SOME ASPECTS OF IODINE METABOLISM

I. THE EFFECT OF PHYSIOLOGICAL SALINE AND DESOXYCORTICOSTERONE ACETATE ON THYROID I-131 UPTAKE IN RATS

II. THE METABOLISM OF I-131-LABELED THYROXINE IN NORMAL AND THYROIDECTOMIZED DOGS

By

Ellen St. John Monkus

AN ABSTRACT

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

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Experiments were conducted to determine the acute effect of desoxycorticosterone acetate (DCA) and physiological saline (0.9% NaCl) on the thyroid uptake of radioactive iodine. Concomitant plasma I-131 levels were measured to determine whether there was a positive correlation between plasma I-131 level and thyroid I-131 uptake. The three hour thyroid uptake of I-131 was increased in adrenalectomized rats after 1.25 mg. of DCA per 100 gm. body weight was given intraperitoneally immediately after intravenous injection of 20 uc. of I-131 with 8.5 ugm. of carrier I-127 as NaI. The plasma level and the urinary output of radioactivity were not changed by the treatment. The one and one-half hour and three hour uptake of I-131 in adrenalectomized rats were increased after 10 cc. of 0.9% NaCl per 100 gm. body weight was given under the same conditions. In this case the blood level of radioactivity was decreased and the urinary output of radioactivity was increased.

A study was made to measure the absolute thyroid circulation of the rat. The arterio-venous I-131 difference for the thyroid was found to be ten per cent of the arterial level under the conditions of the experiment. The estimated

absolute thyroid circulation of these rats was calculated to be 23 mg. of blood per mg. of gland per minute.

A comparison was made of the metabolism of I-131 labeled L-thyroxine in intact and thyroidectomized dogs in an attempt to determine the mechanism of the tolerance to exogenous thyroactive materials seen in normal human beings and intact dogs as compared to the athyreotic. A comparison of the rate of fall of radioactivity in the blood and the rate of its appearance in urine and feces after an intravenous injection of 60 ugm. of I-131 labeled L-thyroxine was made in normal and thyroidectomized dogs, which had been pretreated for ten days with 6 ugm. of non-radioactive thyroxine per day. No difference was seen in total fecal output of radioactivity at seven days after injection of the labeled thyroxine. Combined total thyroidal-urinary I-131 content of the intact dogs equaled urinary I-131 output of the thyroidectomized dogs at seven days. The plasma levels of radioactivity of both groups were described by the sum of three semi-logarithmic regression lines having half-times of 0.94, 8.7 and 69 hours in intact dogs and 0.94, 9.4 and 63 hours in thyroidectomized dogs. The second half-time of 8.7 hours found in intact dogs was statistically significantly less than the 9.4 hours of the thyroidectomized dogs by a "t" test. No difference between the groups was seen in the final blood levels and in the final rate of disappearance of the radioactivity.

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To Frank and John Monkus.

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GENERAL INTRODUCTION

More is known about the thyroid than about any other endocrine gland. Since the early clinical discoveries, investigations of thyroid physiology have passed through the important milestones of the first use of replacement therapy by Murray (1891), the discovery of the high iodine content of the gland by Baumann (1896), and the observation that thyroid feeding enhances metabolic rate by Magnus-Levy (1895). Following these studies came the first observation that injected iodine accumulates in the thyroid gland (Marine, 1915) and the discovery (Kendall, 1915) and synthesis (Harington and Barger, 1927) of the thyroid hormone thyroxine.

In the past two decades the newer methods of modern research have been focused on the thyroid gland. In this thesis two aspects of iodine metabolism have been studied using I-131; therefore, a summary of the metabolism of iodine is desirable in order to understand how the problems investigated fit into the total scheme of thyroid function.

Metabolic Circuit of Iodine.

A number of investigators, among them Brownell (1951), Albert (1952), Riggs (1952) and Gross and Pitt-Rivers (1953) have reviewed the various facets of iodine metabolism and their interaction with each other. Their conclusions can be outlined as follows:

Iodide Metabolism: The thyroid gland is capable of extracting iodide from the blood and maintaining a gland to

blood ratio of approximately twenty-five (in normal rats, Vanderlaan and Vanderlaan, 1947). It uses this iodide to make its hormone. If I-131 is injected intravenously, it is distributed into a volume which approximates the extracellular fluid space (Wallace and Brody, 1937). Besides this, it is rapidly taken up by the thyroid, and it is also excreted in the urine.

The mechanism by which the thyroid gland concentrates iodide is not known. It is believed that the iodide must be oxidized before it can be bound into organic form but free iodine has never been positively identified in the thyroid gland.

Intrathyroidal Metabolism of Iodine: After the iodide has been concentrated in the gland in inorganic form, it is oxidized and combined into a series of iodine containing amino acids. Two of these, diiodotyrosine and thyroxine, were identified chemically many years ago. Recently investigations using paper chromatography and radioautography have resulted in the discovery and identification of two other amino acids, monoiodotyrosine (Fink and Fink, 1948) and triiodothyronine (Gross, Leblond, Franklin and Quastel, 1950; Gross and Pitt-Rivers, 1952a).

The iodine containing amino acids in the thyroid gland are chiefly found in a protein, thyroglobulin, but recently small amounts of them have also been located in free form (Gross, Leblond, Franklin, and Quastel, 1950).

It is believed that the amino acid moiety of these compounds is already in peptide linkage at the time of its iodination. The iodination of tyrosine occurs quite readily; diiodotyrosine can be formed from tyrosine and iodine in vitro at approximately body pH. Then the formation of thyroxine is believed to occur from two molecules of diiodotyrosine. This is thought to be an oxidative process, but the enzyme systems involved have not been definitely determined.

Extrathyroidal Metabolism of Organic Iodine: The thyroid hormone is released directly into the blood stream. In the adult animal the major effect of the hormone is the maintenance of the normal basal metabolic rate. It also has a role in growth and development.

The major iodine containing amino acid found in the blood is thyroxine. Until recently it was believed to be the active form of the thyroid hormone. Small quantities of other iodine containing amino acids including triiodothyrone have been found in the blood by the use of paper chromatography and radioautography. Triiodothyronine has been found to be more potent than thyroxine by several assay techniques (Gross and Pitt-Rivers, 1952b; Gross, Pitt-Rivers and Trotter, 1952). The problem of what is the active form of the thyroid hormone is now being reconsidered, and a final decision in regard to it has not yet been made.

The plasma organic iodine is not dialysable and is loosely bound to one of the plasma proteins. Investigation by the method of paper electrophoresis (Gordon, Gross, O'Conner and

Pitt-Rivers, 1952) showed that organically bound I-131 travels with one of the α-globulins.

The thyroid hormone is de-iodinated in the process of exerting its hormonal effect. The resulting iodide is either reused by the thyroid gland or excreted.

