood in two dogs were 13 and 12.5 minutes and for its transfer from the peritoneal cavity to the blood in the same dogs were 135 and 195 minutes when Co^{60} was given as cobaltous sulfate. Both rates were much faster when an equivalent amount of Co^{60} was administered in the form of cysteine-Co^{60} complex. In the latter case, the half-times for removal from the blood and from the peritoneal cavity in two other dogs were 12 and 8 minutes, and 52 and 45 minutes, respectively. After four hours, the blood Co^{60} concentration leveled off and remained relatively constant up to at least 20 hours.

Reabsorption of Co^{60} from the Urine and Bile Samples:

The data are presented in Table 13. When inorganic Co^{60} was injected into the lumen of the gizzard of three-day-old chicks, the intestinal recovery, one-half hour after injection, was 70.8 ± 4.3% of the injected dose. Corresponding figures for the recoveries of Co^{60} injected as urine were 38.6 ± 2.3, 37.1 ± 4.6 and 40.9 ± 4.3% with an average of 38.9 ± 4.1%. Similar recoveries from bile samples were 41.1 ± 3.6, 45.1 ± 3.5 and 45.5 ± 2.9%, respectively, with an average of 43.9 ± 3.9%. The difference in recoveries between inorganic Co^{60} and Co^{60} from urine or bile samples is highly significant. However, there was no significant difference between the recoveries from urine and bile samples or from the samples obtained during different periods. I_t will be noted, accordingly, that the half-time of disappearance of Co^{60} from the intestine was longer in the group which received inorganic Co^{60} than in those which received urine or bile samples. Statistically, there was no difference between the half-times of the intestinal disappearance of Co^{60} from urine and bile samples or from samples collected during different periods.

Paper partition chromatography using radioautograms to locate the spots containing Co^{60} was employed in an attempt to identify the forms of Co^{60} in urine and bile. There were no conclusive findings. Although the inorganic Co^{60} accounted for a large majority of the radioactivity in both the samples, additional radioactive component or components were present in both the urine and bile samples. In these experiments, Co^{60}-labeled vitamin B_{12}* standards moved approximately one and one-half inches whereas the standards of inorganic

*Kindly supplied by Dr. C. Rosenblum of Merck and Co., Inc., Rahway, New Jersey.

Co^{60} remained at the origin. The other radioactive component or components moved distances varying between one and one and one-half inches from the origin. However, in no specific case was it possible to conclude that more than a very minute trace of the Co^{60} in urine or bile samples was in the form of vitamin B_{12}.

Renal Clearance, Tubular Reabsorption, Hepatic Clearance Rates of Urinary Excretion and Biliary Excretion of Co^{60}:

Renal clearances of plasma Co^{60} in sodium pentobarbital anesthetized dogs are summarized in Tables 14 and 15.

As shown in Table 14, between two and two and one-half hours after a single injection of 20 micrograms of Co^{60} per kg. body weight, the renal clearance was found to average 26.9 $\pm$ 6.5 ml. per minute per square meter of surface area. In the 20 urine collection periods, the average urinary excretion of Co^{60} was 0.99 $\pm$ 0.25 micrograms per minute. The Co^{60} concentration in plasma during these periods averaged 31.50 $\pm$ 5.86 micrograms per liter with a glomerular filtration load of 1.44 $\pm$ 0.41 micrograms per minute. From the difference between the Co^{60} load and the rate of urinary excretion, the tubular reabsorption was calculated to be 69.5 $\pm$ 5.5% of the load. These five dogs had an average glomerular filtration rate of 88.4 $\pm$ 16.2 ml. per minute per square meter of surface area and an average effective renal plasma flow of 225.6 $\pm$ 94.7 ml. per minute per square meter of surface area. The filtration fraction was 0.43 $\pm$ 0.14. The average tubular secretory mass for PAH (T_m) was 1.86 $\pm$ 0.94 mg. per minute.

Additional data for renal clearance, obtained at 6-7½ and 10½-12 hours after the initial Co^{60} injection are summarized in Table 15. It will be noted

that the renal clearance of Co^{60} decreased during the acute experiment of 12 hours' duration. Between ½-2 hours after the Co^{60} priming injection, the average renal clearance was 21.0 ml. per minute per square meter of surface area. It decreased to 14.8 ml. per minute at 6-7½ hours and further to 11.5 ml. per minute 10½-12 hours after the Co^{60} priming injection. Accordingly, the calculated tubular reabsorption increased from an average value of 71.7% at the beginning to 84.1% at the intermediate time and further to 88.5% toward the end of the experiment. Both the glomerular filtration rate (C_{cr}) and the effective renal plasma flow (C_{PAH}) increased with the result that there was little change in the filtration fraction during the entire period. The tubular secretory mass for PAH (T_m) showed a continuous increase in one dog and fluctuated in the other. The average plasma Co^{60} concentration and filtration load increased slightly. The amount of Co^{60} recovered from the urine decreased with time.

Renal and hepatic clearances of blood Co^{60} (based upon the whole blood Co^{60} concentration) in six other dogs are summarized in Tables 16 and 17. It will be noted that the renal clearance increased rapidly and reached a maximum of 69 to 133 ml. per minute per square meter of surface area (39 to 67 ml. per minute per dog) between one-half and three hours after a single injection and fell gradually to 4 to 31 ml. per minute per square meter of surface area (2 to 17 ml. per minute per dog). In case of Co^{60} infused continuously, a maximum renal clearance of 61 and 105 ml. per minute per square meter of surface area was reached within two hours after the priming injection and fell more slowly toward the termination (Table 16). Hepatic clearances showed an intial rapid increase and then leveled off to about 3 to 7.5 ml. per minute per square meter of surface area in both the group of dogs which received

a single injection and in the group which received a priming injection followed by continuous infusion of Co^{60} (Table 17). The renal and hepatic clearances of whole blood Co^{60} of the dogs which received a single injection are shown in Figures 12 and 13. Dog No. 3 was not a satisfactory preparation. A peak renal clearance of 53 ml. per minute was reached 45 minutes after injection but the flow of urine and bile nearly ceased about three hours after injection. The hepatic clearance of this dog remained below 0.5 ml. per minute.

The ratios for clearances of Co^{60} in dogs receiving a single injection to that of dogs which received a constant infusion are also presented in Tables 16 and 17. The ratio of renal clearances was found to be 0.86 at one hour after Co^{60} injection (Table 16). This ratio declined consistently to about 0.34 at eight hours after injection, with a half-time of about four and one-half hours. On the other hand, the ratio for hepatic clearances was 1.31 at one hour after injection (Table 17). This ratio reached a maximum value of 2.06 at three hours after injection and declined to 0.98 at eight hours after injection. The half-time of this decline was also four and one-half hours.

It will be seen in Figures 14 and 15 that the blood level of Co^{60} declined rapidly in the first ten minutes after a single intravenous injection of inorganic Co^{60}. Thereafter, a fairly steady fall in blood levels took place.

Radioactive Co^{60} was detectable in both urine and bile samples within ten minutes after injection (Tables 16 and 17). Figures 16 and 17 showed the Co^{60} concentrations in urine and bile, respectively, during the entire experimental period after the single intravenous injection. Maximal urinary

concentrations of 4.5 to 30.3 micrograms per ml. were usually reached within the first hour. In the most rapid case (Dog No. 4), the peak of 30.3 micrograms per ml. was attained in 20 minutes. Except in Dog No. 3, urinary Co^{60} than dropped to levels between 0.7 and 1.7 micrograms per ml. at the end of the experiment. Concentrations in bile increased rapidly during the first hour and then more slowly until the experiments were terminated. Final levels were between 0.4 and 1.3 micrograms per ml. of bile, about the same as the final concentrations in urine.

The rates of urinary and biliary excretion of Co^{60} in dogs receiving a single intravenous injection, in terms of micrograms of Co^{60} per minute, are summarized in table 18. The rate of urinary Co^{60} excretion declined in much the same way as the urinary Co^{60} concentration. However, the maximal rate of excretion was not reached until twenty minutes to three hours after injection. It then declined toward the end of the experiment. The rate of biliary Co^{60} excretion continued to increase during the first half of the experiment and dropped slightly during the last half.

From Table 19, it will be noted that between 40 and 70% of the injected dose of Co^{60} was recovered in urine plus bile during the course of the experiments in which a single intravenous injection was given. A large majority (nearly 90%) of this was recovered during the first six hours. Only one-seventh to one-tenth of the total recovery was found in the bile. In Dog No. 3, since the flow of urine and bile nearly ceased three hours after injection, a total of only 23% of the injected dose was recovered at the end of six hours.

Blood concentrations, rates of infusion, rates of urinary, biliary and total excretion of Co^{60} in dogs which received a priming injection plus a constant infusion of inorganic Co^{60} are shown in Figures 18, 19, 20, and 21.

Blood concentrations of Co^{60} in three dogs (No. 6, 7 and 25) which received an infusion at constant rates (between varying dogs from 0.627 to 0.390 microgram per minute) increased very slightly during the entire experimental period of twelve hours. In Dog No. 24, there was a very slight decrease in blood concentrations of Co^{60} during the same time.

The rate of biliary excretion of Co^{60} increased in all cases except in Dog No. 6 which showed a slight drop during the last two hours of the experiment. The rise in the rate of biliary excretion continued throughout the first half of the experiment and reached a plateau four to seven hours after the priming injection of inorganic Co^{60}. There was a fairly constant rate of urinary excretion of Co^{60} beginning half an hour after the priming injection in three of the four dogs (Dogs No. 6, 7 and 25). Two of these three dogs showed a slight drop toward the end of the experiment. In the remaining dog, Dog No. 24, the rate of urinary excretion fluctuated and declined slightly during the course of the experiment. In this dog, there was a similar slight decrease in blood concentration of Co^{60}.

The rate of total excretion of Co^{60} paralleled the rate of infusion in Dogs No. 6 and 7. However, Co^{60} was retained in Dogs No. 24 and 25, since the rate of excretion was somewhat less than the infusion rate.

Tissue Distribution of Co^{60} in Dogs:

Twelve hours after inorganic Co^{60} was injected intravenously, a high concentration of Co^{60} was found in the liver, intestinal tract, kidney, urine and bile (Table 20). The Co^{60} activity in each gm. of liver (wet weight) was found to be about 24 times as great as that found in each ml. of plasma. The corresponding ratio for the intestinal wall or for the intestinal contents was 2 to 7, for the bladder urine, kidney or bladder bile was 2 to 3.5. Except

in the first loop of small intestine, the concentrations of Co^{60} were higher in the contents than that in the wall. The concentration was about the same in the wall and in the contents of the caecum and lower in the contents than in the wall of the large intestine.

When Co^{60} was injected into the lumen of the second loop of tied small intestine, only about 50% of the injected Co^{60} was absorbed within twelve hours. As in the case of intravenous injection, Co^{60} was concentrated in the liver, in the intestinal tract, in the urine, in the kidney and in the bile. However, the concentration ratios of tissues to plasma were only 10.5 times in the liver, 1.5 to 4 times in the wall or in the contents of small intestine and of caecum, 2 times in the urine and in the kidney, and about one time in the bile and in the large intestinal wall or contents.

Plasma concentration in the dog which received an intravenous injection was about twice as high as in the dog which received intraintestinal injections. In both cases, the spleen contained a smaller amount of Co^{60} than in plasma, when computed on an equivalent wet weight basis. The total Co^{60} recoveries from extracellular fluid at the end of twelve hours after injection were the same, e.g. 5.3% and 5.1% for the dog receiving intravenous and intraintestinal injections, respectively. These calculations were based upon the plasma concentration of Co^{60} and the assumption that the extracellular fluid is 25% of the body weight in the dog (Greenberg et al., 1943a; Winkler et al., 1943).

TABLE 11

Distribution of Co^{60} in Blood at Various Times After Initial Intravenous Injection

Time After Co^{60} Injection, hrs.	No. of Samples	Co^{60} Percent of Whole Blood		
		In Blood Cells	In Protein-free Plasma Filtrate	In Plasma Protein Precipitate
0.5 - 2.0	19	6.2 ± 7.3*	15.7 ± 2.4	78.1 ± 7.7
6.0 - 7.5	7	0.0 ± 2.9	8.9 ± 1.8	91.1 ± 3.4
10.5 - 12.0	8	-1.6 ± 2.7	4.4 ± 2.3	97.2 ± 3.5
Mean	34	3.1 ± 1.6		

* Mean ± standard error.

Radioactive measurements were made separately on whole blood, plasma and protein-free plasma filtrate as indicated in the text. Conversion of recorded counts to the actual amount of Co^{60} present in each sample was made by comparing the sample with its standards. The following formulae were used to compute the partition of Co^{60} in the blood.

$$1.\quad A = \frac{W-P\,(1-Hcr)}{W} \times 100$$

$$2.\quad B = \frac{F\,(1-Hcr)}{W} \times 100$$

$$3.\quad C = 100 - A - B$$

where A = Percentage of Co^{60} present in the red blood cells
B = Percentage of Co^{60} present in the protein-free plasma filtrate
C = Percentage of Co^{60} present in the plasma protein
W = Amount of Co^{60} in whole blood (ug/ml)
P = Amount of Co^{60} in plasma (ug/ml)
F = Amount of Co^{60} in undiluted protein-free plasma filtrate (ug/ml)
Hcr = Hematocrit

TABLE 12

Half-times for the Absorption into the Blood and Removal from the Circulation of Co^{60} When Administered Intraperitoneally as Cobaltous Sulfate and as Cysteine-Cobalt Complex

	Dog No.	Blood $T_{\frac{1}{2}}$ Min.	Peritoneal $T_{\frac{1}{2}}$ Min.
Cobaltous Sulfate	8	13	135
	9	12.5	195
Cysteine-Cobalt Complex	10	12	52
	11	8	45

TABLE 13

Co^{60} Recovery (Per Cent of Injected Dose) from the Intestinal Tract and Absorption Half-times When Injected as Urine or Bile

Injection	Amount of Co^{60} Injected ug.	Intestinal Co^{60} Recovery %	$T\frac{1}{2}$ Disappearance from the Intestine, Min.
$Co^{60}SO_4$	0.813	70.8 ± 4.3*	62.5 ± 13.3
0 - 4 Hour Urine	0.606	38.6 ± 2.3	21.9 ± 1.4
4 - 8 Hour Urine	0.596	37.1 ± 4.6	21.2 ± 2.5
8 - 12 Hour Urine	0.446	40.9 ± 4.3	23.5 ± 2.6
Mean		38.9 ± 4.1	22.2 ± 4.2
0 - 4 Hour Bile	0.350	41.1 ± 3.6	23.5 ± 2.2
4 - 8 Hour Bile	0.532	45.1 ± 3.5	26.3 ± 2.4
8 - 12 Hour Bile	0.618	45.5 ± 2.9	26.6 ± 2.2
Mean		43.9 ± 3.9	25.5 ± 4.8

* Mean ± standard error

TABLE 14

Renal Clearances of Creatinine, PAH and Co^{60} in Sodium Pentobarbital Anesthetized Dogs. All data are the average of 3 or 8 consecutive urine collection periods of 15 minutes duration beginning half an hour after the intravenous injection of 20 ug. of Co^{60} per kg. body weight.

Dog No.	No. of Period	C_{cr}/M^2 S.A.	C_{PAH}/M^2S.A.	T_m PAH	Filtration Fraction	Co^{60} Plasma Conc.	Co^{60} Load	Co^{60} Urine Vol.	Co^{60} Excreted	Co^{60} Reabsorbed	Co^{60} Reabsorption	C_{co}/M^2S.A.
		ml/min	ml/min	mg/min		ug/L	ug/min	ml/min	ug/min	ug/min	%	ml/min
12	3	56.8	151.2	3.17	0.38	38.27	1.27	1.32	0.32	0.94	73.7	14.4
13	3	104.5	282.4	0.53	0.55	24.45	0.89	0.24	0.23	0.66	73.6	27.2
16	3	94.9	203.7	1.49	0.46	32.96	2.21	0.59	0.82	1.39	63.0	34.8
18	8	90.3	252.0	2.32	0.36	33.27	1.45	3.98	0.44	1.01	69.9	27.1
21	3	92.2	195.0	1.04	0.47	25.60	1.39	0.41	0.46	0.93	67.1	30.5
Mean		88.4 ±16.2	225.6 ±94.7	1.86 ±0.94	0.43 ±0.14	31.50 ±5.84	1.44 ±0.41	1.98 ±2.25	0.45 ±0.19	0.99 ±0.25	69.5 ±5.5	26.9 ±6.5

C_{cr} : Renal clearance of creatinine

C_{PAH}: Renal clearance of PAH

T_m PAH : Tubular secretory mass of PAH

C_{co} : Renal clearance of Co^{60}

TABLE 15

Renal Clearances of Creatinine, PAH and Co^{60} in Sodium Pentobarbital Anesthetized Dogs. All data are the average of 3 consecutive urine collection periods of 30 minutes duration observed beginning 1/2, 6 and 10 1/2 hours after the priming injection of 10 ug. of Co^{60} per kg. body weight.