Control of Thyroid Secretion: The levels of these different substances and the rates of their conversion from one to another are gauged to supply an adequate amount of hormone to the animal. The rate at which hormone is released from the thyroid is controlled by the anterior pituitary. The pituitary produces thyroid stimulating hormone (TSH) which controls both the rate of release and the rate of manufacture of thyroid hormone. A reciprocal relationship has been shown to exist between the thyroid and the pituitary. If the blood level of thyroid hormone falls, the pituitary is stimulated to produce more TSH which in turn stimulates the thyroid to produce more hormone.

The mechanism by which the level of thyroid hormone in the blood controls the rate of TSH secretion is not understood nor has the precise timing involved been investigated.

Thus it can be seen that the basic facts regarding what happens to iodine in the body have been established. The major forms it takes are known. Nevertheless, there are many questions which have not been answered. Continual investigations are being made in search of a deeper understanding of the function of the thyroid gland in the manufacture, storage, and release of the thyroid hormone and the function of the

thyroid hormone in growth and development and in the control of metabolic rate.

The work reported in this thesis concerns two aspects of iodine metabolism which are discussed in the following sections.

The Effect of Physiological Saline and Desoxycorticosterone Acetate on Thyroid I-131 Uptake in Rats.

Although the thyroid gland has the triple function of manufacture, storage and release of its hormone in response to the demands of the whole animal, its most unique property is its ability to concentrate iodine.

Since the first use of radioactive iodine in the study of thyroid physiology by Hertz, Roberts and Evans in 1938, many studies have been made of the thyroid uptake of radioactivity, chiefly with I-131, and the effect of different agents and procedures upon it. Thyroid I-131 uptake is one expression of the gland's capacity to manufacture its hormone. Thyroid uptake is controlled at least in part by the anterior pituitary since TSH increases thyroid uptake (Keating, Rawson, Peacock and Evans, 1945) and hypophysectomy brings about a sharp decrease in it (Leblond, Sue and Chamorro, 1940). Certain chemical substances are also believed to affect the thyroid gland directly to alter its uptake of radioactivity. Most explanations of the effect of such agents upon thyroid uptake have been made by use of one of the two mechanisms just mentioned.

There are also, however, extrathyroidal mechanisms which theoretically could influence thyroid uptake. One of these is the level of radioactivity in the blood. Another is the thyroid circulation. Even though the functional status of the thyroid remained the same, a decreased thyroid I-131 uptake might occur under these circumstances. Although Albert, Tenney, and Ford (1952) suggested that decreased availability of I-131 to the thyroid gland due to decrease in blood level of I-131 might be a factor in the decreased thyroid uptake seen after cortisone, no study has been made of the effect that changes in blood level of radioactivity might in practice have on thyroid uptake.

Decreased levels of I-131 in the blood could arise either because of an increase in the urinary I-131 output or an increase in the volume of dilution of I-131 in the body without known effect on thyroid gland function. No studies have been made of the direct effect of changes in urinary I-131 clearance or volume of dilution of I-131 on thyroid uptake.

Iodide is known to distribute itself in a volume which is approximately equal to the extracellular fluid volume. For this reason a preparation was sought which would have either an expanded or a contracted extracellular fluid volume. It was decided to use physiological saline (0.9% NaCl) intraperitoneally in fairly large quantity to expand extracellular fluid volume. It was thought that this treatment would also partition an increased amount of I-131 to

the urine. These two factors should result in lowered plasma I-131 levels which might lead to decreased thyroid I-131 uptake.

Desoxycorticosterone acetate (DCA) was selected for study because it is a steroid hormone active in the maintenance of salt and water balance. Boatman et al. (1952) observed increased thyroid I-131 concentration ratios after chronic treatment of rats with DCA. They also found increased circulating levels of I-131 in their DCA treated animals and suggested that the increases in I-131 uptake seen were the result of increased availability of the isotope to the gland due to increased blood levels.

By determining I-131 uptake by the thyroids of normal rats and of rats after physiological saline and DCA, it was proposed to study the correlation between I-131 uptake by the thyroid and plasma levels of radioactivity.

The Metabolism of Thyroxine in Normal and Thyroidectomized Dogs.

The major circulating thyroactive substance is thyroxine (Taurog and Chaikoff, 1948; Laidlaw, 1949). Investigations in regard to its metabolism under different conditions are fundamental in elucidating the peripheral action of the thyroid hormone.

Clinicians have suspected for many years that there is a difference in the effect of exogenous thyroid materials in normal and athyreotic individuals (Means, 1937). This observation was carefully studied by Winkler and his co-workers

(1943) who found that myxedematous individuals could be maintained in good health and normal metabolic status for many years by a daily dose of one to three grains of U.S.P. thyroid. If more was given to them, they would develop excessive metabolic rates, cardiac symptoms, and nervousness. On the other hand, certain normal individuals could tolerate daily doses of from three to six grains without elevation of basal metabolic rate or symptoms of toxicity.

Danowski, Man and Winkler (1946) attempted to confirm this observation in dogs and to determine the mechanism of the tolerance in normal animals. They found no significant difference between normal and thyroidectomized dogs. On the other hand, Borgman (1949) saw distinct differences in the response of normal and thyroidectomized dogs to considerably smaller doses of exogenous thyroprotein or thyroid substance.

A study was set up to compare the metabolism of thyroxine in intact and thyroidectomized dogs. This was done by a comparison of the rate of fall of radioactivity in the blood and the rate of its appearance in urine and feces after intravenous injection of I-131 labeled L-thyroxine.

I-131 COUNTING METHODS

Radioactive I-131 was counted with an end-window (1.9 mg. per sq. cm.) Geiger-Muller tube mounted in a two-inch thick lead shield. The output of the tube was connected to the input of a laboratory scaler (Nuclear Instrument and Chemical Corporation, Model 163 or 165). Chiefly the beta component of the radioactivity was counted in these experiments since the G-M tube is a highly efficient detector of beta particles but an inefficient detector of gamma rays.

Sample Preparation.

Samples were prepared in round pyrex glass dishes (2.2 cm. inside diameter, 0.6 cm. deep). All samples were set up by weight using the gramatic balance. The samples were put into the weighed counting dish; dish plus sample was weighed; and the weight of the sample was obtained by difference. To all samples, with the exception of the thyroids, a few drops of a mixture consisting of 2.5% casein, 1% phenol, 1% $NaHSO_3$, and 1% KI were added as an empirical precaution against losses of iodide.

Plasmas, Whole Bloods, and Urines: Plasma, whole blood, and urine samples varied between 300 and 1000 mg. in wet weight. Weights between 300 and 600 mg. were preferred but the 600 mg. limit was exceeded in some cases in which it was known that the counting rate would probably be low.

It was found that, if a few drops of distilled water were added to the whole blood samples, the hemolysed samples

resulting had a tendency to dry into a flat layer instead of an uneven crust which cracked and shattered. It was also found that more uniform samples resulted if whole blood was dried at room temperature.

Thyroids: Rat thyroids were placed intact in the center of the counting dish. Dog thyroids were placed on the dish, snipped into small pieces with scissors, and distributed evenly.

Muscles: Rat muscle samples were prepared in a manner similar to that used for dog thyroids.