Dog No.	Time After Priming Co^{60}	C_{cr}/M^2 S.A.	C_{PAH}/M^2 S.A.	T_m PAH	Filtration Fraction	Co^{60} Plasma Conc.	Co^{60} Load	Co^{60} Urine Vol.	Co^{60} Excreted	Co^{60} Reabsorbed	Co^{60} Reabsorption	C_{co}/M^2 S.A.
	hr.	ml/min	ml/min	mg/min		ug/L	ug/min	ml/min	ug/min	ug/min	%	ml/min
24	0.5-2.0	89.5	197.7	1.58	0.45	37.61	2.08	0.53	0.51	1.57	75.1	22.0
	6.0-7.5	89.7	236.0	3.35	0.38	38.01	2.11	0.91	0.44	1.67	79.1	18.7
	10.5-12.0	102.4	236.4	1.76	0.46	27.60	1.76	0.50	0.20	1.56	88.6	11.7
25	0.5-2.0	64.5	176.8	0.99	0.37	22.52	0.85	0.13	0.26	0.59	68.8	20.0
	6.0-7.5	99.4	268.2	1.55	0.38	32.79	1.92	0.36	0.21	1.71	89.1	10.8
	10.5-12.0	99.7	319.8	2.00	0.31	35.64	2.09	0.58	0.24	1.85	88.4	11.3
Mean	0.5-2.0	77.0	187.3	1.29	0.41	30.06	1.47	0.33	0.39	1.08	71.7	21.0
	6.0-7.5	94.6	252.1	2.45	0.38	35.40	2.01	0.63	0.32	1.69	84.1	14.8
	10.5-12.0	101.1	278.1	1.88	0.39	31.62	1.92	0.54	0.22	1.70	88.5	11.5

C_{cr} : Renal clearance of creatinine

C_{PAH} : Renal clearance of PAH

T_m PAH : Tubular secretory mass of PAH

C_{co} : Renal clearance of Co^{60}

TABLE 16

Renal Clearance of Whole Blood Co^{60} of Experimental Dogs. Dogs 1, 2, 3 and 4 received a single injection of 20 ug. of Co^{60} per kg. intravenously. Dogs 6 and 7 received a priming dose of 10 ug. per kg. followed by a constant infusion of Co^{60} in saline. These clearances were calculated on the basis of whole blood cleared of Co^{60} in each minute.

Time After Co^{60} Injection	Dog 1		Dog 2		DOg 3		Dog 4		Dog 6	Dog 7	Average of Dogs 1,2 & 4	Average of Dogs 6 & 7	Ratio (C_{co}, Single Injection) / (C_{co}, Injection & Infusion)
	ml/min	ml/min /M^2S.A.	ml/min	ml/min /M^2S.A.	ml/min	ml/min /M^2S.A.	ml/min	ml/min /M^2S.A.	ml/min /M^2S.A.	ml/min /M^2S.A.	ml/min /M^2S.A.	ml/min /M^2S. A.	
5Min					0.3	0.5	1.7	3.4	6.1	1.8		4.0	
10	0.1	o.1	o.1	0.1	3.7	5.7	27.9	55.5	45.9	49.2		47.6	
20					17.5	27.5	59.8	118.9	55.7	61.4		58.6	
30			4.9	8.7			66.7	133.1	68.0	47.2		57.6	
45			27.9	47.8	41.7	65.6	20.9	41.6	85.4	50.8		68.1	
1 Hr	10.6	12.3	11.2	19.6	28.1	44.2	47.2	93.8	60.1	36.9	41.9	48.5	0.86
2	57.5	66.6	10.2	17.9	15.1	23.8	22.6	44.8	104.9	34.0	53.1	69.4	0.66
3	25.3	29.3	39.3	68.9	1.0	1.6	17.0	33.8	80.1	45.1	44.0	62.6	0.70
4	27.2	31.4	22.3	39.1			13.5	26.8	67.2	43.7	32.4	55.5	0.58
5	20.6	23.9	19.1	33.5			14.2	28.1	68.1	41.5	28.5	54.8	0.52
6	11.6	13.4	13.4	23.4			8.3	16.5	77.7	41.9	17.8	59.8	0.30
7	9.0	10.4	10.1	17.8	0.9	1.3	6.2	12.4	44.2	36.7	13.5	40.5	0.33
8	6.3	7.3	17.5	30.7			5.2	10.4	50.3	44.8	16.1	37.6	0.34
9	7.6	8.8							58.2	36.0		47.1	
10							6.6	13.1	45.6	36.9		41.2	
11									37.0	24.5		30.8	
12							2.1	4.1	25.5	7.3		5.7	

C_{co} : Renal clearance of whole blood Co^{60}

TABLE 17

Biliary Clearance of Whole Blood Co^{60} in Experimental Dogs. Dogs 1, 2, 3 and 4 received a single injection of 20 ug. of Co^{60} per kg. intravenously. Dogs 6 and 7 received a priming dose of 10 ug. per kg. followed by a constant infusion of Co^{60} in saline. These clearances were calculated on the basis of whole blood cleared of Co^{60} in each minute.

Time After Co^{60} Injection	Dog 1		Dog 2		Dog 3		Dog 4		Dog 6	Dog 7	Average of Dogs 1,2 & 4	Average of Dogs 6 & 7	Ratio (C_{co}, Single Injection) / (C_{co}, Injection & Infusion)
	ml/min	ml/min /M^2S.A.	ml/min	ml/min /M^2S.A.	ml/min	ml/min /M^2S.A.	ml/min	ml/min /M^2S.A.	ml/min /M^2S.A.	ml/min /M^2S.A.	ml/min /M^2S.A.	ml/min /M^2S.A.	
5Min					0.01	0.01	0.001	0.003	0.01	0.02			
10	0.0003	0.0004	0.2	0.3	0.001	0.002	0.001	0.003	0.09	0.03		0.06	
20					0.2	0.3	0.2	0.3	0.1	0.4		0.3	
30			0.7	1.2			0.5	1.0	0.6	1.1		0.9	
45			1.8	3.1	0.4	0.6	1.3	2.7	1.0	2.2		1.6	
1 Hr.	0.2	0.2	1.2	2.1	0.2	0.4	2.0	4.0	1.1	2.0	2.1	1.6	1.31
2	0.3	0.3	1.7	2.0	0.2	0.3	2.3	4.6	1.2	2.0	2.3	1.6	1.43
3	1.9	2.2	2.3	3.9	0.3	0.4	2.5	5.0	1.1	2.4	3.7	1.8	2.06
4	3.0	3.5	3.5	6.0			3.2	6.3	2.3	3.5	5.3	2.9	1.83
5	3.9	4.5	5.3	9.2	0.3	0.5	3.8	7.5	3.9	5.7	7.1	4.8	1.48
6	3.7	4.4	4.9	8.5			4.8	9.6	5.9	7.3	7.5	6.5	1.15
7	3.7	4.3	4.0	7.0	0.2	0.3	4.1	8.1	6.3	5.2	6.4	5.8	1.10
8	3.8	4.4	3.5	6.1			2.9	5.7	5.6	5.4	5.4	5.5	0.98
9	3.4	4.0					3.6	7.2	6.5	5.0		5.8	
10							3.3	6.6	5.8	6.2		6.0	
11							3.1	6.1	4.2	4.7		4.4	
12							3.8	7.5	3.1	5.4		4.3	

C_{co} : Biliary clearance of whole blood Co^{60}

TABLE 18

The Rates of Urinary and Biliary Excretion of Co^{60} After Single Intravenous Injection

Time After Co^{60} Injection	Urinary Co^{60} Excretion ug/min					Biliary Co^{60} Excretion ug/min				
	Dog 1	Dog 2	Dog 3	Dog 4	Mean*	Dog 1	Dog 2	Dog 3	DOg 4	Mean*
5 min			0.01	0.12				0.001	0.001	
10	0.01		0.15	1.06	0.53	0.002		0.001	0.001	0.001
20			0.56	1.88				0.006	0.005	
30		1.16		1.63	0.90		0.023		0.013	0.018
45		0.66	1.14	0.44	0.56		0.042	0.010	0.028	0.035
1 hr.	0.30	0.24	0.75	0.87	0.47	0.004	0.026	0.006	0.037	0.022
2	1.11	0.17	0.37	0.34	0.54	0.004	0.028	0.005	0.034	0.022
3	0.43	0.70	0.22	0.21	0.45	0.032	0.040	0.006	0.031	0.034
4	0.38	0.31		0.15	0.28	0.043	0.048		0.034	0.042
5	0.28	0.20		0.13	0.20	0.053	0.055	0.005	0.036	0.048
6	0.16	0.13		0.08	0.12	0.052	0.047		0.045	0.048
7	0.13	0.10	0.01	0.06	0.10	0.054	0.038	0.003	0.036	0.043
8	0.08	0.15		0.04	0.09	0.048	0.031		0.022	0.034
9	0.10			0.01	0.06	0.045			0.027	0.036
10				0.04					0.021	
11									0.019	
12				0.01					0.023	
13									0.023	

* Except Dog No. 3.

TABLE 19

Urine and Bile Volumes and Co^{60} Recoveries (in Per Cent of Injected Dose) After a Single Intravenous Injection

DOg No.			1	2	3	4
Body Weight, kg.			21.4	11.5	13.5	9.5
Duration of Exp., hrs.			9	8	7	13
At End of 1 Hour	Urine	Volume, ml.	5.1	3.6	5.8	3.3
		Co^{60}, %	3.1	6.8	15.0	29.0
	Bile	Volume, ml.	7.3	5.6	1.9	4.8
		Co^{60}, %	0.1	0.6	0.1	0.6
At End of 6 Hour	Urine	Volume, ml.	77.8	56.5	9.1	21.2
		Co^{60}, %	32.9	44.8	22.6	55.2
	Bile	Volume, ml.	38.9	30.3	7.0	19.3
		Co^{60}, %	2.4	6.1	0.6	5.8
At End of Exp.	Urine	Volume, ml.	89.8	73.9	9.4	32.5
		Co^{60}, %	36.9	60.9	23.2	60.1
	Bile	Volume, ml.	50.8	38.2	7.4	27.0
		Co^{60}, %	4.2	7.8	0.6	10.9

TABLE 20

Tissue Distribution of Co^{60} in Two Dogs Twelve Hours After Injection. The percentage recovery in extracellular fluid was computed from plasma activity as indicated in the text.

Tissues		Conc. of Co^{60}, ug/mg, Wet Wt.	
		Dog No. 22 Injected Intravenously	Dog No. 23 Injected Intraintestinally
Blood		0.0101*	0.0042*
Plasma		0.0152*	0.0079*
Extracellular Fluid		5.34**	5.12**
Urinary Bladder Urine		0.0298*	0.0159*
Gall Bladder Bile		0.0554*	0.0088
Liver		0.3529	0.0842
Right Kidney		0.0380	0.0146
Spleen		0.0125	0.0033
First Loop of Small Intestine	Wall	0.0327	0.0175
	Contents	0.0320	0.0140
Second Loop of Small Intestine	Wall	0.0441	49.6**
	Contents	0.0502	
Third Loop of Small Intestine	Wall	0.0652	0.0110
	Contents	0.1062	0.0310
Caecum	Wall	0.0353	0.0166
	Contents	0.0337	0.0153
Large Intestine	Wall	0.0315	0.0069
	Contents	0.0248	0.0085

* ug per ml

** Recovery of Co^{60} (per cent of the injected dose)

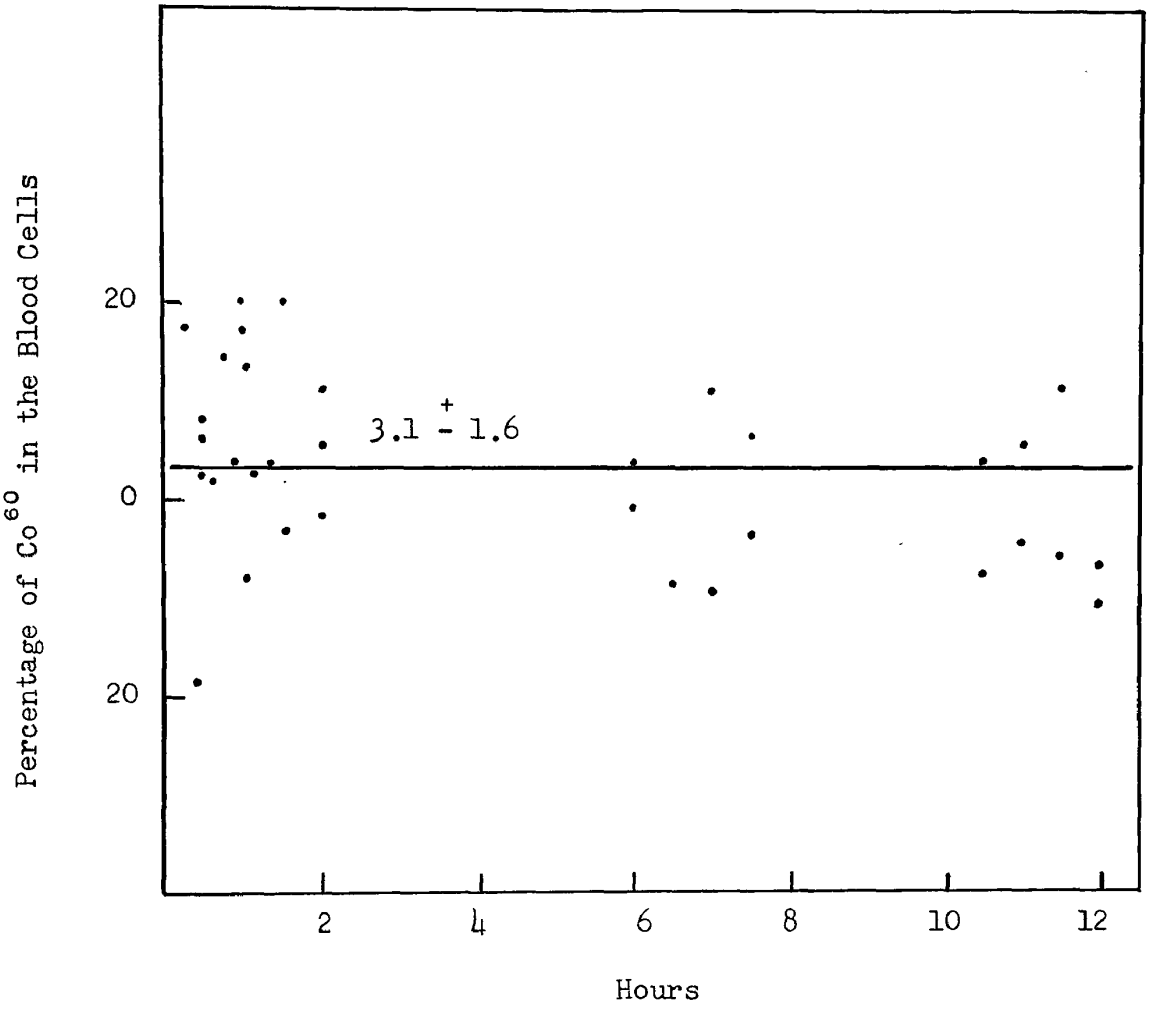


Figure 10. The Amount of Co^{60} Present in the Blood Cells at Various Time After Initial Intravenous Injection.

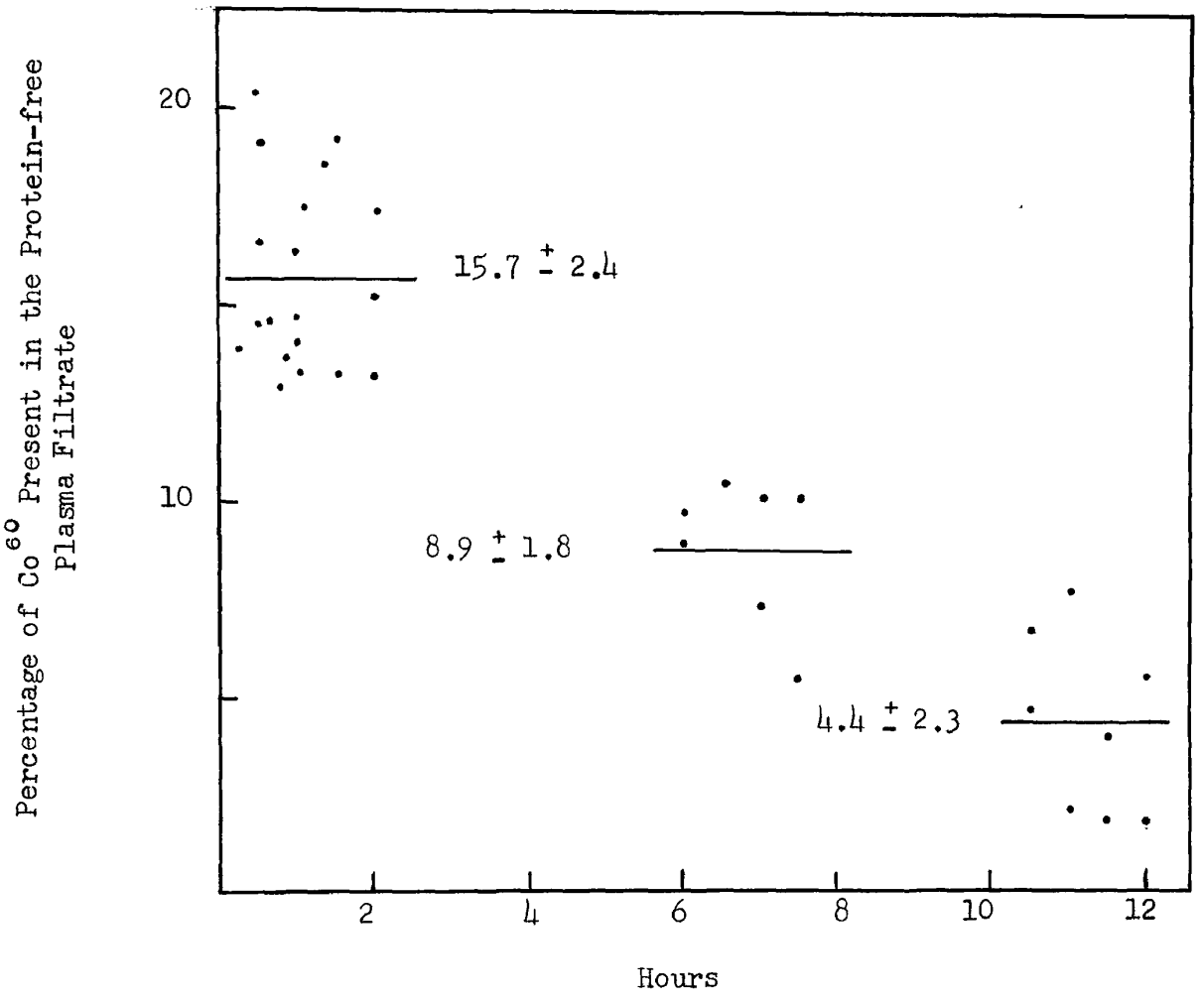


Figure 11. The Amount of Co^{60} Present in the Protein-free Plasma Filtrate at Various Time After Initial Intravenous Injection.