Feces: Dog feces were homogenized in a Waring Blendor with a minimum of water before an aliquot was removed for counting. Fecal samples varied from 300 to 1000 mg. In this case also a limit of 600 mg. was preferred but was exceeded where extremely low counts might have been anticipated.

All samples, with the exception noted, were placed in an oven at less than 80 degrees Centigrade until they appeared to be dry. They were never left there for a period of more than two hours. They were then stored, exposed to air in the laboratory, and were weighed and counted at a later time.

Counting Time.

Counting was done for varying lengths of time. The standards cited by Calvin et al. (1949) were used to determine the number of counts which must be recorded to

obtain a given statistical accuracy. Whenever it was feasible, counts were made to better than 5 per cent accuracy. Background counts were made at least daily and for sufficient time to give 5 per cent statistical accuracy to the longest count of the day.

Regardless of the counting level all counts were continued for at least 100 seconds.

Comparison with an Aliquot of Injected Dose.

Aliquots of the injection solution were set up with added plasma and treated in the same fashion as plasma samples. On the basis of these aliquots all counts were expressed in terms of per cent of injected dose. Some of the counts were calculated in terms of per cent of injected dose per mg. wet weight of the sample.

Counting Correction Factors.

All of the counting in these experiments was relative, i. e., in comparison with an aliquot of the dose prepared and counted under identical conditions. With two exceptions, which are discussed when they occurred, all counting was beta counting. Whitehouse and Putnam (1953) discuss the errors involved in this type of counting. They list the order in which corrections should be applied as follows:

(1) Losses of counts in the dead time of the counter

(2) Background rate

(3) Geometry factor

(4) Self-absorption and back-scattering.

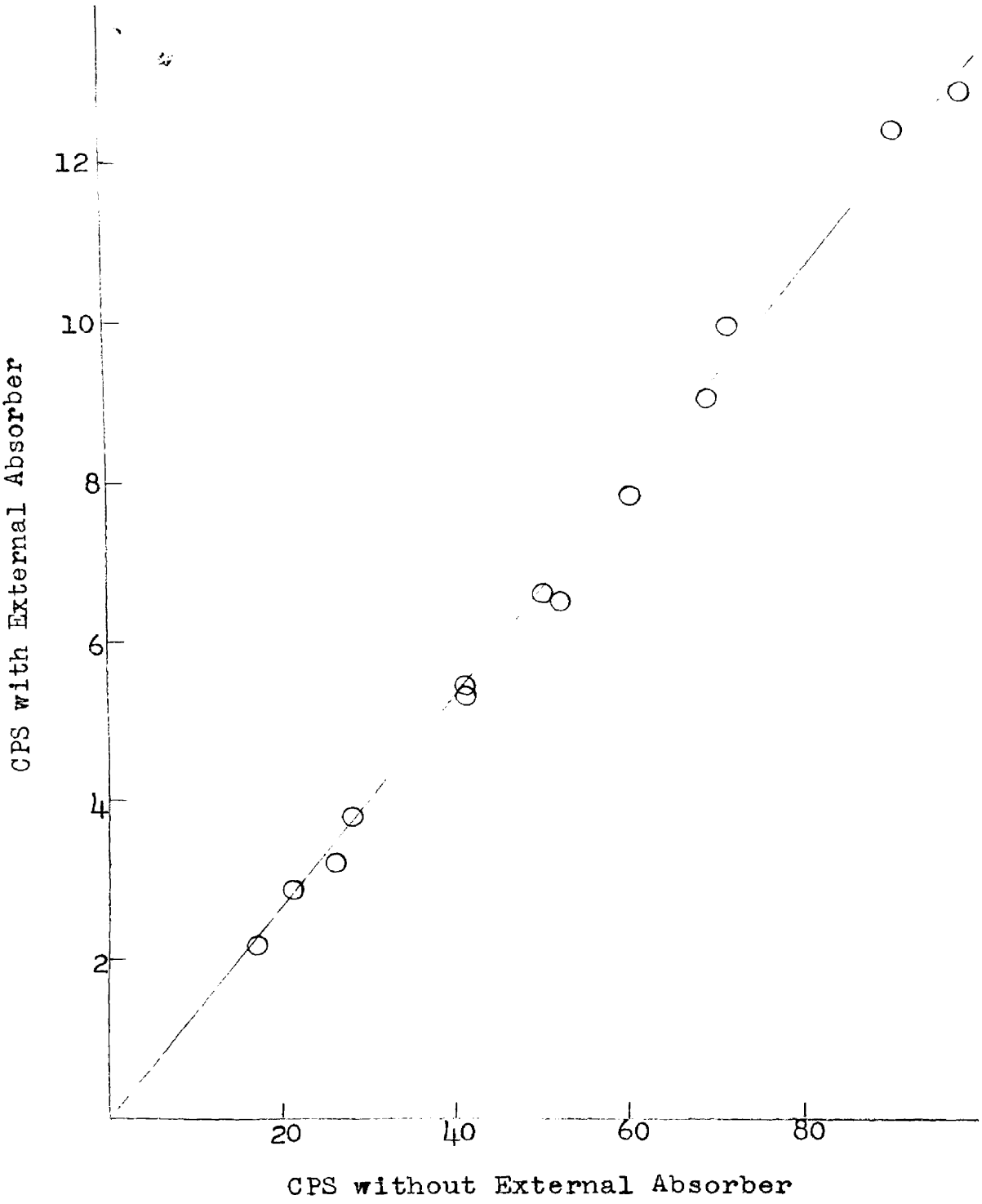
Each of these factors had to be evaluated under the conditions of this laboratory.

Losses of Counts in the Dead Time of the Counter: Counting was restricted to rates of less than 100 counts per second. It was thought that below these levels counting rates would be linear. Graph 1 shows that this prediction was correct.