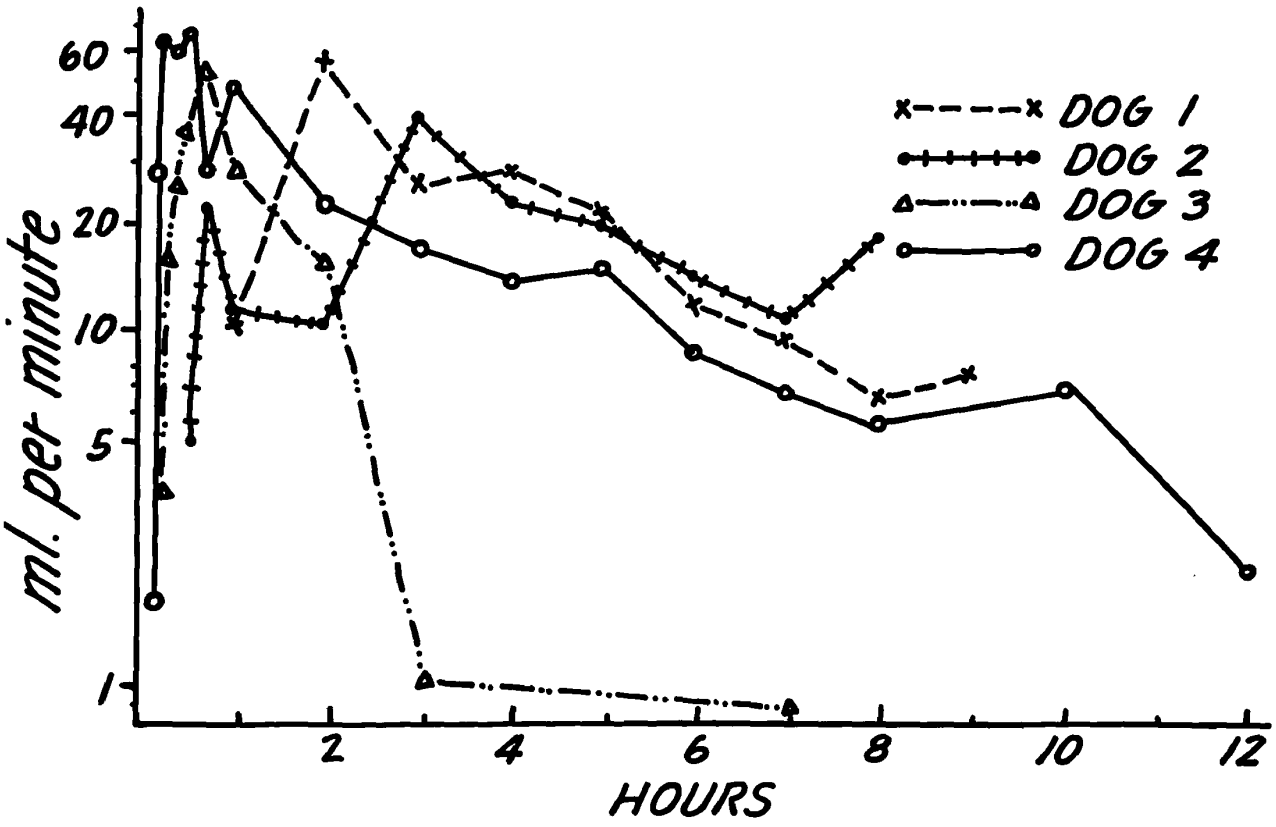


Figure 12. Renal Clearance of Whole Blood Co^{60} During the Entire Experimental Period After A Single Intravenous Injection.

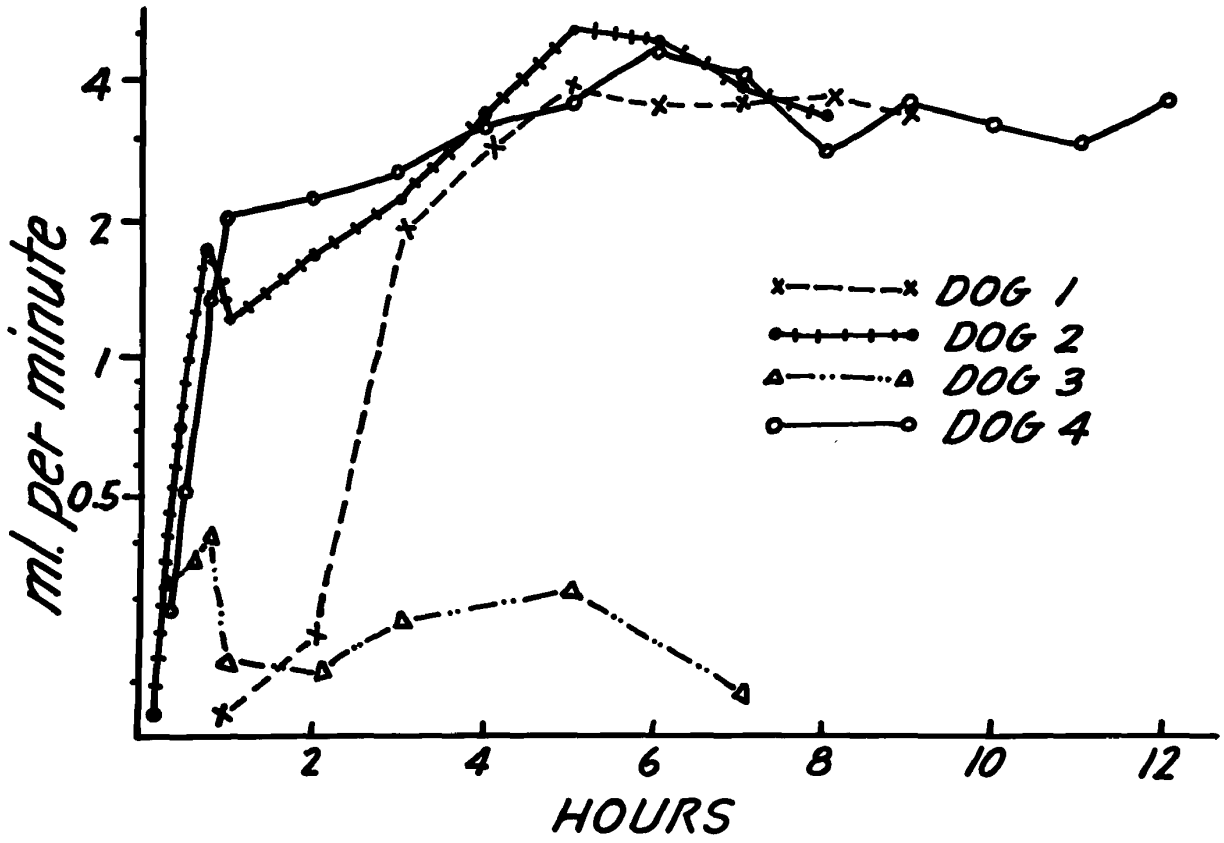


Figure 13. Hepatic Clearance of Whole Blood Co^{60} During the Entire Experimental Period After A Single Intravenous Injection.

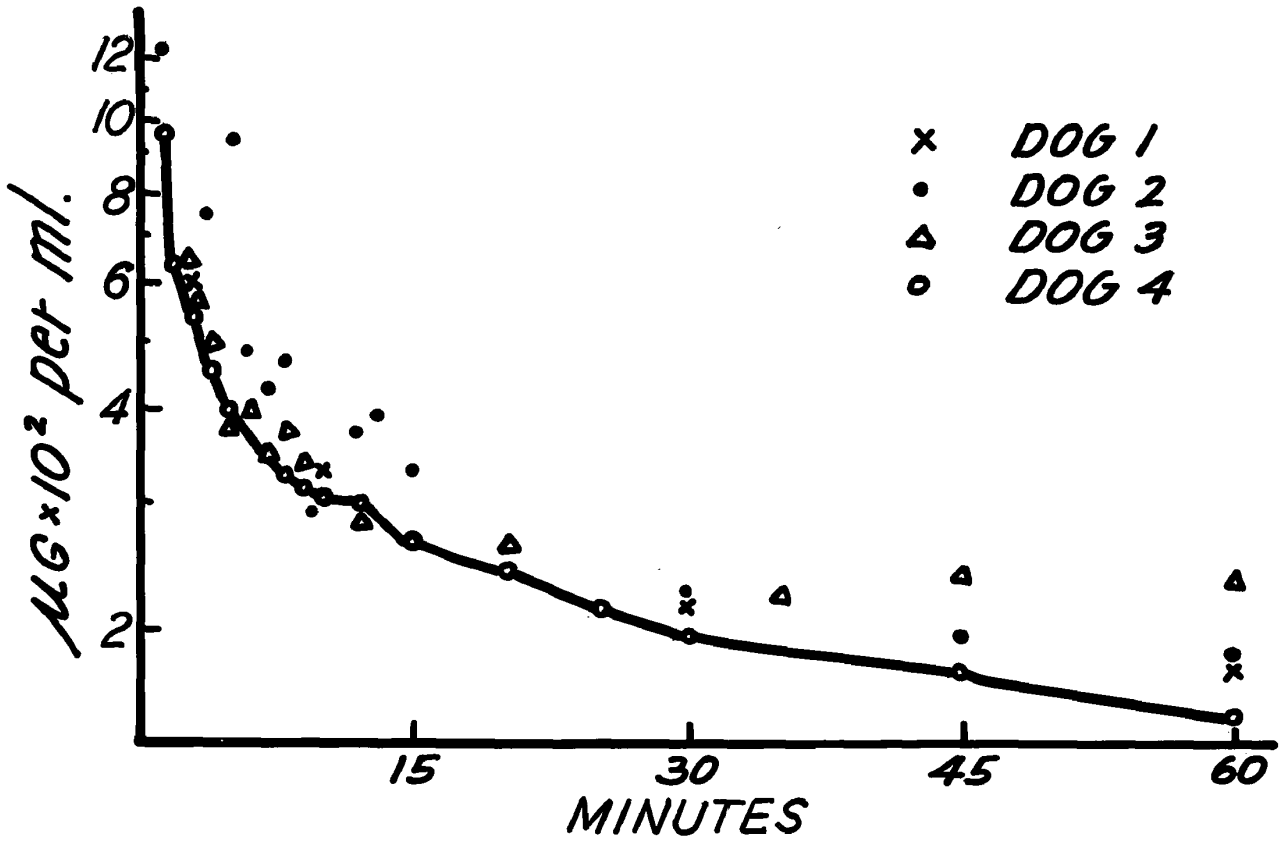


Figure 14. The Co^{60} Concentrations in Blood During the First Hour After A Single Intravenous Injection.

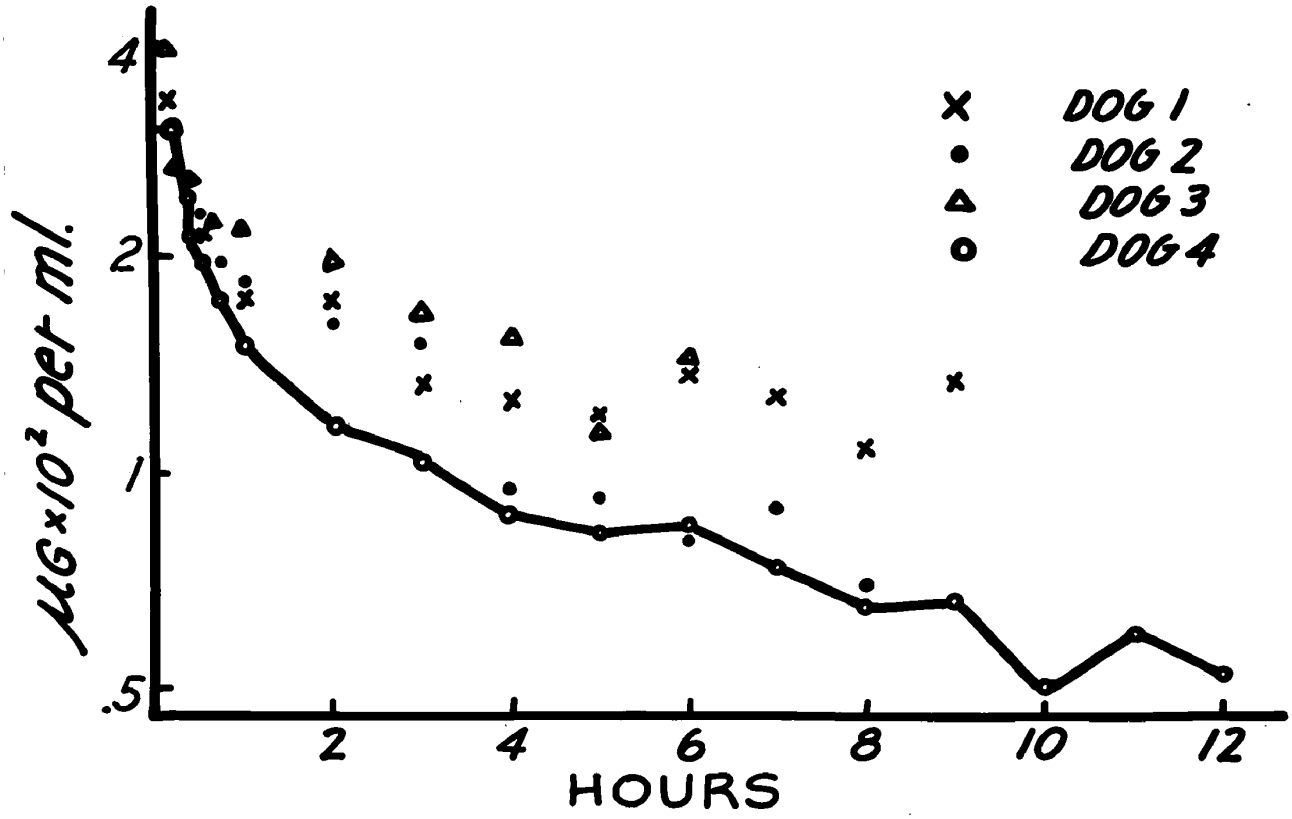


Figure 15. The Co^{60} Concentrations in Blood During the Entire Experimental Period After A Single Intravenous Injection.

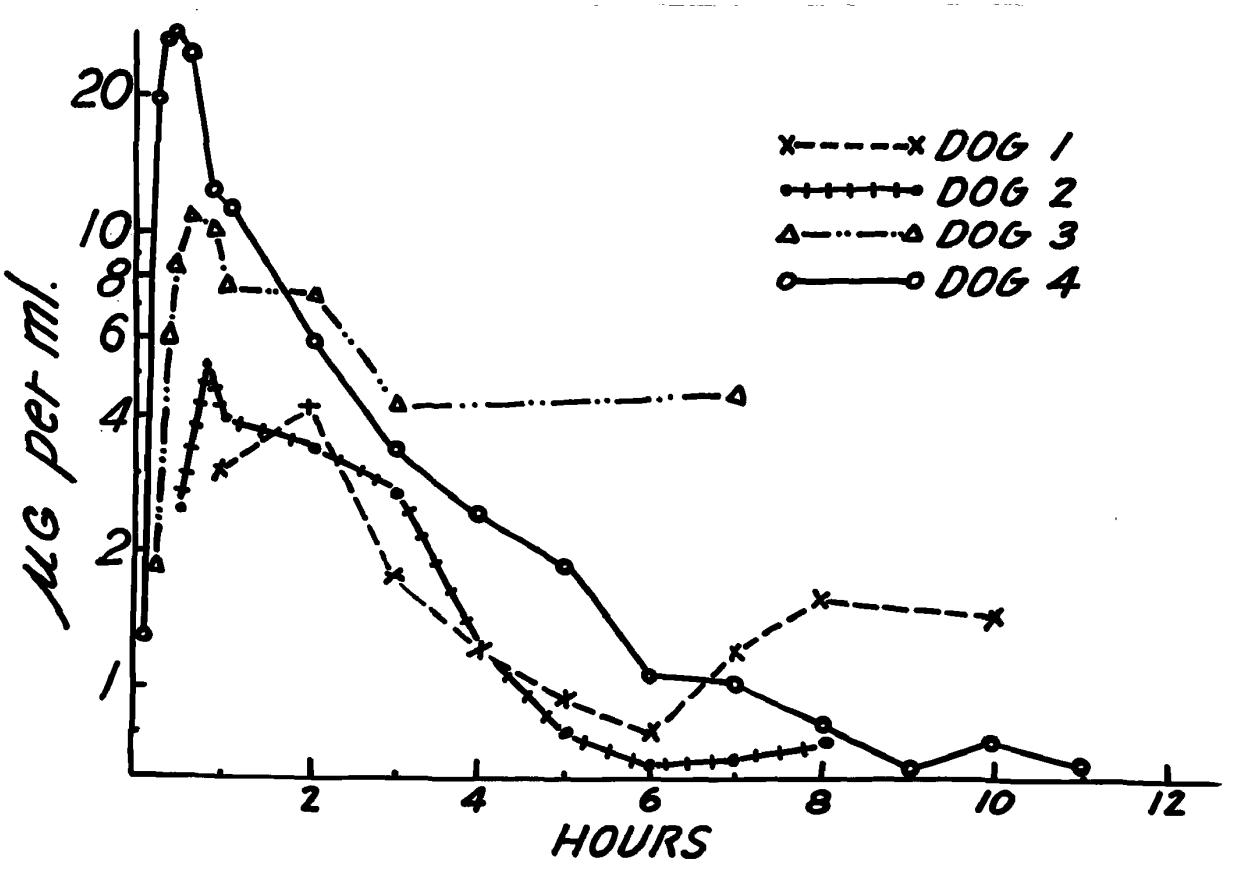


Figure 16. The Co^{60} Concentrations in Urine During the Entire Experimental Period After A Single Intravenous Injection.