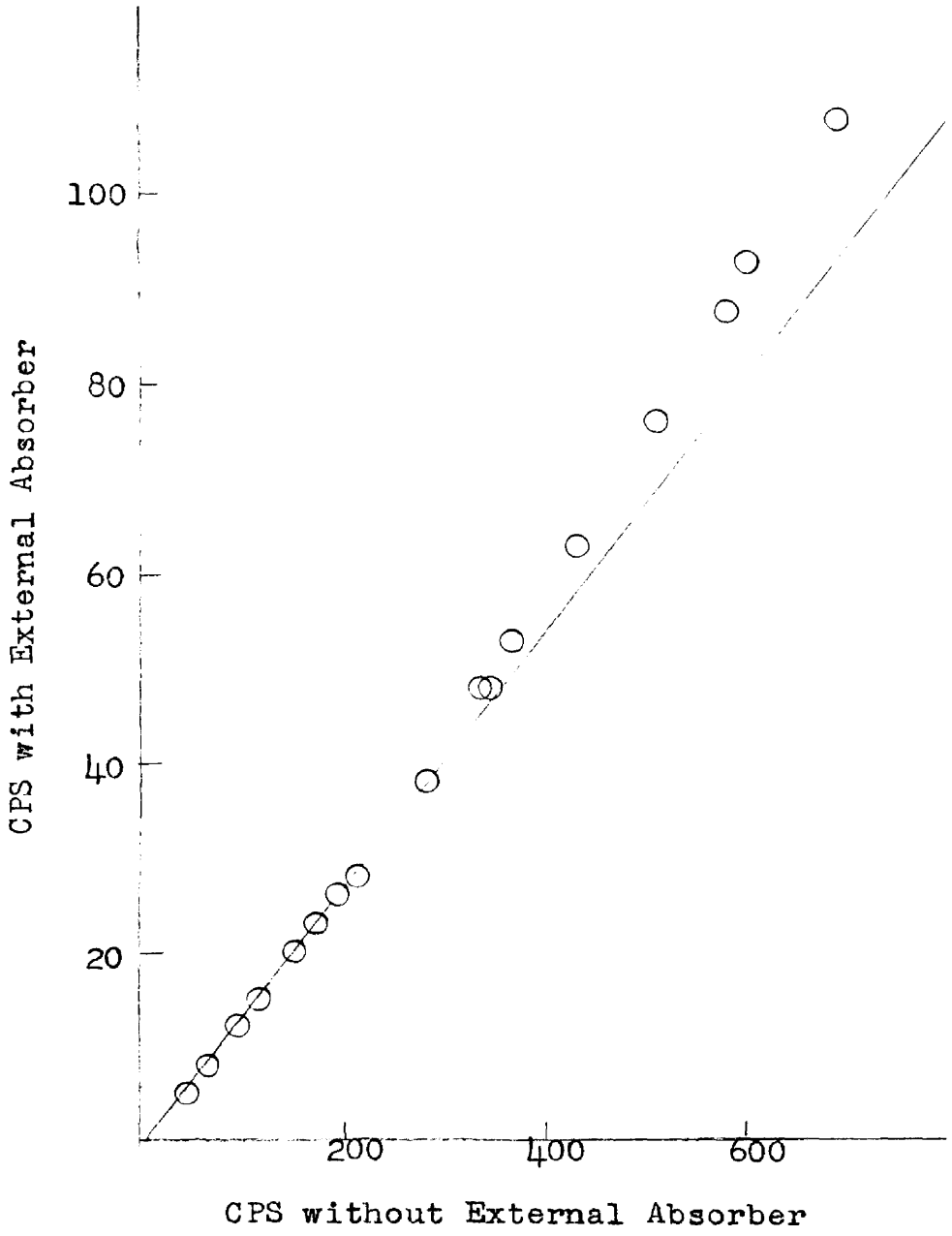
These data were collected by recounting six different rat thyroids over several half lives. Each count was done with and without an external absorber. The count with an external absorber was approximately one-eighth the count without it. (This was the only occasion on which external absorbers were used in these experiments.) Graph 2 shows the losses of counts which occur at high counting rates. Graph 1, however, shows that a restriction of counting to rates of less than 100 counts per second permits the assumption of a negligible loss of counts in the dead time of the counter.

Geometry: In these data all samples have been counted on the same shelf during any given experiment. This was either the second or the third shelf of the plastic sample rack within the lead shield. The first shelf was avoided because random distribution, either horizontal or vertical, of samples on the dish can introduce an excessive error in counting when the sample is placed relatively close to an end window G-M tube (Calvin et al., 1949).

Self-Absorption: Corrections for the self-absorption of beta particles were made according to the data given in



Graph 1: Linearity of counting up to 100 cps.

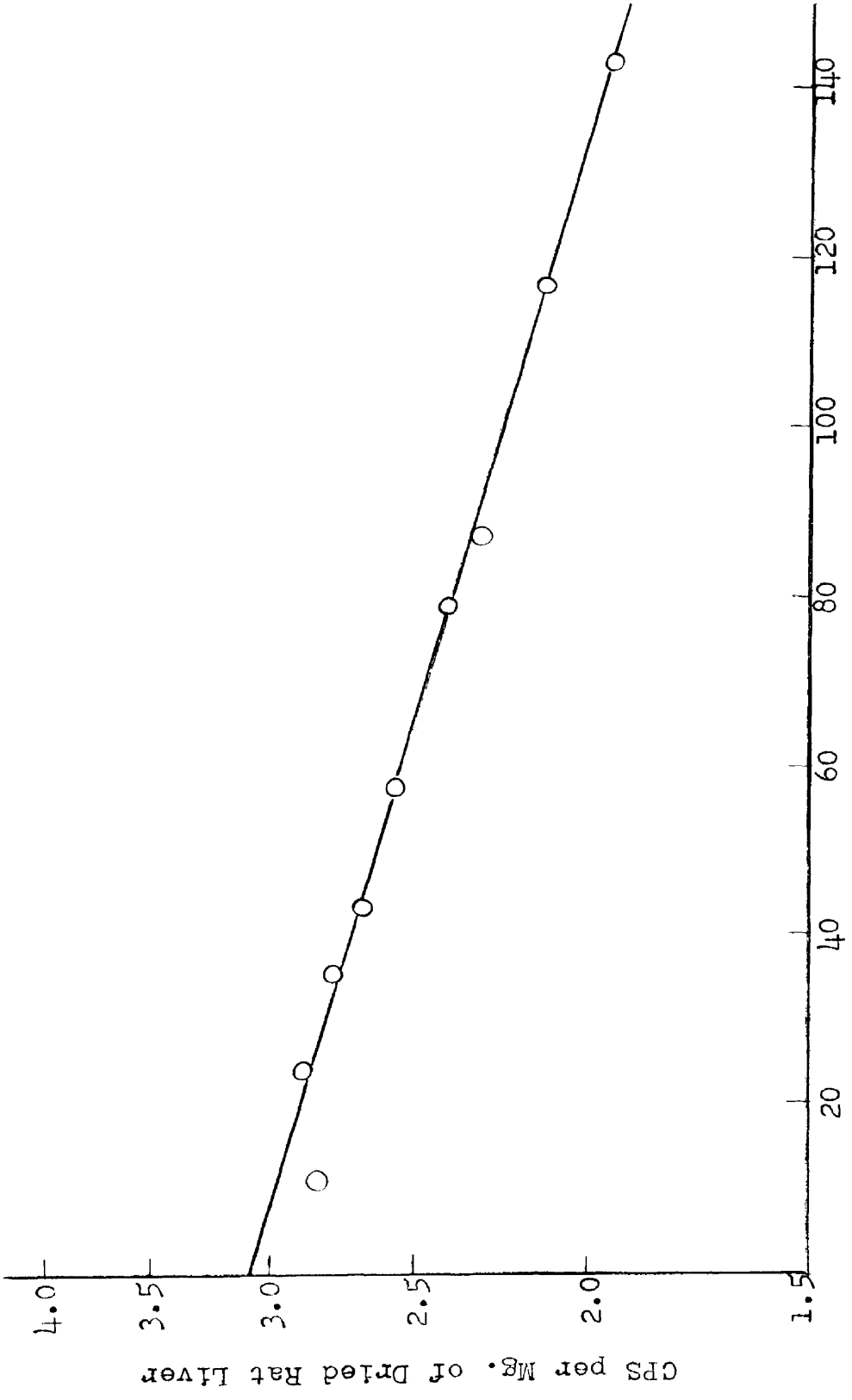


Graph 2: Loss of counts at high counting ratio.

table 2 of the appendix. These correction factors were calculated from a series of samples of different weights of rat liver labeled with I-131. The liver had been dried and powdered; the portion which would pass through an 180 mesh screen was used. Each of the points of the curve on which the self-absorption correction factors are based was established by a triplicate count of the sample, which was carefully stirred and redistributed between counts. The formula for the best semi-logarithmic straight line through the points between 40 and 140 mg. was used to set up the correction factor table. The method used to determine this line is discussed later in this thesis. Then samples for counting were restricted to 20 to 120 mg. dry weight; this was achieved by limiting the wet weight of samples.