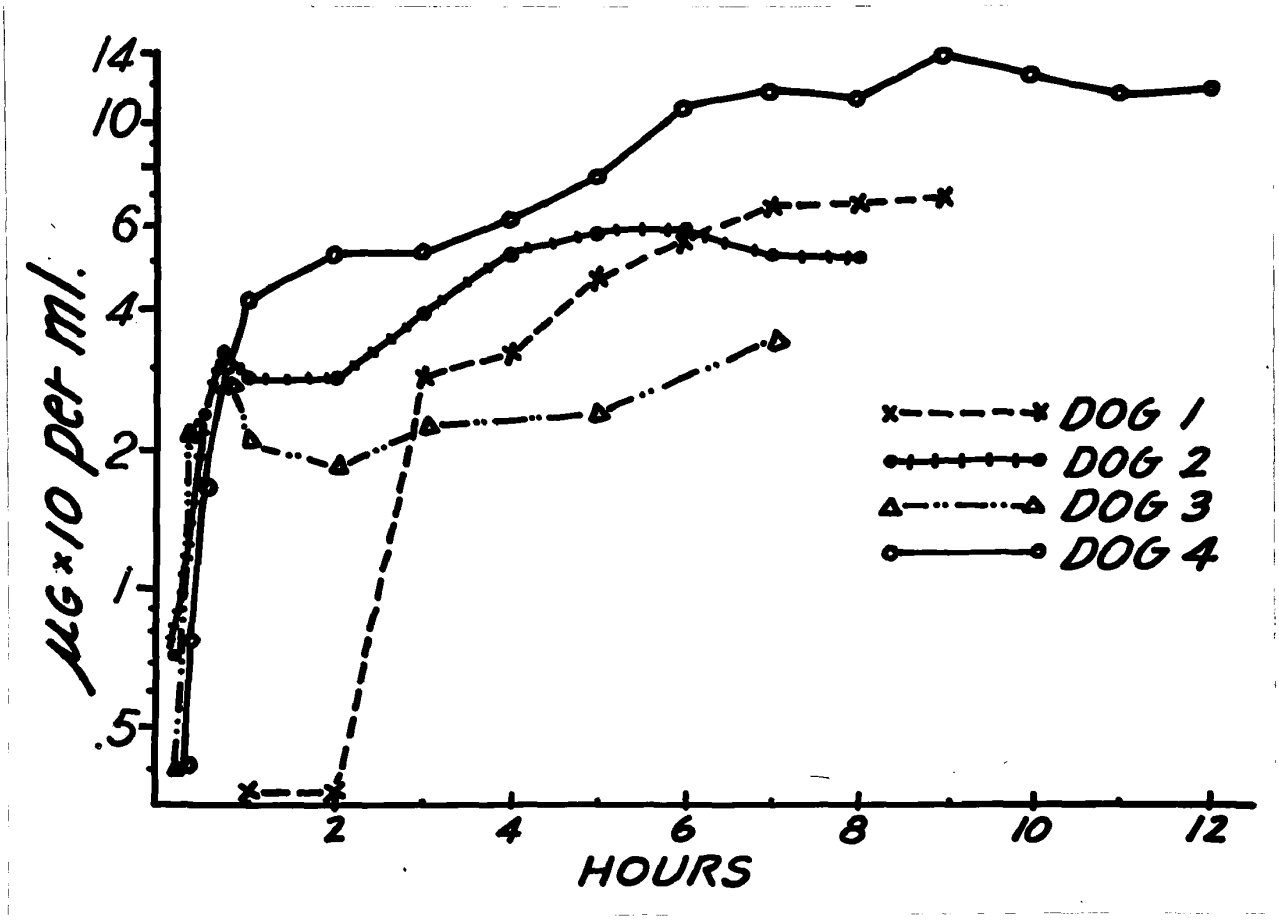


Figure 17. The Co^{60} Concentrations in Bile During the Entire Experimental Period After A Single Intravenous Injection.

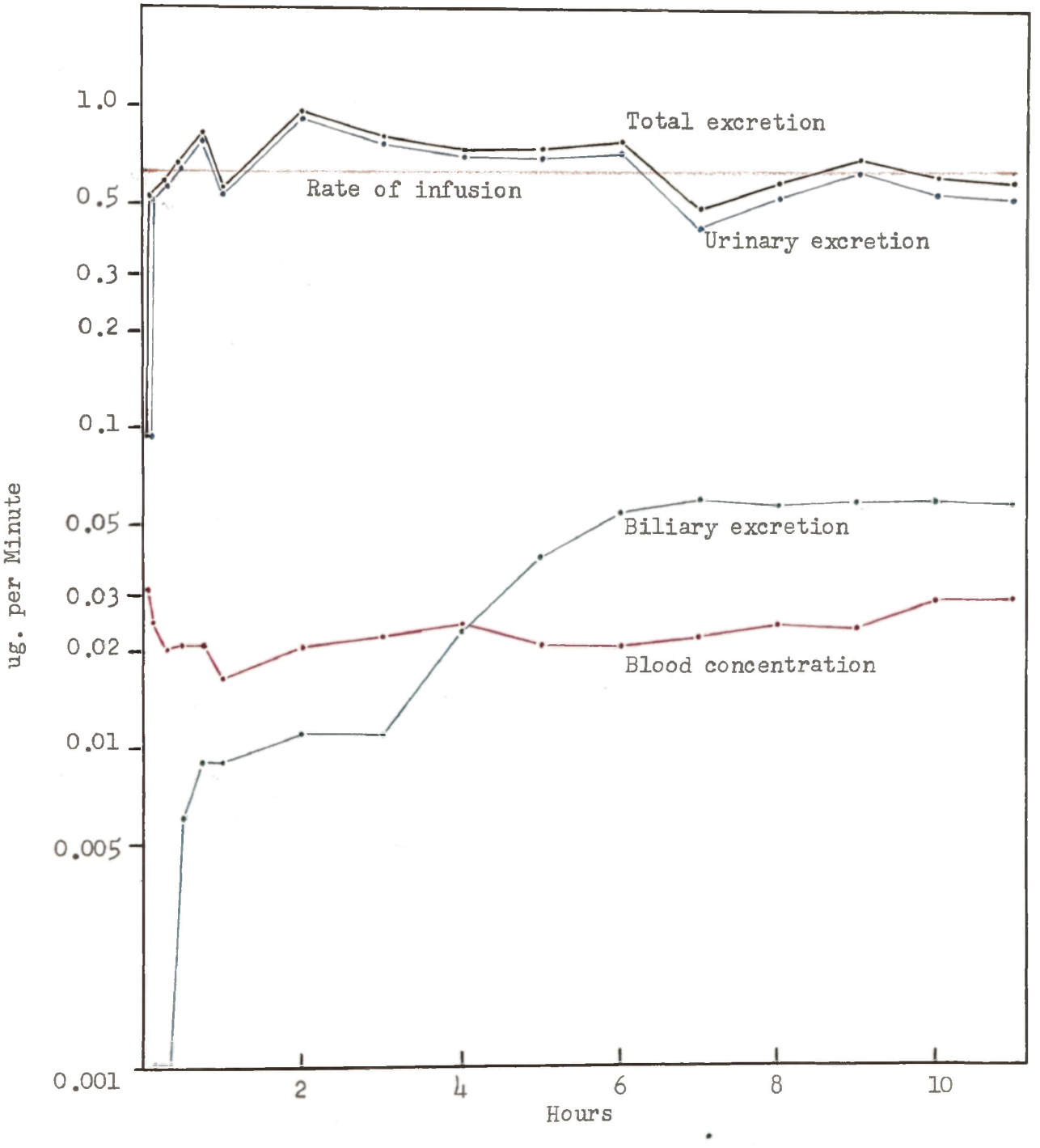


Figure 18. Rate of Infusion, Blood Concentration, Urinary, Biliary and Total Excretion of Co^{60} in Dog No. 6.

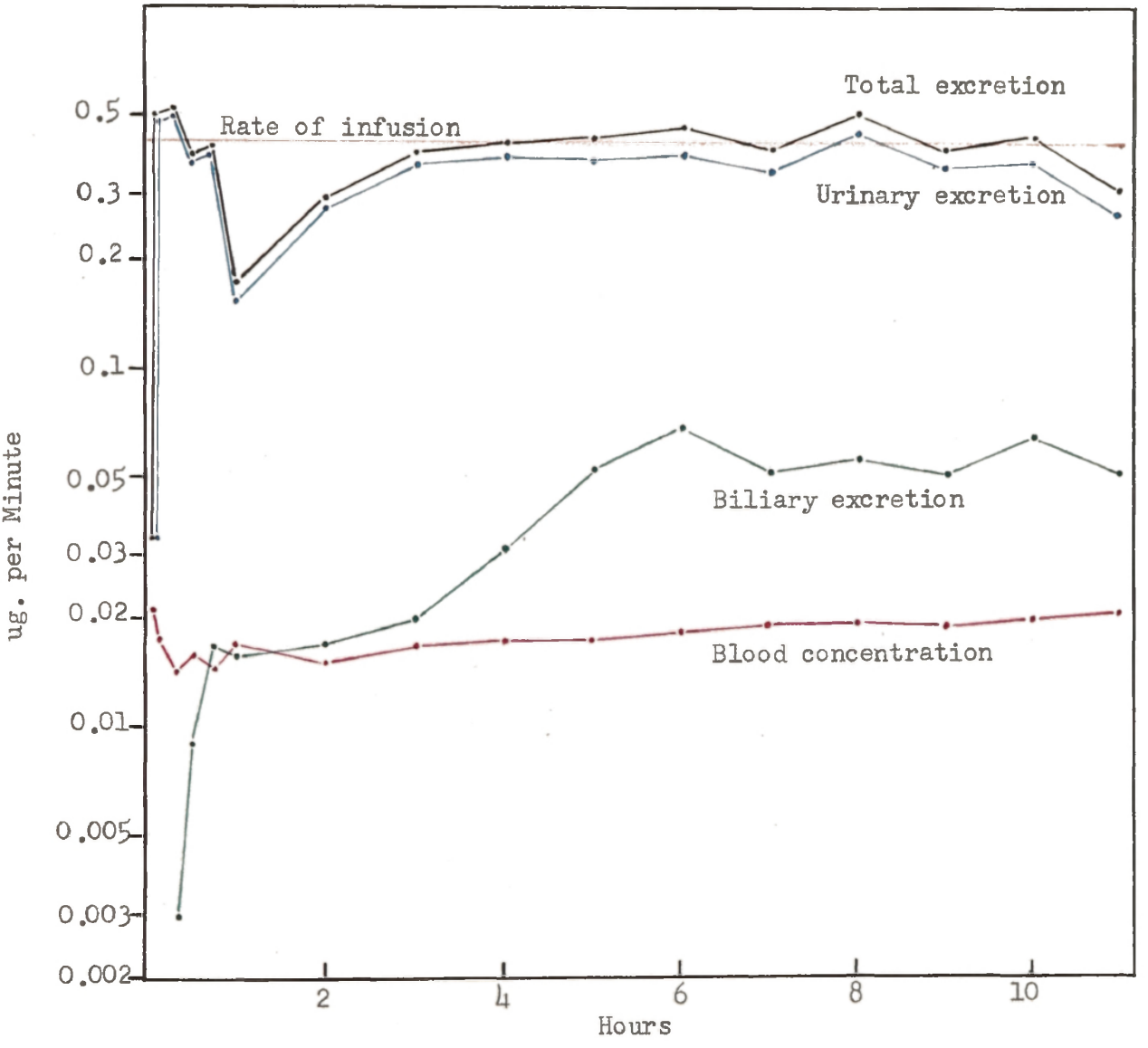


Figure 19. Rate of Infusion, Blood Concentration, Urinary, Biliary and Total Excretion of Co^{60} in Dog No. 7.

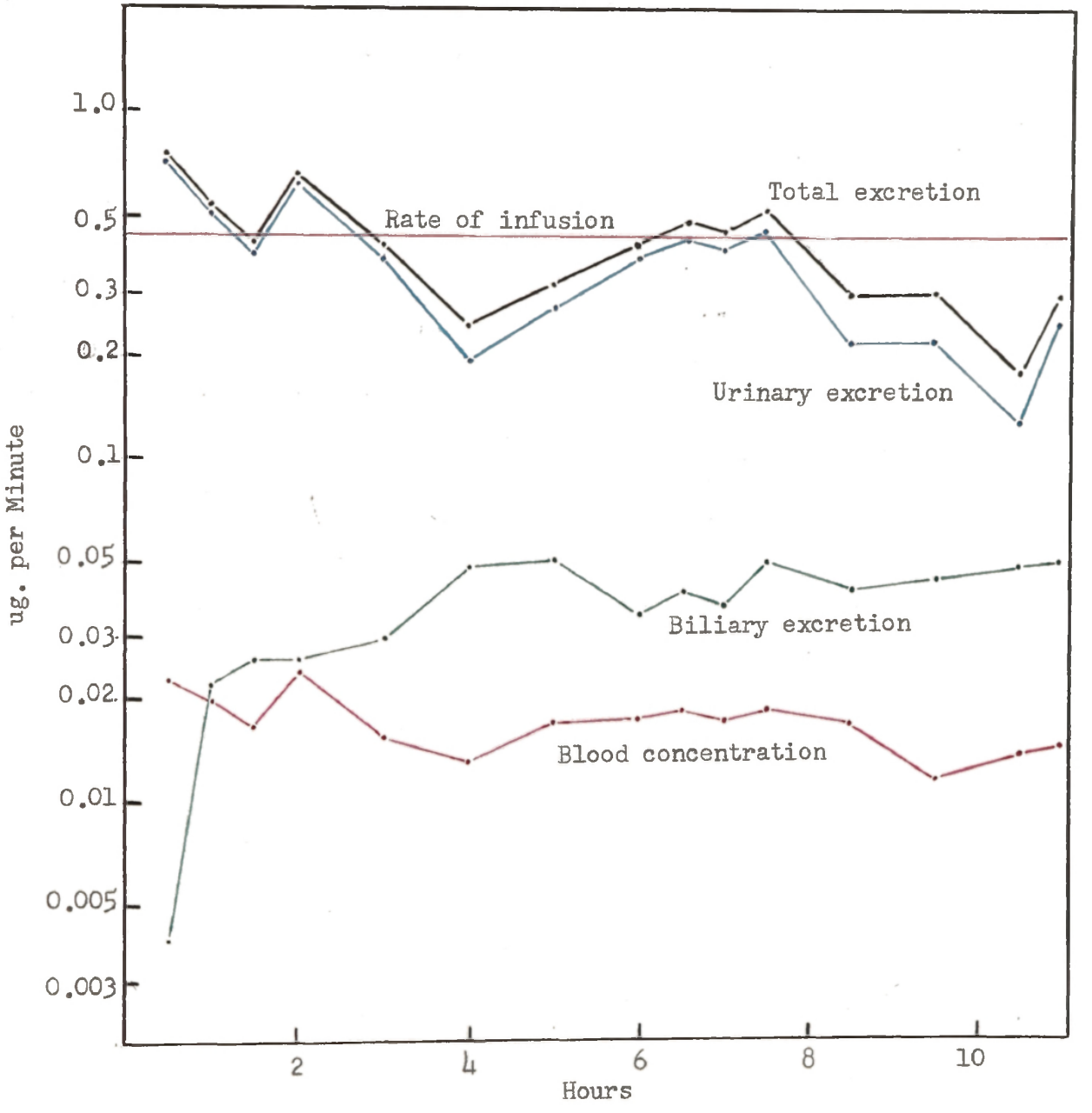


Figure 20. Rate of Infusion, Blood Concentration, Urinary, Biliary and Total Excretion of Co^{60} in Dog No. 24.