Some of the dog fecal samples exceeded 120 mg. dry weight so correction factors based on the empirical data were added to include higher weights. All samples except these fecal ones fit the restrictions imposed by the theoretical curve. Table 2 in the appendix also gives the empirical correction factors used.

An idea of the accuracy of the self-absorption and backscattering corrections used was obtained when the laboratory acquired a scintillation counter. Gamma counts were made for a series of rat plasmas and thyroids which had already been counted with the G-M tube. The counts of these samples were rather low and it was not practical to attain better than 10 per cent counting accuracy in some cases; however, a study



Graph 3: I-131 self-absorption correction curve. Pyrex glass dishes 1.19 cm. in diameter. Log y = +0.5144 -0.00138x.

made of the comparative counts answered the following questions: (1) Was there an error introduced by the use of self-absorption corrections which showed a trend as sample thickness changed? This question arose because the dried plasma samples tended to stick to the walls of the dish and correction factors were based on ideally arranged rat liver powder. (2) Was there an error, which showed a trend with thyroid weight, introduced by not using self-absorption corrections for rat thyroids? Both of these questions can be answered in the negative.

Graphs 1 and 2 of the appendix show a plot of ratios of the individual beta counts to their respective gamma counts against dry weight of plasma and wet weights of thyroids (thyroid dry weights are too small to be measured accurately by the methods used). The beta counts were corrected for self-absorption before the ratios were calculated. Tables 1 and 2 show that there is no correlation between ratio of beta-to-gamma count and either plasma wet weight or thyroid dry weight. Since there is no error due to self-absorption of gamma rays at these thicknesses, this means that there is no trend in the counts of these samples with change in either plasma or thyroid weight.

Physical Decay: Corrections for physical decay of I-131 were made according to factors listed in table 1 in the appendix. Counts were corrected to a zero time which was arbitrarily established in each experiment. These correction factors have been calculated for an 8.00 day half life. The

most recent information found for the half life of I-131 was that of Lockett and Thomas (1953). They give 8.06±0.02 days. This means that the use of 8.00 days involves introduction of an error no greater than one per cent per half life.

The local counting methods then are such that the observed counts per second were corrected in the following order:

(1) The background rate was subtracted.

(2) The value obtained in (1) was multiplied by the proper correction for physical decay from table 1 in the appendix.

(3) The value obtained in (2) was multiplied by the proper correction for self-absorption from table 2 in the appendix. Rat thyroids were not corrected for self-absorption.

(4) The value obtained in (3) was calculated in terms of per cent of dose.

THE EFFECT OF PHYSIOLOGICAL SALINE AND DESOXYCORTICOSTERONE ACETATE ON THYROID I-131 UPTAKE IN RATS

Introduction.

Plasma levels of radioactivity may be one of the factors which influence the uptake of I-131 by the thyroid gland. The purpose of this study was to evaluate the effect of plasma levels of radioactivity on thyroid uptake in rats which had been given excess physiological saline intraperitoneally and in rats which had received desoxycorticosterone acetate (DCA).

Physiological Saline: No information was found regarding the effect of a large intraperitoneal dose of physiological saline on thyroid I-131 uptake.

Storlaasi, Rosenberg and Friedell (1953) however considered a related problem when they investigated the effect of food and water on thyroid uptake of radioiodine. Their rats had been maintained on iodine deficient diet and distilled water for four weeks. They measured six and twenty-four hour uptake and excretion of a tracer dose of I-131 under four different conditions: (1) no food and water for 36 hours before and after administration of I-131, (2) no food and water after administration of I-131, (3) only water ad libitum, and (4) food and water ad libitum. They used small numbers of animals (three per group). They found that food and water or even water alone tended to increase urinary output and to decrease thyroid uptake at both six and twenty-four hours.

Desoxycorticosterone Acetate: The first study made regarding the effect of DCA on thyroid uptake of I-131 was that of Paschkis et al. (1950). Their primary aim was the study of thyroid function in the "alarm reaction," but they included a group of rats which had been pretreated with 40 mg. of DCA at forty-eight and twenty-four hours. In their experiment DCA failed to influence significantly the four hour uptake of a tracer dose of I-131 in adult male rats on a normal diet.

Money and his associates studied the effects of several adrenal and gonadal steroids on the twenty-four hour uptake of a carrier-free dose of I-131 in 250 to 300 gm. male rats which had been on an iodine deficient diet for twenty days. They injected 1.0 mg. (1950) or 15 mg. (1951) of DCA per rat per day for ten days. The rats had increased thyroid gland weights and body weights at both DCA levels. They found that 1.0 mg. had no significant effect on thyroid I-131 uptake expressed as per cent of dose per gland or per cent of dose per mg. gland per 100 gm. body weight. They showed however that, although thyroid uptake in terms of per cent of dose per gland was not altered, a significant decrease in thyroid uptake expressed as per cent of dose per mg. gland per 100 gm. body weight was seen after the 15 mg. dose of DCA.

Boatman et al. (1952) found increased concentration ratios (which they define as per cent of administered activity found in an organ divided by the organ's per cent of the body weight) in DCA treated rats three hours after

the injection of carrier-free I-131. They used approximately 200 gm. rats, half of which had been hemithyroidectomized. The experimental rats were given one mg. DCA intramuscularly in oil three times a week for a period of four weeks. Intact and hemithyroidectomized rats which had received DCA each showed increased thyroidal concentration ratios when compared with untreated animals of the same type. They found the whole blood, plasma, and erythrocyte content of I-131 to be about thirty per cent higher in the DCA treated animals than in the controls and hypothesized that the increased concentration ratios found in the thyroids of these animals could be a reflection of the increased circulating levels of radioactivity.

In 1952, Gabrilove, Dorrance and Soffer found that goitrogen-treated rats which had received one mg. of DCA per day for a period of either twelve or twenty-two days showed no difference in thyroid weight as compared to control rats.

Zingg and Perry (1953) studied the effect of DCA on thyroid I-131 uptake and some other indicators of iodine metabolism in patients in the psychopathic ward of the Winnipeg General Hospital. Each individual served as his own control. They found decreased thyroid I-131 uptake, two, four, twenty-six, and fifty hours after oral carrier-free I-131, after three days treatment with ten mg. of DCA daily in two five mg. doses twelve hours apart. Treatment was continued during uptake measurements. The uptake of I-127 by the thyroid gland was also significantly depressed.

Plasma iodide-127 and renal I-131 clearance were not significantly altered by the DCA.

No information was found in regard to the effect of intraperitoneal 0.9% NaCl on thyroid I-131 uptake. Money et al. (1951) reported a decreased per cent of dose of I-131 per mg. thyroid gland per 100 gm. body weight in rats after chronic treatment with DCA. A depressed thyroid uptake of I-131 after DCA treatment likewise was revealed in human beings by Zingg and Perry (1953). On the other hand, Boatman et al. (1952) found increased thyroid I-131 concentration ratios, along with increased plasma and blood levels of radioactivity, after chronic treatment of rats with DCA.

Materials and Methods.

Several groups of male albino rats from Carworth Farms, New City, New York, were used in this study. Experiments 1 and 2 were preliminary experiments; experiments 3 and 4 were the main ones. Experiment 5 was exploratory towards a method for explaining the results found in 3 and 4.

Diet: In experiments 1, 2, and 5, the animals were maintained on a ration consisting of:

Yellow Corn Meal	35%
Ground Whole Wheat	25%
Whole Milk Powder	20%
Linseed Oil Meal	10%
Alfalfa Leaf Meal	6%
Brewer's Yeast	3%
Table Salt (Iodized)	1%

In experiments 3 and 4, the animals were given an iodine deficient diet (Remington, 1937) for ten days prior to the experiment, "Iodine Deficient Diet," General Biochemicals Inc., Chagrin Falls, Ohio, consisting of:

Yellow Corn Meal (grown in iodine deficient area)	78%
Wheat Gluten	18%
Brewer's Yeast	2%
Calcium Carbonate	1%
Sodium Chloride	1%

All animals had tap water to drink.

Food was removed from four to six hours before the injection of I-131. The rats had free access to tap water up until the time of I-131 injection. From then until the time of sacrifice one and one-half to three hours later, they had neither food nor water available. Although starving the animals overnight before performing the experiments would have been desirable, it was found that a large percentage of the adrenalectomized animals passed into a coma if deprived of food for such a long period of time.

Body Weights: The rats of the first four experiments, as can be seen from table 3, were of approximately the same weight. In each of these experiments the rats were carefully matched for weight. The experiments were conducted so that animals were injected in the following order: animal No. 1, group 1; animal No. 1, group 2; animal No. 1, group 3; etc., throughout the total number of groups; then,

animal No. 2, group 1; animal No. 2, group 2; etc. . . . . All of the animals numbered 1 were matched for weight and so were all the animals numbered 2, 3, etc. Running totals of the weight sums were kept for each group and animals were assigned to the different groups so as to keep the sums as close to each other as possible.

The body weights of rats in experiment 5 are to be found in the appendix, tables 3 and 4.

Injection of I-131: A dose of approximately twenty uc. of I-131 per rat was injected intravenously. An incision about two cm. long was made under light ether anesthesia on the medial side of the thigh; the I-131 was injected into the femoral vein; and the incision was closed with a metal skin clip. Injections were made in a total volume of one-half cc. per rat.

In experiments 1 and 2, the I-131 was carrier-free. In experiments 3, 4, and 5, the I-131 was prepared so that each rat received 8.5 ugm. of carrier I-127 in the form of NaI.

I-131 injections were made in the order described under body weights. The conditions of these experiments made it necessary to inject the animals over a period of ten to fifteen hours. The order of injections used would have minimized the effect of any diurnal variation in thyroid uptake. Dougherty, Gross and Leblond (1951) found no differences in four hour I-131 uptake by the thyroids of rats, both on normal and iodine deficient diet, injected at 1 p.m., 5 p.m., 9 p.m., 1 a.m., 5 a.m., and 9 a.m.

Injection of 0.9% NaCl and DCA: Saline, warmed to approximate body temperature, was injected intraperitoneally. DCA was administered intraperitoneally as a suspension in corn oil. DCA injections were 0.2 cc. per rat in experiments 1 and 2 and 0.1 cc. per 100 gm. body weight in experiments 3 and 4. The injections were given as soon as possible after the I-131.

Adrenalectomy: Adrenalectomies were performed by a dorsal approach from eight to ten days before the experiment. Animals were maintained on 0.25 mg. DCA given subcutaneously in solution in corn oil in a single daily dose. They had tap water to drink.

All animals were examined grossly for the presence of adrenal tissue at the time of autopsy. Thanks is due to Dr. Joseph Meites for assistance with part of these examinations. The few animals in which adrenal tissue was found were discarded.

Samples for Counting: Blood was obtained by two methods. In experiments 1, 2, and 5, the rats were anesthetized with ether and blood was removed from the abdominal aorta with a syringe. The animals were sacrificed immediately by opening the chest cavity and cutting the heart. In experiments 3 and 4, in which larger numbers of animals were used, the rats were sacrificed by a guillotine method and freely flowing blood was collected in a small beaker. Heparin was used as an anticoagulant.

Thyroid glands were dissected free of visible fat and connective tissue and weighed on a 125 mg. capacity Roller-Smith balance. Wollman and Scow (1953) found a progressive loss of radioactivity from the thyroid glands of thiouracil treated mice between the time of death and the time of removal of the thyroid glands five to eighty minutes later. If such effects occur in these normal animals in which organic binding of the iodine occurs, they should have been equalized among the groups by the method of handling used. As soon as possible after sacrifice, the animals were placed in the deep freeze. They were removed at a later time and were autopsied as soon as possible in the same order in which they had been injected.

Muscle samples were taken from the thigh opposite to the injection site, cleaned of visible fat, connective tissue, blood vessels and nerves and weighed on a 100 mg. capacity Roller-Smith balance. Samples of 400 through 600 mg. were used.

It was not thought to be possible to collect accurate urine samples for only one and one-half hours without special arrangements. In experiment 3, which lasted three hours, the animals were placed in metabolism cages by groups and the total urinary radioisotope output of the group measured. In experiment 4 an attempt was made to collect urine from the rats individually at one and one-half hours by placing a purse string ligature on the penis (Friedman and Livingstone, 1942) and removing the bladder intact while the rat was still

frozen. A few trial rats done by this procedure were successful. However, when this method was used in experiment 4, some of the rats, even those which had received saline, were found to have empty bladders at the time of autopsy. Therefore, these data have been used only as an indication of the maximum amount of urinary radioactivity to be found at one and one-half hours.

Special Conditions and Methods of Experiment 5: The last experiment of this group was conducted in rats which were considerably larger, and therefore older, than those of the other four experiments. The rats were anesthetized with nembutal in contrast to the other experiments in which rats were conscious during the uptake period aside from the short period of light ether anesthesia necessary for intravenous injection. These were intact, not adrenalectomized, rats.