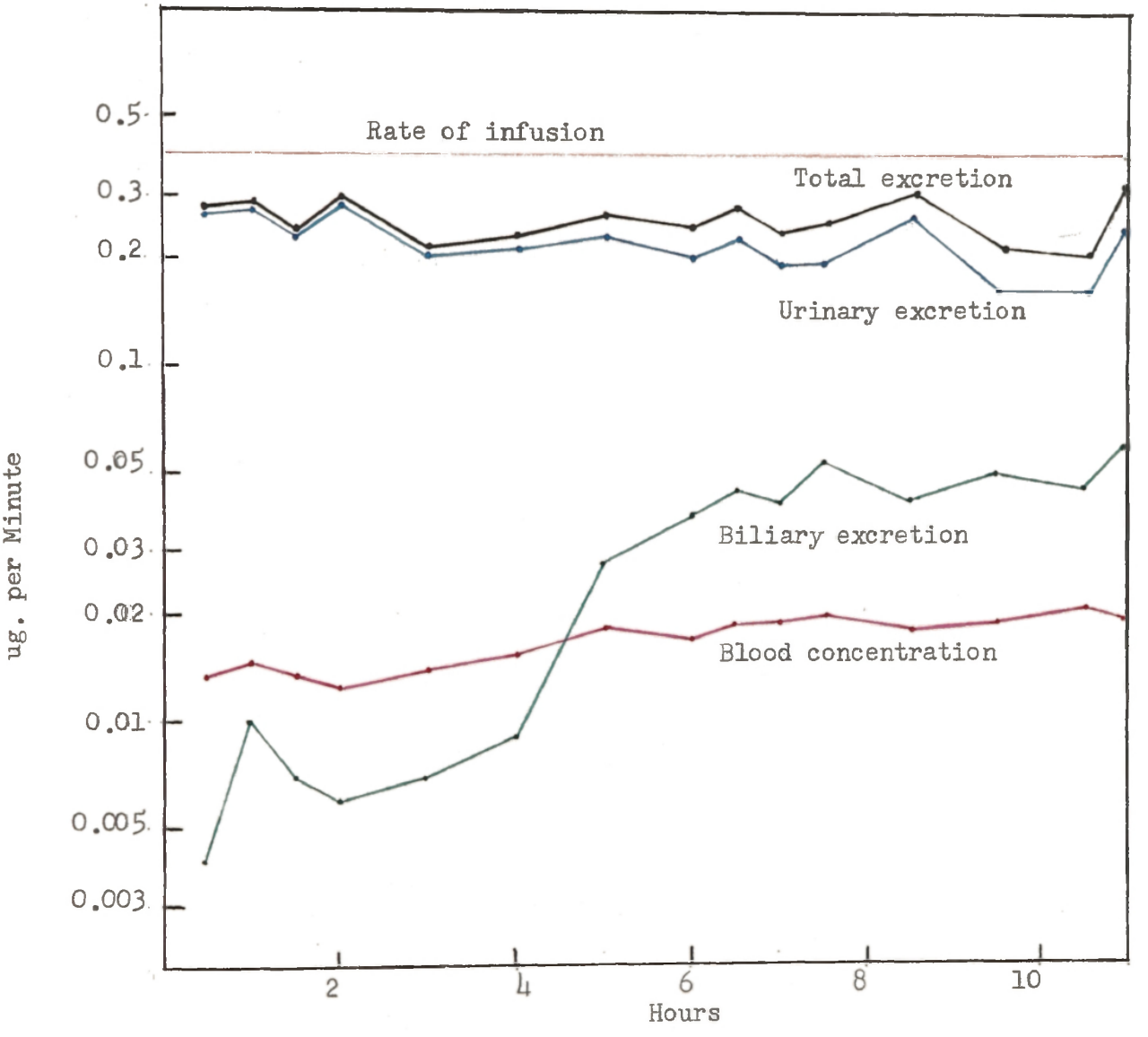


Figure 21. Rate of Infusion, Blood Concentration, Urinary, Biliary and Total Excretion of Coeo in Dog No. 25.

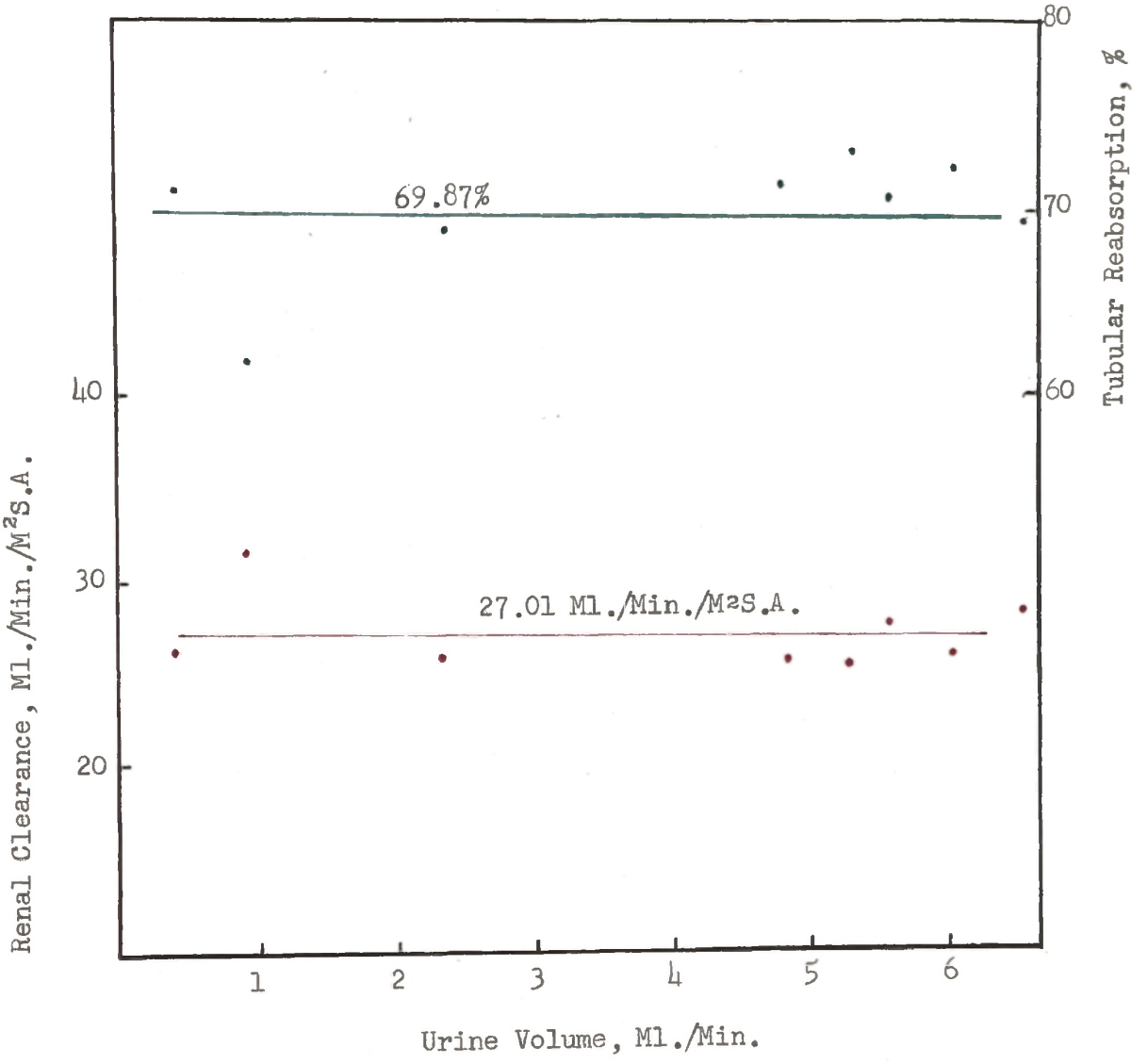


Figure 22. Relationship between Urine Volume, Tubular Reabsorption and Renal Clearance of Co^{60} (Data from Dog No. 18).

C. Discussion

Distribution of Co^{60} in Blood and Probable Protein-Bound Forms:

Within twelve hours after intravenous injection of Co^{60}, the blood cells contained less than 5% of the Co^{60} present in the whole blood (Table 11 and Figure 10). This suggests that Co^{60} does not penetrate the red blood cells or penetrates to only a very minute degree. This result is in contrast to the findings of Braude et al. (1949) in pigs and Cuthbertson et al. (1950) in rats following oral administration of Co^{60} in the feed for periods of 40 to 90 days. These authors reported that up to 80% of the activity in whole blood was present in the blood cell fraction. This high level in the red blood cells may be due either to a slow penetration over a long period, or to actual incorporation into the blood cells during hematopoiesis. From the data of the present experiments, the second mechanism would appear the most probable.

It will be noted that the initial blood levels of Co^{60} were in the neighborhood of 0.1 microgram per ml. after a single intravenous injection of 20 micrograms per kg. body weight of inorganic Co^{60} (Figure 14). This indicates an immediate dilution of the injected dose by about 20% of the body weight. In other words, within the first few minutes, the tracer was no longer confined to the plasma but had mixed rapidly into the entire extracellular compartments. The blood level then declined rapidly for about ten minutes, indicating either removal by specific organs or entrance into tissue cells generally. Cobalt 60 bound to plasma constituents would, of course, have been counted in the blood. Therefore, the rapid removal rate must be due to loss from the plasma of "unbound" forms of Co^{60}.

Schubert et al. (1950) have suggested that cations injected into the blood stream may form complex, colloid-like particles. It has also been reported that as much as 75% of the Co^{60} in the whole blood of sheep is in the bound and vitamin B_{12}-like forms seven days after oral administration of inorganic Co^{60} (Monroe et al., 1952b).

That the Co^{60} may be in bound form in the plasma of the dogs studied here is indicated by three observations. First, less than 20% of the blood Co^{60} can be recovered from the protein-free plasma filtrate within two hours after injection (Table 11 and Figure 11). If a majority of the Co^{60} is bound to the plasma protein, it would be precipitated by treatment with cadmium hydroxide, leaving only a small amount of Co^{60} in the protein-free filtrate. Second, the renal clearance of Co^{60} decreased with time after a single intravenous injection (Tables 15 and 16). At the beginning of the experiment, a large fraction of the injected inorganic Co^{60} is quickly cleared through the kidney. However, toward the end of the experiment, a different form of Co^{60} (presumably, some protein-bound form which is cleared less readily by the kidney) may be present in plasma to account for the decline in the clearance values. Third, when plasma was dialyzed in vitro, a much smaller amount of Co^{60} was diffusible from the plasma procured ten hours after a single intravenous injection than from the plasma procured one hour after injection. This is direct evidence for a change in the ratio of diffusible to non-diffusible Co^{60} with time after injection. If the kidney can clear only the diffusible Co^{60}, the renal clearance should decline, as was actually observed.

It is not certain, from these data, what form or forms of Co^{60} are actually cleared by the kidney. Inorganic Co^{60} is undoubtedly removed from the plasma. In addition, Co^{60} complexed with amino acids is probably cleared to a certain

extent. The fact that Co^{60} dialyzed out from the cadmium hydroxide precipitate of plasma indicates that at least 10% of what might be called "protein-bound" Co^{60} may be "available" for renal excretion. Thus, the Co^{60} brought down by cadmium hydroxide is in at least two forms, one of which, since it is dialyzable, is presumably capable of being cleared by the kidney. It is possible that the dialyzable Co^{60} was merely adsorbed on the protein precipitate and subsequently eluted in dialysis by exposure to the large volume of external solvent.

In the protein precipitate of the blood sample obtained one hour after Co^{60} injection, 9% of its Co^{60} was diffusible in a dialyzing period of eight hours. Since the Co^{60} in the protein precipitate represents 78% of the whole blood Co^{60} in the first two hours (Table 11), it may readily be computed that 72% of the whole blood Co^{60} is non-diffusible (78% - 9% x 78%). Similarly, in the blood sample obtained ten hours after injection, 23% of the protein precipitate Co^{60} was diffusible. About 97% of the whole blood Co^{60} in the sample collected ten and one-half hours after injection was precipitated in the protein fraction (Table 11). Thus, about 75% of the whole blood Co^{60} was non-diffusible (97% - 97% x 23%), a figure identical with that found at two hours. This means that the total amount in the non-diffusible Co^{60} form in the blood apparently does not change with time after a single intravenous injection.

Boulanger et al. (1952) have reported that cadmium hydroxide brings down all proteins and polypeptides in the urine and that all amino acids remain in the filtrate and in the washing solution of the precipitate,

It is interesting to note here that the form of Co^{60} which will diffuse through the dialyzing membrane does not diffuse through the membrane of the red blood cell. As will be pointed out later, it appears that some fraction of the Co^{60} which will not dialyze through the cellophane membrane will, however, diffuse across the glomeruli of the kidney.

The turnover rate of Co^{60} within the biological system is faster when it is in the form of amino acid complexes than when it is present in inorganic form (Table 12 which confirms the general conclusion of Berlin and Siri, 1951). The fact that the Co^{60} in urine and bile samples has a more rapid rate of absorption from the chick intestine than has inorganic Co^{60} (Table 1e) suggests that the Co^{60} in the urine and bile, or a portion of it, may exist in amino acid complexes.

It has been reported that 7 to 10% of the total Co^{60} in the urine of sheep is recoverable as a vitamin B_{12}-like substance (calculated over a period of seven days after administration of inorganic Co^{60})(Monroe et al., 1952b). Both bound and vitamin B_{12}-like forms other than inorganic Co^{60} were found in all tissues tested by these investigators. On the basis of these findings, it might be presumed that certain fractions of the total Co^{60} in the bile could be in some organic form. Accordingly, preliminary paper partition chromatography and radioautography was undertaken on selected urine and bile samples obtained from the dogs employed in the clearance studies. The results indicate that inorganic Co^{60} is not the only radioactive form present in these samples. Comparison with a standard preparation of Co^{60}-labeled vitamin B_{12} failed to reveal significant amounts of vitamin B_{12} in either the bile or urine samples. The present data do not confirm the findings of Monroe et al. (1952b) in sheep. The failure to find vitamin

B_{12} Co^{60} in either the urine or the bile from dogs may be a true species difference from the sheep. On the other hand, the quantity of the urine or the bile used in these studies may have contained less than the minimal quantity of Co^{60} detectable by radioautographic methods.

Urinary and Biliary Excretion of Intravenously Injected Inorganic Co^{60}:

Marston (1952) has summarized the fairly extensive data leading to the conclusion that parenterally injected cobalt is excreted mainly in the urine, but in part by the bile. The total Co^{60} recoveries in urine and bile obtained in the dog under the present acute experimental conditions and after single intravenous injection (Table 19) are in general agreement with those reported previously in other species (Comar and Davis, 1947a; 1947b; Comar et al., 1946a, 1946b; Copp and Greenberg, 1941; Greenberg et al., 1943b; Sheline et al., 1946; Monroe et al., 1952b). The rate of excretion (in micrograms per minute) by the kidney was very rapid during the first few hours after intravenous injection. The total Co^{60} recovery from the urine was much higher than that from the bile. Since the Co^{60} in bile is reabsorbable in the intestinal tract, a large fraction of the Co^{60} recovered from bile must normally be recirculated through the body. Thus, the net rate of Co^{60} loss in bile fistula dogs should be somewhat greater than that in intact dogs whose biliary Co^{60} passes into the intestine from which it will be reabsorbed. In the bile fistula dogs, fecal loss of Co^{60} may be chiefly composed of that fraction of the intestinal Co^{60} fixed by the intestinal bacteria.

The rapid increases both in the urinary concentration and in the rate of urinary excretion of Co^{60} soon after a single intravenous injection (Figure 16 and Table 18) indicate that the plasma Co^{60} rapidly filters

through the glomeruli. The maximal values were reached within one-half to three hours. As the plasma Co^{60} concentration rapidly dropped and then more slowly declined, corresponding changes in the urinary concentration and in the rate of urinary excretion followed. However, changes in the biliary concentration and in the rate of biliary excretion were much more gradual. A maximal rate of biliary Co^{60} excretion was not reached until five to seven hours after a single intravenous injection (Table 18 and Figure 17). At this time, the blood concentration and the rate of urinary excretion of Co^{60} were far below their maximal values. Thereafter, the rate of biliary excretion of Co^{60} decreased only slightly. This suggests that the delay of Co^{60} in the liver may be much longer than in the kidney. The first appearance of Co^{60} both in the urine and in the bile was five to ten minutes after intravenous injection. There is, then, no greater delay in the initial time of appearance of Co^{60} in the bile than in the urine. However, the peak of the hepatic excretion curve is delayed about four hours in comparison with that of the urinary curve. A similar delay of phosphate (Kleiber, 1952a) and of acetate (Kleiber, 1952b) by the mammary gland of the cow has been reported.

The delay in the liver is slightly less, however. The ratio of hepatic clearances of Co^{60} in dogs receiving a single injection to that of dogs which received a constant infusion increased during the first three hours after injection (Table 17). It reached a maximum value of two and fell off thereafter. On the other hand, the ratio of single injection to constant infusion for renal clearances in these same dogs was less than one at the beginning and declined gradually (Table 16). It should be noted that from three hours on, the half-times for the decline in these ratios were the same for the renal clearance curve as for the hepatic clearance. Finally, in dogs receiving a constant infusion, the rate of biliary excretion increased gradually and

reached a plateau within four to seven hours after injection (Figures 18 to 21). However, the rate of urinary excretion was relatively constant throughout the experiment.

It would appear that the kidney and the liver clear the same form of Co^{60}, at least after the third hour after Co^{60} injection, although these two organs handle this tracer at a different rate. This conclusion is based on two types of findings. First, the Co^{60} from urine and bile was absorbed at the same rate by the intestine of young chicks (Table 13). Second, half-times for the decline in the ratios for renal and hepatic clearances in dogs receiving a single injection to dogs which received a constant infusion were both about four and one-half hours. In each case, one would expect different half-times if different forms of Co^{60} were cleared by the kidney and the liver.

The biliary excretion rate for Co^{60} in four dogs which received a constant infusion reached plateau values of 0.045 to 0.06 microgram per minute within four to seven hours after injection. That these values were reached and then maintained for seven to four hours infour different animals indicates that some maximal, or saturation output rate exists. In experiments in which fluctuation in the perfusion rate occurred, corresponding variations in the rate of urinary excretion of Co^{60} were noted (Figure 20). However, the rate of biliary excretion was not influenced.

A low rate of total excretion resulted in retention of Co^{60} in two of the four dogs which received a constant infusion of Co^{60}. The blood concentration of Co^{60} increased in one of these two dogs and declined in the other one. Since no tissue sample from these dogs was analyzed, how the retained Co^{60} was distributed in the body is not known.

Renal Clearance and Tubular Reabsorption of Co^{60}:

It has been noted in a previous section that a large fraction of the whole blood Co^{60} is in some "non-diffusible" form or forms throughout the experiment. Since the total blood Co^{60} decreased from an initial concentration of 0.1 microgram per ml. to about 0.01 microgram per ml., a reduction of ten times, at least a part of the "non-diffusible" Co^{60} must then have been "available" to the glomeruli of the kidney for clearance.