Just prior to sacrifice, blood was taken from one of the thyroid veins. The rat has a vein leading from the posterior end of the thyroid gland along the side of the trachea. The sharp angle at which the chest rises and the small size of the vein make it impossible to draw blood from it with a hypodermic needle and syringe. It is possible to expose this vein at a distance of perhaps one cm. posterior to the gland, cut the vein with a small pair of scissors, and collect around 0.2 ml. of blood as it flows from the vein in a syringe. This procedure results in sufficient blood for analysis in approximately two-thirds of the rats. About one cm. length of the vein was exposed and cleaned

free of fascia; the vein was then tied off at the posterior end of the exposed portion and cut anterior to the suture. A detailed report of the gross anatomy of the circulation of the rat thyroid gland has not been found. The thyroidal-tracheal area of several rat necks has been examined with a hand lens by Dr. E. P. Reineke and the author. All gross appearances lead to the conclusion that the vein from which blood was taken drains the thyroid gland. If it drains any other sites, it is probably only the small amount of fat attached posterior to the gland.

In these rats arterial blood was removed from the abdominal aorta after the thyroid blood. In some of them general systemic venous blood was also removed from the vena cava. The arterial and thyroid venous bloods were counted under the same conditions in order to obtain the thyroid I-131 arteriovenous difference.

Statistics: Comparisons between the groups in these experiments were made by Analyses of Variances. A probability of less than 0.05 was accepted as being statistically significant.

Experimental and Results.

The aim in this study was to perform the experiments under such conditions that the amount of I-131 found in the thyroid gland would represent as nearly as possible the amount of radioactivity that the gland had extracted from the blood up to the time of sacrifice. The per cent of I-131 found in the thyroid gland, called "uptake" here, is actually

the difference between the per cent of I-131 extracted from the blood and the per cent of I-131 returned to the blood.

Since the release of I-131-labeled hormone is a slow process relative to the rate of extraction of I-131 from the blood, the shorter the time period chosen, the more nearly the measured "uptake" represents amount extracted from the blood. Albert (1951) found a mean exponential rate of disappearance of thyroidal I-131 in rats, between 50 and 165 hours after injection of I-131, of 28 per cent per day. This would represent a loss of only 5 per cent for three hours. In these experiments there is no evidence whether or not the rate of labeled hormone release had been changed by the treatments used; in any case a rate double that found by Albert would increase the output to only 8.5 per cent for three hours. Thus it was felt that any reasonable change of output would not quantitatively effect the per cent of dose in the gland if uptake was measured at three hours or less.

It was also desired to have the same amount of I-127 labeled in the different groups. Without attendant measurements of I-127, a decrease in uptake of I-131 does not necessarily mean that a decreased amount of iodine has been concentrated - it might mean only that the I-131 was diluted by more I-127. Perry and Hughes (1952) found such a mechanism occurring in renal disease in humans. In a study in which uptake of a carrier-free dose of I-131 is measured in animals which have been treated chronically with a hormone which is known to have effects on salt and water balance,

there is no guarantee that this situation, or its reverse, has not arisen. It was therefore decided to give the saline and DCA after I-131 was administered.

Another condition of these experiments was the use of adrenalectomized animals. As has been mentioned, intact animals show decreased thyroid I-131 uptake after various stresses, e. g. formalin injections (Williams, Jaffe and Kemp, 1949), anoxia and trauma (Van Middlesworth and Berry, 1951), and tourniquet shock (Hamolsky, Gierlach and Jensen, 1951), and after ACTH and cortisone (Perry, 1951; Soffer, Gabrilove, and Dorrance, 1951). It was not known how much of a stress the etherization and intravenous injections constituted. Neither was it known how great a stress the intraperitoneal physiological saline would be. It was also not known what effect on the adrenal-pituitary axis would result from the DCA injection. In order to eliminate the need to evaluate the importance of these parameters in the I-131 uptake picture, it was decided to perform the experiments in adrenalectomized animals.

Table 1 presents the thyroid I-131 uptakes and plasma I-131 levels of the rats in experiments 1, 2, 3, and 4.

The observations of experiment 5 are reported in tables 3, 4, and 5 of the appendix.

Experiments 1 and 2: In these two experiments the one and one-half hour thyroid I-131 uptake after a carrier-free intravenous dose of radioactive iodine was measured in adrenalectomized rats which had been fed the standard laboratory

Table 1: Thyroid and plasma I-131 levels in rats treated with physiological saline and desoxycorticosterone acetate. Experiments 1, 2, and 4 at one and one-half hours and experiment 3 at three hours. All rats except groups 4, 5 and 6 of experiment 4 have been adrenalectomized.

Group	Treatment	No. of Animals	Mean Body Weight gm. (range)
Experiment 1			
1	Control	7	154(132-168)
2	10 cc. 0.9% NaCl	7	149(134-164)
Experiment 2			
1	Control	9	149(136-169)
2	0.1 mg. DCA	9	152(132-171)
3	0.5 mg. DCA	8	149(132-162)
4	2.5 mg. DCA	10	153(134-167)
Experiment 3			
1	Control	10	161(138-174)
2	10 cc. 0.9% NaCl/100 gm.	10	164(128-182)
3	5 cc. 0.9% NaCl/100 gm.	10	163(132-202)
4	1.25 mg. DCA/100 gm.	10	162(141-193)
Experiment 4			
1	Control	21	123(114-140)
2	1.5 mg. DCA/100 gm.	20	120(106-142)
3	10 cc. NaCl/100 gm.	20	120(106-138)
4	Control	21	133(112-152)
5	1.5 mg. DCA/100 gm.	20	129(116-146)
6	10 cc. NaCl/100 gm.	19	131(116-158)

*Indicates significantly different from mean of controls $P < 5\%$.

1. Muscle iodide space in $\% = \frac{\% \text{ dose/mg. muscle}}{\% \text{ dose/mg. plasma}} \times 89.2$ (Hastings & Eichelberger, 1937)
2. A value of 56.1% has been omitted. No statistics were done for muscle space in these rats.

Mean Thyroid Weight mg.	Muscle I-131 Space[1] %	Urine I-131 % of Dose Per Rat	Plasma I-131 % of Dose/mg. x 10^{-3}	Thyroid I-131 % of Dose
Experiment 1				
13.7	21.5[2]		1.09	1.88
14.3	23.9		0.854*	1.66
Experiment 2				
14.1	25.0		1.10	1.37
14.1	28.4		1.08	1.42
12.0	29.7		1.10	1.21
12.2	31.4*		1.03	1.13
Experiment 3				
19.4	19.3	10.0	0.747	5.61
18.3	24.2*	19.4	0.448*	9.60*
19.7	24.4*	22.0	0.515*	7.64
17.4	19.4	9.5	0.685	8.78*
Experiment 4				
12.3			1.20	0.66
11.5			1.16	0.65
12.1			0.907*	0.95*
12.9			1.11	0.46
11.7			0.986	0.45
12.1			0.849*	0.55

ration prior to the experiment. Plasma I-131, muscle I-131, and the I-131 level in the part of the saline infusion which was still present intraperitoneally were measured.