Since the "non-diffusible" form or forms of Co^{60} in the blood may be in some "protein-bound" form or forms, and since the protein moiety of the complex is not cleared by the kidney, it appears that the protein-Co^{60} complex is readily dissociated. The rate of dissociation for this protein-Co^{60} complex is not known.

Half of the plasma calcium is combined with plasma protein. Calcium and magnesium proteinates are much less completely dissociated than are the corresponding sodium and potassium salts (Smith, 1951). Nevertheless, formation of the calcium proteinate "does not appear to interfere with the transcapillary movement of calcium" (Armstrong et al., 1952). A similar situation may well exist with respect to the cobalt.

Since the Co^{60} is cleared, all renal and hepatic clearances were calculated on the basis of whole blood or whole plasma Co^{60} concentration rather than on the basis of that portion not precipitated by cadmium hydroxide. Thus, the calculated clearance values may be smaller than the true values by whatever fraction of the whole blood Co^{60} is not "available" for filtration.

In experiments of short duration, the barbiturates have no effect on renal function (Corcoran and Page, 1943); but for experiments of long duration (more than three hours), a reduction in effective renal plasma flow

and in the maximal T_m for PAH have been reported (Glauser and Selkurt, 1952). The glomerular filtration rate observed in five dogs in these present experiments within three hours during sodium pentobarbital anesthesia (Table 14) fell within the normal range for the 75 normal, trained, female dogs not under anesthesia tabulated by Houck (1948). It is reasonably certain that these five dogs were normal in the presence of mild surgical trauma and that their renal function was not deteriorating during periods up to three hours.

In these present experiments of short duration (up to three hours) an average renal clearance of 27 ml. per minute (Table 14) indicates a high degree of tubular reabsorption of Co^{60}, as was calculated. It is important to note the uniformity in the renal clearance values and in the percentages of tubular reabsorption in each of the five dogs (Appendix 4).

Renal handling of strong electrolytes has been summarized by Smith (1951). Sodium and chloride are completely reabsorbed by tubules under normal conditions. Extensive potassium reabsorption occurs in the proximal tubules, but resecretion takes place in the distal tubules. Renal clearance studies of calcium remain ambiguous. Renal handling of magnesium and strontium is unknown. Iodide and thiocyanate clearances are very low and influenced by the sodium and chloride concentrations. There is, then, little information in the literature concerning the handling of electrolytes which is of aid in evaluating the cobalt data. It is even possible that cobalt might be excreted as an anionic complex ion, rather than as a cation. As has been noted previously, the renal behavior of the Co^{60} precipitated by cadmium hydroxide is not clear. This type of uncertainty is the present limitation to the interpretation of the clearance data. Until the chemistry of the cobalt complex in blood is known, the true extent of tubular reabsorption cannot be accurately determined.

Since a certain fraction of the blood Co^{60} may be in the form of amino acid complexes which are diffusible on dialysis, these amino acid complexes may then be present in the glomerular filtrate. Total amino acid clearances have been reported to be very low, usually less than 10 ml. (Kirk, 1936). The essential amino acids are very effectively reabsorbed by the tubules (Beyer et al., 1946; Doty, 1943; Goettsch et al., 1944; Harvey and Horwitt, 1949; Kirsner et al., 1949; Pitts, 1943; Russo et al., 1947; Sheffner et al., Wright et al., 1947). This suggests a mechanism which could account for a portion of the low clearance values and of the relatively high percentage of tubular reabsorption of Co^{60} in the present studies.

In these experiments of long duration (up to thirteen hours), a relatively constant renal clearance of Co^{60} was maintained in dogs which received a constant infusion (Table 16). This directly indicates that, in the dog, the surgical operation employed in these experiments does not significantly affect the function of the kidney as far as the handling of the Co^{60} is concerned. Thus, the reduction in the renal clearance of Co^{60} following a single injection (Tables 15 and 16 and Figure 12) must be primarily due to the changes of the renal function (including the clearance of a different form of cobalt) rather than to surgical trauma. This is directly indicated by the fact that the percentage of tubular reabsorption of Co^{60} increased with time after injection (Table 15).

It has been reported that variations in urine flow have no effect on glomerular filtration rate in rabbits under controlled experimental conditions (Forster, 1952). In these acute experiments, a 16-fold variation in urine flow (0.40 to 6.57 ml. per minute in Dog No. 18 weighing 9 kg.) failed to change the relatively constant renal clearance and tubular reabsorption of Co^{60} (Figure 22).

Tissue Distribution, Intestinal Absorption and "Secretion" of Co^{60}:

Data from the dog are in agreement with conclusions previously obtained in the chicken. First, inorganic Co^{60} is readily absorbed by the small intestine of the dog. A segment of the first part of small intestine, isolated from the rest of the tract by ligation, absorbed half of the injected Co^{60} from its lumen within twelve hours. This is much slower than the absorption rate observed in chicks. Second, a relatively large amount of intravenously injected inorganic Co^{60} gets into the intestinal tract of the dog through the intestinal wall, in addition to the amount reaching the tract through the bile. Whether this active passage of Co^{60} across the intestinal wall is a process of "secretion" or merely "continuous diffusion" remains uncertain. Third, Co^{60} must be in some "non-diffusible" forms in the contents and also in the wall of the intestinal tract of the dog, since the Co^{60} concentration in the contents or in the wall were greater than the plasma concentration (Table 22).

Considerable biosynthesis of vitamin B_{12} or B_{12}-like substance has been reported to take place in the intestinal tract of sheep after Co^{60} ingestion, and in the tissues after intravenous injection (Monroe et al., 1952b). According to these investigators, the vitamin B_{12}-like Co^{60} in the small intestine is from 6 to 15%, and the bound Co^{60} in this organ is from 41 to 53%. This bound Co^{60} may be present within the bacterial cells.

The fact that the contents and the wall itself in the first tied segment of the small intestine contained the same low concentration of Co^{60} indicates either less binding or greater absorption of Co^{60} in this segment. It should be noted that the bile duct empties into this loop. Hence, the increasing concentration of Co^{60}, both in the contents and in the wall, as

one passes from the first to the third loop suggests increased retention of Co^{60} in the lower segments. The more activity in lower segments may be due either to some "binding" agent (for example, bacteria or intestinal contents) or to increased secretion into the intestinal lumen.

Unlike the chicken, the concentration of Co^{60} was about the same in the contents as in the wall of the caecum in the dog. The Co^{60} concentration ratios of caecal wall or caecal contents to blood were much less in the dog than in the chicken.

When one considers tissue retention of Co^{60}, the liver has been reported to be one of the tissues in which cobalt is concentrated in the largest amount (Braude et al., 1949; Comar and Davis, 1947a; 1947b; Comar et al., 1946a; 1946b; Copp and Greenberg, 1941; Cuthbertson et al., 1950; Greenberg et al., 1943b; Lawrence, 1947; Monroe et al., 1952a; 1952b; Ulrich and Copp, 1951). It has also been known that liver is a very rich source of vitamin B_{12} in ruminants (Lewis et al., 1949; Thompson et al., 1950). However, no evidence for the biosynthesis of vitamin B_{12} or B_{12}-like substance was shown in the liver of sheep (Monroe et al., 1952b).

The following points emphasize the role of the liver in the metabolism of Co^{60} in the dog: First, in the acute bile filuta dogs, 4 to 11% of the intravenously injected inorganic Co^{60} was excreted in the bile during a period of seven to thirteen hours (Table 19). Second, a form or forms of Co^{60} other than its inorganic form is present in the bile. This conclusion is based on two lines of evidence. (1) The absorption rate of the bile Co^{60} was faster than that of its inorganic form (Table 13); (2) paper partition chromatographic and radioautographic separation studies revealed the presence in the bile of a radioactive component or components containing Co^{60} other than in its inorganic form. Third, twelve hours after intravenous or intraintestinal

injection, Co^{60} was much more concentrated in the liver than in either the kidney, the contents or the wall of the intestinal tract, the bladder urine, the gall bladder bile or the spleen.

From these observations, it is certain that the liver in the dog plays an important part in the metabolism of Co^{60} and that its physiological role in handling this tracer is apparently different from that of the kidney. The kidney apparently serves only as an excretory organ for Co^{60}. The liver, on the other hand, may be a storage organ for various types of cobalt compounds, or may even synthesize certain of these compounds.

Comar and Davis (1947b) have reported that cobalt is concentrated 100 times in the liver of a young calf than in the whole blood seventeen hours after intravenous injection of labeled cobalt. The cobalt concentration ratio of the liver to whole blood in ruminants is much higher than that found in dogs. This indicates the greater importance of liver in ruminants in the metabolism of cobalt than that in non-ruminants. It also accounts for the fact that the liver of ruminants is a very rich source of vitamin B_{12} (Lewis et al., 1949; Thompson et al., 1950).

The relatively high concentration of Co^{60} in the kidney may reflect the excretion of this element through that organ. Since the concentration of Co^{60} in the spleen was lower than the plasma level, the biosynthesis of vitamin B_{12} or vitamin B_{12}-like substance in this organ in the dog (as reported by Monroe et al., 1952b, in the sheep) remains doubtful.

SUMMARY

1. Cobalt 60, as $Co^{60}SO_4$, is both readily absorbed from the intestinal tract and "secreted" or "continuously diffused" into the intestinal tract through its wall in both the chicken and the dog.

Twenty-four hours after Co^{60} is administered intravenously or injected into the gizzard of the chicken, 6% of the injected dose may be recovered from the small intestine, 5% from both caeca, 1% from the large intestine and 5% from the extracellular fluid. During this period, if the Co^{60} is injected intravenously, at least 13% of the injected dose is eliminated in the feces and a large fraction of the remaining 70% is excreted in the urine emptied into the cloaca. If the Co^{60} is injected into the gizzard, at least 51% appears in the feces and a fraction of the remaining 32% appears in the urine by the end of 24 hours.

After a single intravenous injection of Co^{60} into the dog, between 40 and 70% of the injected dose may be recovered in the urine plus the bile during a period of seven to thirteen hours. A large majority of this (nearly 90%) is recovered in the first six hours. Only one-seventh to one-tenth of the total recovery is found in the bile. When Co^{60} is injected into a loop formed at the first part of the small intestine, half of the injected dose is absorbed by the end of twelve hours.

2. In the chicken, most of the Co^{60} found in the caecum enters from the gut. However, under certain experimental conditions (such as complete obstruction of both the large intestine and of the caecum itself), Co^{60} passes into the caecum through the wall in large amounts. In the dog, the caecum plays an insignificant role in the metabolism of Co^{60}.

3. In the chicken, Co^{60} is greatly concentrated in the caecal contents, in the caecal wall, and in the large and small intestines regardless of the route of administration. In these locations, it is present in some "non-diffusible" form or forms. A very small amount of Co^{60} is found in the bladder bile of the chicken. In the dog, the highest concentrations of Co^{60} are found in the liver, in the intestinal wall and in the contents of the first part of the jejunum, in the kidney, in the gall bladder bile and in the bladder urine. The spleen contains a very low amount of Co^{60}.

4. Cobalt 60 passes into the extracellular fluid soon after intravenous injection of its inorganic form in the dog. Blood cells contain an insignificant amount of Co^{60}. About 75% of the whole blood Co^{60} is precipitable with cadmium hydroxide and remains "non-diffusible" on dialysis. This "non-diffusible" Co^{60} in the protein precipitate appears to be "dissociable" and "available" for clearance by the kidney and the liver.

5. In the dog, the plasma Co^{60} quickly filters through the glomeruli following a single intravenous injection. The maximal values for the rate of urinary excretion are reached within one-half to three hours. The urinary excretory rate then decreases as the plasma Co^{60} concentration drops. On the other hand, the rate of biliary Co^{60} excretion reaches a maximum about five to seven hours after injection and then drops only slightly.

In dogs receiving a constant infusion, relatively constant plasma Co^{60} concentrations are maintained. The rate of urinary Co^{60} excretion in these dogs remains constant but at a level dependent upon the rate of infusion. However, the rate of biliary excretion increases gradually and reaches a plateau at about four to seven hours.

The first appearance of Co^{60} both in the urine and in the bile is within five to ten minutes after intravenous injection. There is a "delay" of

about four hours in the peak of the biliary excretion curve when compared with the peak of the urinary excretion curve.

6. The renal clearance of Co^{60} within three hours after intravenous injection averages 27 ml. per minute per square meter of surface area in five sodium pentobarbital anesthetized dogs in the presence of mild surgical trauma. About three-fourths of the filtration load is reabsorbed by the tubules, when the calculation is made on the basis of whole plasma Co^{60} concentration. In acute experiments of long duration (up to thirteen hours), renal clearances of Co^{60} decline due to an increase in the tubular reabsorption.

7. In the dog, the half-time for the removal of Co^{60} from the blood is 20% faster when injected as the cystein-Co^{60} complex than when injected as $Co^{60}SO_4$. The transfer of Co^{60} from the peritoneal cavity to the blood is 3.3 times faster when injected as the cysteine-Co^{60} complex than when injected as $Co^{60}SO_4$. Four hours after injection, the blood Co^{60} concentration levels off in both cases and remains relatively constant for at least 20 hours.

8. A form (or forms) of Co^{60} other than its inorganic form is found in the bile and in the urine samples collected from these experimental dogs. The Co^{60} in these samples is reabsorbed from the gut of young chicks at a considerably faster rate than is inorganic Co^{60}.

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Appendix I

The Cobalt 60 Mass Absorption Coefficient

The external absorber method of Aten (Aten, Jr. 1950) was used to construct a self-absorption curve for Co^{60}. About 80 mg. of ashed intestines in which Co^{60} was uniformly distributed were evenly mounted on a crucible cover. The activity was measured under the same geometry as employed throughout the chicken and dog experiments. The activities of the same sample covered by a series of thin aluminum absorbers were then counted. The following formula was employed to calculate the absorption coefficient (u):

$$\frac{A_a}{A_m} = e^{-ux_a}$$

where A_m is the activity observed without an absorber, A_a is the activity observed through an external absorber of thickness x_a (mg. per square cm.), and u is the mass absorption coefficient (square cm. per mg.).

Four absorbers ranging from 1.75 to 13.70 mg. per square cm. were used. Activities with and without the external absorbers and the calculated values for the mass absorption coefficient are summarized in Table 1.

Table 1. The Mass Absorption Coefficient (u) for Co^{60}.

Thickness of External Absorber, mg/cm^2	Activity cps	Calculated u cm^2/mg
0.00	48.503	
1.75	42.966	0.069
3.23	38.816	0.069
6.04	31.278	0.072
13.70	18.335	0.071

An average value of 0.070 was taken for u. By substituting the value of u and different values of x_a into the formula given above, the activity ratios (A_a/A_m) were calculated and Table 2 and Figure 1 were constructed.

Table 2. Activity Ratios (A_a/A_m) of Co^{60} at Different Thickness of Sample.

Thickness of Sample mg/cm^2	Activity Ratio, %	
	Calculated	Data from Gleason et al. (1951)
1	93.2	91.6
2	87.1	83.9
3	81.3	76.8
4	75.9	70.4
5	70.8	64.5
6	66.7	59.0
7	61.7	54.1
8	57.5	
9	53.7	
10	50.1	
15	35.5	
20	25.1	
25	17.8	
30	12.6	

In practice, the thickness of a sample is obtained by dividing the ash weight of the sample (mg.) by its surface area (cm^2). The plot given as Figure 1 is entered with this value on the abscissa. The corresponding value of the activity ratio may then be read on the ordinate. The measured activity of the sample is then divided by the activity ratio, the result being the true activity of the sample.

Schweitzer and Stein (1950) reported good agreement between three different methods, including this method of Aten (1950), when used to correct for the effect of self-absorption of beta particles. Gleason et al. (1951) computed a u value of 0.088 cm^2/mg for Co^{60} from the equation:

$$u = 0.017\ E_{max}^{-1.43}$$

where u is the mass absorption coefficient near zero thickness, and E_{max} is the maximum energy of the beta spectrum in Mev. With their counting geometry, the computed value for u was experimentally confirmed. The lower u found in the present case is presumably to be ascribed to scattered and secondary radiation.

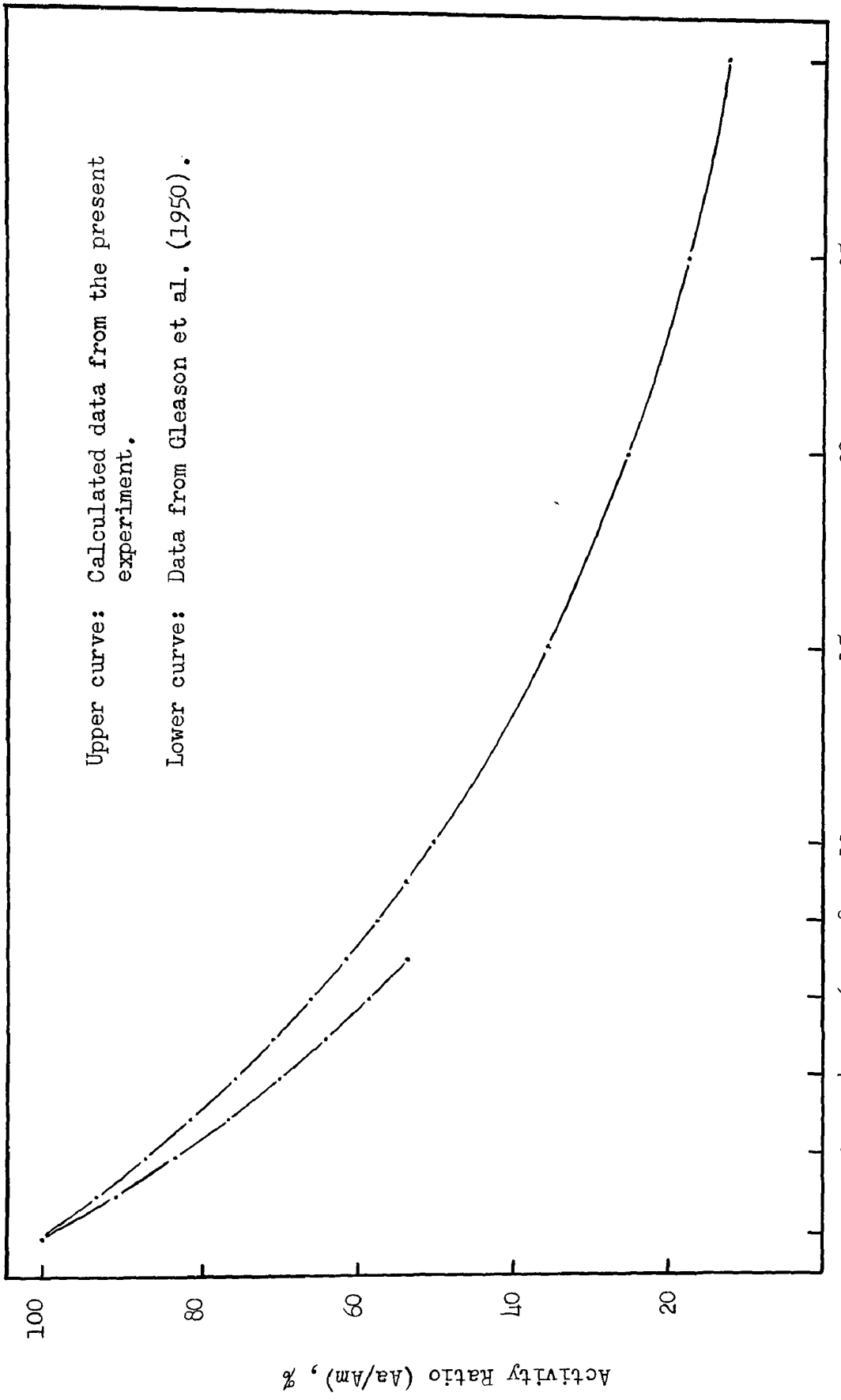

Figure 1. Self-absorption Curve for Co^{60}.

Appendix II

Methods and Calculations for the Study of Renal Function in the Dog

1. Protein-free Plasma Filtrate: .

   1. Centrifuge the blood sample to obtain plasma.
   2. Pipette 2 ml. of plasma into 10 ml. of water in a clean 50 ml. centrifuge tube (do this in duplicate for each blood sample).
   3. Add 16 ml. of acid cadmium sulfate* from a burette and mix.
   4. Add 2 ml. of 1.1 N NaOH, stopper and shake well.
   5. Let stand for ten minutes and centrifuge.

   The water-clear supernatant layer is used for chemical analysis.

2. Urine Creatinine:

   1. Pipette 3 ml. of the final diluted urine into a 5 ml. colorimeter tube.
   2. Add 1 ml. of saturated picric acid and mix.
   3. Add 1 ml. of 0.75 N NaOH and mix.
   4. Let stand exactly fifteen minutes and read in the colorimeter using filter of 540 mu.

   Do this in duplicate.

   Reagent blank: Three ml. of distilled water is used.

   Standards: Three tubes containing known amounts of creatinine (20, 40 and 60 micrograms) in 3 ml. of distilled water solution will be made. Do

*Acid cadmium sulfate is made from the following formula:

$CdSO_4 \cdot 8H_2O$	13 gm.
1 N H_2SO_4	63.5 ml.
Distilled water, q.s.	1000 ml.

this in duplicate. A standard curve is then made by plotting the amounts of creatinine versus the readings on the colorimeter.

3. Plasma Creatinine:

Pipette 3 ml. of the protein-free plasma filtrate (water-clear supernatant layer) into a colorimeter tube and proceed as for the urine creatinine described above. Sometimes, when the 0.75 N NaOH is added, a faint flocculant precipitate forms which must be thrown down in the centrifuge. To do this it is probably best to develop the plasma creatinine color in a conical centrifuge tube. Centrifuge during the 15-minute color-development period, then pour the contents into a colorimeter tube for reading. As before a reagent blank and three standard tubes will be made.

4. Urine PAH:

1. Pipette 3 ml. of the final diluted urine into a large test tube containing 7 ml. of water.
2. Add 2 ml. of 1.2 N HCl from a burette and mix.
3. Also from burettes add the following:
   a. One ml. of $NaNO_2$ (100 mg.%), shake vigorously.
   b. After standing not less than three nor more than five minutes, add 1 ml. of ammonium sulfamate (500 mg.%) and mix thoroughly.
   c. After standing not less than two nor more than five minutes, add 1 ml. of N(1-naphtyl) ethylenediamine dihydrochloride (100 mg.%) and mix well.
4. Let stand for ten minutes and read in the colorimeter tubes using filter of 540 mu.

   Do this in duplicate. This color is stable. There is no need for undue hurry.

Reagent blank: Ten ml. of distilled water is used.

Standard: One tube containing a known amount of PAH (20 micrograms) in 10 ml. of distilled water will be made. Do this in duplicate. There is a direct proportion between the amount of PAH present in the solution and the reading from the colorimeter.

5. Plasma PAH:

Pipette 5 ml. of the protein-free plasma filtrate (water-clear supernatant layer) into a large test tube containing 5 ml. of distilled water. Proceed as for the urine PAH determination described above. As before, a reagent blank and a standard tube will be made.

6. Formulae Used in Calculations:

$$1.\quad C_{cr} = \frac{U_{cr}V}{P_{cr}}$$

where C_{cr} is the renal clearance of creatinine in ml. per minute, U_{cr} is the urine concentration of creatinine in mg. per ml. (of undiluted urine), P_{cr} is the plasma filtrate concentration of creatinine in mg. per ml. (undiluted filtrate) and V is the urine volume in ml. per minute.

$$2.\quad C_{PAH} = \frac{U_{PAH}\ V}{P_{PAH}}$$

where C_{PAH} is the renal clearance of PAH in ml. per minute, U_{PAH} is the urine concentration of PAH in mg. per ml. (undiluted urine), P_{PAH} is the plasma filtrate concentration of PAH in mg. per ml. (undiluted filtrate) and V is the urine volume in ml. per minute.

$$3.\quad C_{Co}60 = \frac{U_{Co}60\ V}{P_{Co}60}$$

where $C_{Co}60$ is the renal clearance of Co^{60} in ml. per minute, $U_{Co}60$ is the urine concentration of Co^{60} in micrograms per ml. (undiluted urine),

$P_{Co^{60}}$ is the plasma concentration of Co^{60} in micrograms per ml. and V is the urine volume in ml. per minute.

$$4.\quad T_m = U_{PAH}\ V - C_{cr} \times P_{PAH} \times FW$$

where T_m is the tubular secretory mass of PAH in mg. per minute, U_{PAH} is the urine concentration of PAH in mg. per ml. (undiluted urine), V is the urine volume in ml. per minute, C_{cr} is the renal clearance of creatinine in ml. per minute, P_{PAH} is the plasma filtrate concentration of PAH in mg. per ml. (undiluted filtrate) and the FW factor is taken as 0.917 as suggested by Smith (1951).

$$5.\quad FF = \frac{\text{Renal Clearance of PAH}}{\text{Renal Clearance of Creatinine}}$$

where FF is the filtration fraction.

6. Co^{60} Load (ug/min.) =
Glomerular Filtration Rate or C_{cr} (ml/min.) x
Plasma Concentration of Co^{60} (ug/ml)

7. Co^{60} Excreted (ug/min.) = Urine Volume (ml/min.) x
Urine Concentration of Co^{60} (ug/ml)

8. Co^{60} Reabsorbed (ug/min.) =
Co^{60} Load (ug/min.) - Co^{60} Excreted (ug/min.)

$$9.\quad Co^{60}\ \text{Reabsorption}\ (\%) = \frac{Co^{60}\ \text{Reabsorbed}}{Co^{60}\ \text{Load}}$$

Appendix 3

Concentration of Co^{60} in Whole Blood, Plasma, Protein-free Plasma Filtrate and Relative Distribution of Co^{60} in Blood

Dog No.	Time After Co^{60} Injection	Corrected Hematocrit	Plasma Co^{60} Conc.	Whole Blood Co^{60} Conc.	Amount of Co^{60} in Blood Cells	Protein-free Plasma Filtrate Co^{60} Conc.*	Amount of Co^{60} in Protein-free Plasma Filtrate	Amount of Co^{60} in Plasma Protein Precipitate
		%	ug/ml	ug/min	%	ug/ml	%	%
15	1 hr.	34.5	0.0359	0.0271	13.3	0.00585	14.1	72.6
	2	34.5	0.0354	0.0227	-1.4	0.00535	15.3	86.1
16	25 min.	39.4	0.0337	0.0172	-18.7	0.00580	20.4	98.3
	37.5	44.7	0.0363	0.0205	2.2	0.00534	14.4	83.4
	53	44.3	0.0325	0.0187	3.3	0.00458	13.7	83.0
	67.5	46.6	0.0301	0.0165	2.6	0.00554	17.9	79.5
	80	45.6	0.0313	0.0176	3.4	0.00604	18.6	78.0
21	15 min.	47.0	0.0289	0.0186	17.7	0.00486	13.8	68.5
	30	48.0	0.0256	0.0142	6.2	0.00394	14.4	79.4
	45	48.0	0.0236	0.0143	14.2	0.00352	12.8	73.0
	60	46.2	0.0262	0.0170	17.1	0.00417	13.2	69.7
24	0.5 hr.	46.1	0.0387	0.0227	8.1	0.00705	16.7	75.2
	1.0	45.7	0.0397	0.0200	-7.9	0.00603	16.4	91.5
	1.5	47.5	0.0330	0.0168	-3.1	0.00616	19.3	83.8
	2.0	45.2	0.0415	0.0241	5.7	0.00580	13.2	81.1
	6.0	50.8	0.0364	0.0178	-0.6	0.00306	8.9	91.7
	7.0	50.3	0.0387	0.0176	-9.3	0.00356	10.1	99.2
	7.5	50.8	0.0392	0.0187	-3.2	0.00206	5.5	97.7
	10.5	51.3	0.0301	0.0137	-7.1	0.00136	4.8	102.3
	11.0	49.9	0.0279	0.0148	5.4	0.00061	2.1	92.5
	11.5	51.3	0.0273	0.0126	-5.4	0.00046	1.8	103.6
	12.0	51.7	0.0253	0.0115	-6.3	0.00042	1.8	104.5

Appendix 3 --- Continued

25	0.5 hr.	50.8	0.0263	0.0133	2.6	0.00518	19.2	78.2
	1.0	40.9	0.0229	0.0147	20.5	0.00423	14.7	64.8
	1.5	48.5	0.0209	0.0135	20.2	0.00344	13.2	66.6
	2.0	48.0	0.0211	0.0124	11.3	0.00413	17.4	71.3
	6.0	43.7	0.0305	0.0178	3.5	0.00314	9.9	76.6
	6.5	43.8	0.0366	0.0190	-8.3	0.00354	10.5	97.8
	7.0	43.3	0.0300	0.0191	11.0	0.00244	7.3	81.7
	7.5	43.3	0.0329	0.0200	6.8	0.00354	10.1	83.1
	10.5	42.1	0.0350	0.0210	3.6	0.00245	6.7	89.7
	11.0	43.3	0.0351	0.0191	-4.2	0.00264	7.9	96.3
	11.5	42.4	0.0338	0.0220	11.5	0.00156	4.1	84.4
	12.0	42.0	0.0409	0.0215	-10.2	0.00210	5.6	104.6

Appendix 4

Renal Clearances of Creatinine, PAH and Co^{60} in Sodium Pentobarbital Anesthetized Dogs of Short Duration. (up to three hours)

Dog No.	Duration of Period	C_{cr}/M^2 S.A.	C_{PAH}/M^2 S.A.	T_m PAH	Filtration Fraction	Co^{60} Plasma Conc.	Co^{60} Load	Co^{60} Urine Vol.	Co^{60} Excreted	Co^{60} Reabsorbed	Co^{60} Reabsorption	C_{co}/M^2 S.A.
	min.	ml/min	ml/min	mg/min		ug/L	ug/min	ml/min	ug/min	ug/min	%	ml/min
12	16	46.0	106.7	2.83	0.43	39.38	1.06	0.99	0.39	0.67	63.3	16.9
	14	58.5	153.0	3.34	0.38	41.13	1.41	1.69	0.30	1.12	79.0	12.3
	15	66.0	194.1	3.33	0.34	34.31	1.33	1.28	0.28	1.05	78.7	14.0
		56.8	151.2	3.17	0.38	38.27	1.27	1.32	0.32	0.95	73.7	14.4
13	15	106.1	115.4	0.06	0.92	27.23	1.01	0.30	0.26	0.75	73.9	29.7
	15	89.5	168.7	0.36	0.53	24.12	0.75	0.23	0.23	0.52	69.6	27.1
	15	118.0	563.2	1.16	0.21	22.00	0.91	0.19	0.21	0.70	77.4	26.7
		104.5	282.4	0.53	0.55	24.45	0.89	0.24	0.23	0.66	73.6	27.2
16	15	84.2	182.5	1.19	0.46	36.25	2.17	0.65	0.87	1.30	59.8	33.7
	17	101.5	214.2	1.63	0.47	32.55	2.35	0.49	0.86	1.49	63.4	36.9
	15	99.0	214.3	1.66	0.46	30.08	2.12	0.63	0.73	1.39	65.7	33.8
		94.9	203.7	1.49	0.46	32.96	2.21	0.59	0.82	1.39	63.0	34.8
18	16	89.8	188.9	1.50	0.48	39.65	1.73	0.40	0.50	1.22	70.9	26.1
	16	82.9	207.2	1.97	0.40	32.48	1.31	0.89	0.50	0.81	61.9	31.6
	17	83.6	269.6	2.41	0.31	38.23	1.55	2.36	0.48	1.07	69.0	25.9
	17	90.0	239.0	2.21	0.38	37.61	1.64	4.76	0.47	1.17	71.4	25.7
	15	92.0	289.5	2.77	0.32	32.98	1.47	6.57	0.46	1.02	69.1	28.4
	15	95.7	287.3	2.70	0.33	31.25	1.45	6.03	0.40	1.05	72.6	26.3
	15	94.1	271.6	2.62	0.35	27.36	1.25	5.58	0.37	0.88	70.7	27.6
	15	94.1	262.8	2.39	0.36	26.62	1.22	5.26	0.33	0.89	73.3	25.2
		90.3	252.0	2.32	0.36	33.27	1.45	3.98	0.43	1.02	69.9	27.1
21	15	98.8	212.4	1.07	0.46	27.26	1.58	0.35	0.51	1.07	67.6	32.4
	15	87.6	174.6	0.86	0.50	24.61	1.27	0.40	0.37	0.89	70.6	25.7
	15	90.1	198.0	1.20	0.45	24.92	1.32	0.48	0.49	0.83	63.0	33.3
		92.2	195.0	1.04	0.47	26.00	1.39	0.41	0.46	0.93	67.1	30.5
Grand mean		88.4 ±16.2	225.6 ±94.7	1.86 ±0.94	0.43 ±0.14	31.50 ±5.86	1.45 ±0.41	1.98 ±2.25	0.45 ±0.19	1.00 ±0.25	69.6 ±5.5	26.9 ±6.5

C_{cr} : Renal clearance of creatinine
C_{PAH}: Renal clearance of PAH
T_m PAH : Tubular secretory mass of PAH
C_{co} : Renal clearance of Co^{60}

Appendix 5

Renal Clearances of Creatinine, PAH and Co^{60} in Sodium Pentobarbital Anesthetized Dogs of Long Duration. (up to twelve hours)

Dog No.	Time After Priming Co^{60}	C_{cr}/M^2 S.A.	C_{PAH}/M^2 S.A.	T_m PAH	Filtration Fraction	Co^{60} Plasma Conc.	Co^{60} Load	Co^{60} Urine Vol.	Co^{60} Excreted	Co^{60} Reabsorbed	Co^{60} Reabsorption	C_{co}/M^2 S.A.
	hr.	ml/min	ml/min	mg/min		ug/L	ug/min	ml/min	ug/min	ug/min	%	ml/min
24	0.5-2.0	72.5	171.0	1.01	0.42	39.20	1.76	0.23	0.52	1.25	70.7	21.3
		82.2	172.9	1.19	0.48	36.38	1.85	0.53	0.40	1.46	78.5	27.0
		113.9	249.1	2.56	0.46	37.25	2.63	0.83	0.63	2.00	76.1	27.0
		89.5	197.7	1.85	0.45	37.61	2.08	0.53	0.52	1.57	75.1	22.0
	6.0-7.5	97.3	249.1	3.38	0.39	36.91	2.23	0.75	0.44	1.78	80.1	19.3
		85.8	238.9	3.59	0.36	38.16	2.03	1.03	0.41	1.62	79.6	17.5
		85.9	220.2	3.08	0.39	38.95	2.08	0.94	0.47	1.61	77.5	19.3
		89.7	236.0	3.35	0.38	38.01	2.11	0.91	0.44	1.67	79.1	18.7
	10.5-12.0	116.5	352.3	3.13	0.33	28.97	2.09	0.60	0.24	1.86	88.5	13.4
		99.3	207.6	1.18	0.48	27.56	1.70	0.48	0.22	1.48	87.2	12.8
		91.3	159.4	0.96	0.57	26.27	1.49	0.42	0.15	1.34	90.1	9.0
		102.4	236.4	1.76	0.46	27.60	1.76	0.50	0.20	1.56	88.6	11.7
25	0.5-2.0	60.9	151.3	0.75	0.40	24.61	0.88	0.11	0.28	0.60	68.5	19.2
		52.4	140.8	0.79	0.37	21.93	0.68	0.11	0.23	0.45	65.9	17.8
		80.4	238.5	1.42	0.34	21.02	0.99	0.17	0.28	0.71	71.5	22.9
		64.5	176.8	0.99	0.37	22.52	0.85	0.13	0.26	0.59	68.7	20.0
	6.0-7.5	102.5	326.5	1.75	0.31	33.56	2.02	0.31	0.23	1.79	88.6	11.7
		100.4	239.4	1.30	0.42	33.32	1.96	0.37	0.20	1.77	90.0	10.1
		95 .5	238.7	1.62	0.40	31.49	1.77	0.39	0.20	1.57	88.8	10.7
		99.5	268.2	1.55	0.38	32.79	1.92	0.36	0.21	1.71	89.1	10.8
	10.5-12.0	95.2	300.3	2.18	0.32	35.09	1.96	0.55	0.25	1.72	87.4	11.0
		101.9	293.8	1.70	0.35	34.48	2.07	0.56	0.24	1.82	88.3	11.9
		102.0	365.4	2.14	0.28	37.34	2.24	0.64	0.24	2.00	89.5	10.7
		99.7	319.8	2.00	0.31	35.64	2.09	0.58	0.24	1.85	88.4	11.3

C_{cr} : Renal clearance of creatinine

C_{PAH} : Renal clearance of PAH

T_m PAH : Tubular secretory mass of PAH

C_{co} : Renal clearance of Co^{60}

Appendix 6

Blood Concentration, Urinary and Biliary Excretion of Co^{60} in Dog No. 1; S. A., 0.8642 square meters

Time After Co^{60} Injection	Blood Co^{60} Conc. ug/ml	Co^{60} in Urine			Co^{60} in Bile		
		Volume ml/min	Conc. ug/ml	Excreted ug/min	Volume ml/min	Conc. ug/ml	Excreted ug/min
3 min.	0.0658						
10	0.0366	0.270	0.022	9.006	0.120	0.001	0.000
30	0.0240						
1 hr.	0.0194	0.088	3.386	0.298	0.122	0.036	0.004
2	0.0190	0.243	4.562	1.109	0.123	0.036	0.005
3	0.0148	0.215	1.998	0.430	0.102	0.313	0.032
4	0.0140	0.282	1.336	0.337	0.118	0.362	0.043
5	0.0130	0.257	1.086	0.279	0.103	0.510	0.053
6	0.0154	0.182	0.902	0.164	0.080	0.654	0.052
7	0.0138	0.095	1.388	0.132	0.073	0.738	0.054
8	0.0118	0.045	1.798	0.081	0.065	0.745	0.048
9	0.0146	0.060	1.664	0.100	0.060	0.757	0.045

Appendix 7

Blood Concentration, Urinary and Biliary Excretion of Co^{60} in Dog No. 2; S. A., 0.5711 square meters

Time After Co^{60} Injection	Blood Co^{60} Conc. ug/ml	Co^{60} in Urine Volume ml/min	Co^{60} in Urine Conc. ug/ml	Co^{60} in Urine Excreted ug/min	Co^{60} in Bile Volume ml/min	Co^{60} in Bile Conc. ug/ml	Co^{60} in Bile Excreted ug/min
1 min.	0.1384						
3.5	0.0836						
5.0	0.1062						
6.0	0.0544						
7.0	0.0484						
8.0	0.0524						
9.5	0.0328						
11.5	0.0424						
13	0.0440						
15	0.0372	0.020	0.018	0.0004	0.087	0.079	0.007
30	0.0258	0.053	2.942	0.156	0.087	0.259	0.023
45	0.0222	0.113	5.942	0.671	0.120	0.352	0.042
1 hr.	0.0208	0.053	4.566	0.242	0.080	0.324	0.026
2	0.0184	0.043	3.876	0.167	0.088	0.321	0.028
3	0.0174	0.225	3.130	0.704	0.090	0.447	0.040
4	0.0112	0.222	1.404	0.312	0.082	0.587	0.048
5	0.0102	0.217	0.912	0.198	0.082	0.666	0.055
6	0.0092	0.175	0.740	0.130	0.070	0.671	0.047
7	0.0100	0.128	0.758	0.097	0.065	0.585	0.038
8	0.0078	0.162	0.894	0.145	0.067	0.457	0.031

Appendix 8

Blood Concentration, Urinary and Biliary Excretion of Co^{60} in Dog No. 3; S. A., 0.6355 square meters

Time After Co^{60} Injection	Blood Co^{60} Conc. ug/ml	Co^{60} in Urine			Co^{60} in Bile		
		Volume ml/min	Conc. ug/ml	Excreted ug/min	Volume ml/min	Conc. ug/ml	Excreted ug/min
2.5 min.	0.0720						
3	0.0640						
4	0.0556						
5	0.0440	0.021	0.086	0.002	0.025	0.011	0.000
6	0.0450						
7	0.0390						
8	0.0418						
9	0.0376						
10	0.0350	0.070	2.160	0.151	0.020	0.003	0.000
12	0.0326						
15	0.0302						
20	0.0296	0.068	8.166	0.555	0.042	0.131	0.005
35	0.0252						
45	0.0272	0.096	11.836	1.136	0.034	0.307	0.010
1 hr.	0.0264	0.087	8.664	0.754	0.026	0.234	0.006
2	0.0228	0.050	7.460	0.373	0.025	0.212	0.005
3	0.0188	0.045	4.988	0.224	0.022	0.259	0.006
4	0.0174						
5	0.0130				0.019	0.275	0.005
6	0.0162						
7	0.0160	0.003	5.472	0.013	0.007	0.405	0.003

Appendix 9

Blood Concentration, Urinary and Biliary Excretion of Co^{60} in Dog No. 4; S. A., 0.5028 square meters

Time After Co^{60} Injection	Blood Co^{60} Conc.	Co^{60} in Urine			Co^{60} in Bile		
		Volume	Conc.	Excreted	Volume	Conc.	Excreted
	ug/ml	ml/min	ug/ml	ug/min	ml/min	ug/ml	ug/min
1 min.	0.1064						
2	0.0716						
3	0.0620						
4	0.0512						
5	0.0452	0.080	1.458	0.117	0.096	0.009	0.001
8	0.0362						
9	0.0354						
10	0.0346	0.048	22.000	1.056	0.060	0.010	0.001
12	0.0336						
15	0.0302						
20	0.0272	0.062	30.284	1.878	0.090	0.060	0.005
25	0.0240						
30	0.0222	0.060	27.190	1.631	0.070	0.182	0.013
45	0.0198	0.032	13.706	0.439	0.080	0.351	0.028
1 hr.	0.168	0.065	13.326	0.866	0.080	0.463	0.037
2	0.0130	0.053	6.374	0.338	0.058	0.591	0.034
3	0.0116	0.058	3.630	0.211	0.053	0.590	0.031
4	0.0100	0.055	2.648	0.146	0.050	0.687	0.034
5	0.0092	0.066	2.032	0.134	0.042	0.858	0.036
6	0.0094	0.065	1.193	0.077	0.038	1.183	0.045
7	0.0082	0.048	1.142	0.055	0.027	1.326	0.036
8	0.0072	0.043	0.942	0.041	0.018	1.246	0.022
9	0.0074	0.008	0.762	0.006	0.017	1.571	0.027
10	0.0056	0.050	0.846	0.042	0.015	1.429	0.021
11	0.0066				0.015	1.253	0.019
12	0.0058	0.019	0.670	0.013	0.018	1.274	0.023
13	0.0052				0.018	1.277	0.023

Appendix 10

Blood Concentration, Urinary, Biliary and Total Excretion of Co^{60} in Dog No. 6; S. A., 0.4483 square meters

Time After Co^{60} Injection	Blood Co^{60}	Co^{60} in Urine			Co^{60} in Bile			Total Co^{60}
	Conc.	Volume	Conc.	Excreted	Volume	Conc.	Excreted	Excretion
	ug/ml	ml/min	ug/ml	ug/min	ml/min	ug/ml	ug/min	ug/min
1 min.	0.0663							
2	0.0369							
3	0.0329							
4	0.0287							
5	0.0314	0.072	1.312	0.094	0.030	0.003	0.000	0.094
6	0.0254							
7	0.0273							
8	0.0216							
9	0.0265							
10	0.0247	0.064	8.036	0.514	0.072	0.016	0.001	0.515
20	0.0205	0.120	4.711	0.565	0.060	0.022	0.001	0.566
30	0.0212	0.100	6.355	0.636	0.058	0.096	0.006	0.642
45	0.0212	0.120	6.657	0.799	0.063	0.149	0.009	0.808
1 hr.	0.0167	0.140	3.841	0.538	0.053	0.178	0.009	0.547
2	0.0209	0.197	4.734	0.933	0.045	0.236	0.011	0.944
3	0.0226	0.130	6.036	0.785	0.038	0.394	0.011	0.796
4	0.0245	0.077	9.210	0.709	0.033	0.739	0.024	0.733
5	0.0216	0.095	7.420	0.705	0.020	1.994	0.040	0.745
6	0.0213	0.108	6.757	0.730	0.016	3.433	0.055	0.785
7	0.0227	0.095	4.486	0.426	0.016	3.822	0.066	0.487
8	0.0248	0.108	4.963	0.536	0.015	3.958	0.059	0.595
9	0.0241	0.107	5.965	0.638	0.017	3.506	0.060	0.698
10	0.0292	0.107	5.080	0.544	0.017	3.606	0.061	0.605
11	0.0300	0.095	5.573	0.529	0.015	3.958	0.059	0.588
12	0.0341	0.063	5.819	0.367	0.010	4.401	0.044	0.411

Appendix 11

Blood Concentration, Urinary, Biliary and Total Excretion of Co^{60} in Dog No. 7; S. A., 0.5203 square meters

Time After Co^{60} Injection	Blood Co^{60}	Co^{60} in Urine			Co^{60} in Bile			Total Co^{60}
	Conc.	Volume	Conc.	Excreted	Volume	Conc.	Excreted	Excretion
	ug/ml	ml/min	ug/ml	ug/min	ml/min	ug/ml	ug/min	ug/min
1 min.	0.0586							
2	0.0390							
3	0.0334							
4	0.0244							
5	0.0222	0.096	0.351	0.034	0.048	0.007	0.000	0.034
6	0.0210							
7	0.0192							
8	0.0192							
9	0.0178							
10	0.0174	0.100	4.968	0.497	0.036	0.010	0.000	0.497
20	0.0144	0.105	5.867	0.511	0.054	0.056	0.003	0.514
30	0.0160	0.090	4.147	0.373	0.045	0.193	0.009	0.382
45	0.0146	0.080	5.024	0.402	0.056	0.311	0.017	0.419
1 hr.	0.0168	0.070	2.277	0.159	0.046	0.357	0.016	0.175
2	0.0152	0.091	3.107	0.283	0.041	0.415	0.017	0.300
3	0.0170	0.133	2.825	0.367	0.038	0.535	0.020	0.396
4	0.0174	0.108	3.622	0.391	0.058	0.535	0.031	0.421
5	0.0178	0.125	3.038	0.380	0.056	0.926	0.052	0.432
6	0.0186	0.108	3.684	0.398	0.065	1.055	0.069	0.467
7	0.0192	0.083	4.327	0.359	0.046	1.103	0.051	0.410
8	0.0200	0.108	4.228	0.457	0.045	1.233	0.056	0.513
9	0.0196	0.110	3.344	0.368	0.041	1.252	0.051	0.419
10	0.0204	0.100	3.843	0.384	0.045	1.421	0.064	0.448
11	0.0214	0.055	4.818	0.265	0.036	1.416	0.051	0.316
12	0.0200	0.016	4.890	0.078	0.038	1.515	0.058	0.136

Appendix 12

Blood Concentration, Urinary, Biliary and Total Excretion of Co^{60} in Dog No. 24; A. S., 0.6198 square meters

Time After Co^{60} Injection	Blood Co^{60}	Co^{60} in Urine			Co^{60} in Bile			Total Co^{60}
	Conc.	Volume	Conc.	Excreted	Volume	Conc.	Excreted	Excretion
	ug/ml	ml/min	ug/ml	ug/min	ml/min	ug/ml	ug/min	ug/min
10 min.	0.0334							
20	0.0237							
30	0.0227	0.206	3.574	0.736	0.053	0.076	0.004	0.740
1.0 hr.	0.0200	0.227	2.278	0.517	0.057	0.390	0.022	0.539
1.5	0.0168	0.530	0.751	0.398	0.070	0.371	0.026	0.424
2.0	0.0241	0.833	0.755	0.629	0.093	0.277	0.026	0.655
3.0	0.0154	0.773	0.496	0.383	0.097	0.312	0.030	0.413
4.0	0.0131	0.519	0.382	0.198	0.103	0.480	0.049	0.247
5.0	0.0171	0.573	0.484	0.277	0.117	0.433	0.051	0.328
6.0	0.0178	0.675	0.566	0.382	0.123	0.295	0.036	0.418
6.5	0.0187	0.750	0.591	0.443	0.137	0.301	0.041	0.484
7.0	0.0176	1.033	0.401	0.414	0.127	0.299	0.038	0.452
7.5	0.0187	0.943	0.496	0.468	0.143	0.350	0.050	0.518
8.5	0.0167	0.692	0.381	0.264	0.137	0.305	0.042	0.306
9.5	0.0118	0.548	0.482	0.264	0.100	0.439	0.044	0.308
10.5	0.0137	0.465	0.279	0.128	0.068	0.708	0.048	0.176
11.0	0.0148	0.603	0.399	0.241	0.077	0.636	0.049	0.290
11.5	0.0126	0.483	0.452	0.218	0.077	0.633	0.049	0.267
12.0	0.0115	0.413	0.353	0.146	0.080	0.614	0.049	0.195

Appendix 13

Blood Concentration, Urinary, Biliary and Total Excretion of Co^{60} in Dog No. 25; S. A., 0.5875 square meters

Time After Co^{60} Injection	Blood Co^{60} Conc. ug/ml	Co^{60} in Urine Volume ml/min	Co^{60} in Urine Conc. ug/ml	Co^{60} in Urine Excreted ug/min	Co^{60} in Bile Volume ml/min	Co^{60} in Bile Conc. ug/ml	Co^{60} in Bile Excreted ug/min	Total Co^{60} Excretion ug/min
10 min.	0.0186							
20	0.0150							
30	0.0133	0.133	2.029	0.270	0.083	0.044	0.004	0.274
1.0 hr.	0.0147	0.113	2.455	0.277	0.093	0.102	0.011	0.287
1.5	0.0135	0.113	2.033	0.230	0.110	0.067	0.007	0.237
2.0	0.0124	0.167	1.697	0.283	0.127	0.044	0.006	0.289
3.0	0.0140	0.148	1.377	0.204	0.123	0.053	0.007	0.211
4.0	0.0152	0.192	1.124	0.216	0.092	0.093	0.009	0.225
5.0	0.0187	0.397	0.603	0.239	0.087	0.324	0.029	0.267
6.0	0.0178	0.387	0.524	0.203	0.083	0.453	0.038	0.241
6.5	0.0190	0.307	0.753	0.231	0.080	0.561	0.045	0.276
7.0	0.0191	0.373	0.528	0.197	0.067	0.629	0.042	0.239
7.5	0.0200	0.393	0.503	0.198	0.073	0.709	0.052	0.250
8.5	0.0183	0.400	0.659	0.264	0.058	0.743	0.043	0.307
9.5	0.0192	0.567	0.287	0.163	0.067	0.748	0.050	0.213
10.5	0.0210	0.447	0.360	0.161	0.058	0.795	0.046	0.207
11.0	0.0191	0.550	0.449	0.247	0.083	0.663	0.055	0.302
11.5	0.0220	0.560	0.430	0.241	0.053	0.758	0.040	0.281
12.0	0.0215	0.637	0.369	0.235	0.073	0.778	0.057	0.292

Appendix 14

Tissue Distribution of Co^{60} in Two Dogs Twelve Hours After Injection. The percentage recovery in extracellular fluid was computed from plasma activity as indicated in the text.

Tissues		Dog No. 22 Injected Intravenously			Dog No. 23 Injected Intraintestinally		
		Wet Weight	Amount of Co^{60} After Correction for Self Absorption	Conc. of Co^{60} per Wet Weight	Wet Weight	Amount of Co^{60} After Correction for Self Absorption	Conc. of Co^{60} per Wet Weight
		gm	ug	ug/gm	gm	ug	ug/gm
Blood				0.0101*			0.0042*
Plasma				0.0152*			0.0079*
Extracellular Fluid				5.34**			5.12**
Urinary Bladder Urine				0.0298*			0.0159*
Gall Bladder Bile				0.0554*			0.0088*
Liver		0.4276	0.1509	0.3529	0.4587	0.0386	0.0842
Right Kidney		0.5363	0.0204	0.0380	0.5683	0.0083	0.0146
Spleen		0.5262	0.0066	0.0125	0.5519	0.0018	0.0033
First Loop of Small Intestine	Wall	1.4140	0.0459	0.0327	1.5006	0.0262	0.0175
	Contents	2.5148	0.0804	0.0320	1.5036	0.0211	0.0140
Second Loop of Small Intestine	Wall	1.1943	0.0527	0.0441			49.6**
	Contents	2.8832	0.1447	0.0502			
Third Loop of Small Intestine	Wall	0.7669	0.0500	0.0652	1.0746	0.0118	0.0110
	Contents	0.7979	0.0847	0.1062	0.7637	0.0237	0.0310
Caecum	Wall	1.6855	0.0595	0.0353	1.4491	0.0241	0.0166
	Contents	0.9231	0.0311	0.0337	0.9725	0.0149	0.0153
Large Intestine	Wall	0.7848	0.0247	0.0315	0.9190	0.0063	0.0069
	Contents	0.5805	0.0144	0.0248	0.5273	0.0045	0.0085

* ug per ml

** Recovery of Co^{60} (per cent of the injected dose).