In experiment 1, the rats received 10 cc. total of physiological saline intraperitoneally just after intravenous I-131. Plasma I-131 was significantly decreased in these animals as compared to the controls. The saline remaining intraperitoneally appeared to be equilibrated with the plasma; I-131 levels in it averaged 95 per cent of the plasma level, with a range of 68.5 to 120.2 per cent. The thyroid uptake of I-131 was not significantly altered in the saline-treated animals.

In experiment 2, there were four groups of rats, a control group and groups receiving 0.1, 0.5, and 2.5 mg. total of DCA. None of the groups receiving DCA showed any significant difference in plasma I-131 level when compared to the controls. The thyroid uptake of I-131 was not significantly altered in any of the DCA-treated animals. There was no indication that the smaller doses of DCA showed any effect which was qualitatively different than the largest dose.

Considerable thought regarding the situation in these rats lead to the conclusion that if rats were put on an iodine deficient diet for a short period of time in order to decrease their body stores of iodine and then if the I-131 was given with some I-127 as carrier, the desired condition of labeling the same amount of I-127 in each animal would more nearly be attained. An amount of carrier was used which

was less than the 10 ugm. which Wolff and Chaikoff (1948) found to show no signs of depressing thyroidal organic binding of iodine. Since these experiments have been performed, Halmi (1954) found that "Low Iodine Test Diet," made by Nutritional Biochemicals Corporation by the same formula as the iodine deficient diet used here, had no stimulating effect on thyroid morphology in rats which had been eating it for a period of 19 to 20 days. The rats used in experiments 3 and 4 were placed on the iodine deficient diet for a period of 9 to 10 days; they received 8.5 ugm. of carrier iodide.

It was concluded that perhaps more striking differences might be seen at three hours instead of one and one-half without a great sacrifice of the desired experimental conditions. It was anticipated that the data from this experiment would permit determination of whether or not a correlation between thyroid uptake and blood I-131 levels occurred in saline-treated and normal rats. Two doses of saline were chosen so that, between saline-treated and normal rats, a variety of blood levels would be attained.

It was decided that it would be more acceptable to dose by weight rather than to give each animal a predetermined amount. Five and 10 cc. of saline per hundred grams body weight were decided upon; one of these is larger and the other smaller in amount than the dose of experiment 1 of 10 cc. per rat, equivalent to an average of 6.7 cc. per 100 gm. body weight. A dose of 1.25 mg. of DCA per 100 gm. body

weight was chosen; this was slightly less than the largest dose of experiment 3 which averaged 1.6 mg. per 100 gm. body weight.

Experiment 3: The three hour thyroid uptake of I-131, injected with 8.5 ugm. of I-127 as NaI, was measured in adrenalectomized rats which had been on an iodine deficient diet for nine days. Plasma I-131 and muscle I-131 were also measured.

Both 10 cc. of 0.9% NaCl and 1.25 mg. of DCA per 100 gm. body weight produced a significant increase in thyroid uptake at three hours under the conditions of this experiment. In the case of the saline, the plasma I-131 level was decreased; in the case of DCA, no change in plasma level was seen. Five cc. 0.9% NaCl per 100 gm. produced no significant alteration in either uptake or plasma level. The pooled urinary output data suggest that there was an increased urinary output of radioactivity in the saline treated animals as compared to the controls. This effect was not seen with DCA.

Experiment 4: Both adrenalectomized and intact rats were used in this experiment. As in experiment 3, the animals were placed on an iodine deficient diet for ten days and were given I-131 with 8.5 ugm. of I-127 carrier. Thyroid uptake, plasma level and urinary output of radioactivity were measured at one and one-half hours.

The maximum urinary output of radioactivity in these rats was 2.5 per cent of dose. This was determined by averaging the two highest values found in each group.

Adrenalectomized animals given 10 cc. of saline per 100 gm. body weight showed a statistically significantly increased thyroid I-131 uptake which was not demonstrable in intact animals. Both adrenalectomized and intact animals had significantly lower plasma I-131 levels than their respective controls.

Adrenalectomized animals given 1.5 mg. DCA per 100 gm. body weight showed no differences from their controls. Thyroid I-131 uptake and plasma I-131 levels in the two groups were the same. Similar results occurred when intact animals were compared with their controls. No differences in either thyroid uptake or plasma level of radioactivity were seen.

When all three groups of adrenalectomized animals were compared with the three intact groups, the adrenalectomized rats had a statistically significantly greater uptake of I-131 than the intact rats.

Experiment 5: The last of this series was a preliminary experiment. It points a way toward an attempt in a different

direction to determine the mechanism behind the results found in experiments 3 and 4. The observations of experiment 5 have been recorded in tables 3, 4, and 5 of the appendix.

The differences between this and the other four rat experiments have already been pointed out. In brief these were very large intact rats which had not been placed on iodine deficient diet but which had received the 8.5 ugm. of carrier I-127. The rats which received physiological saline were given 10 cc. of 0.9% NaCl per 100 gm. Blood levels, rather than plasma levels, of radioactivity were measured because insufficient thyroid blood was available conveniently to obtain a plasma sample.

These data also can be looked upon as a final control on the influence of circulating I-131 levels on thyroid uptake. Since plasma and blood I-131 levels would be very high early after an intravenous dose of I-131, this experiment was planned to ascertain the behavior of blood levels of radioactivity at early times. Examination of table 5 of the appendix shows that I-131 levels in the blood of normal and saline-treated rats tend to be the same at 6 and 30 minutes but between 60 and 135 minutes after I-131, the NaCl-treated rats fall on an average below the controls. Therefore -- however the increased thyroid I-131 uptake after saline in experiments 3 and 4 may be explained -- there is probably no reason to attribute it to increased blood I-131 levels at any time after I-131 injection.

Experiment 5 also afforded data to calculate a tentative figure for thyroid circulation. All available calculations of thyroid circulation have been based on the assumption of complete plasma clearance of I-131. There is also an implicit assumption that there is no return of radioactivity back to the blood during the time used for calculation. Jones (1945) found a minimum perfusion of the thyroid gland of the rat of three to ten volumes of blood per volume of gland per minute. Myant, Pochin and Goldie (1949) and Stanley (1949) concluded respectively that the minimum circulation of the thyroid gland of human subjects was 0.5 and 0.25 volumes of blood per volume of gland per minute.

Thyroid I-131 clearance can be calculated by the classical method used for kidney clearance.

If t = time in minutes

U = thyroid uptake of radioactivity, expressed as % of dose, between t_1 and t_2

B = average % of dose of I-131 per mg. of blood from t_1 to t_2

C = the clearance, the number of mg. of blood per minute from which I-131 has been completely extracted.

Then $$C = \frac{U}{B \times (t_2 - t_1)}$$

If it is assumed that the blood is completely cleared of I-131 in each passage through the gland, the clearance represents the amount of blood per minute which has passed through the gland between t_1 and t_2.

If the blood is not completely cleared of I-131, then it will require more blood to deliver to the gland the amount of radioactivity which it has collected between t_1 and t_2.

Let P = gland circulation expressed as mg. blood per mg. gland per minute.

w = gland weight in mg.

D = $\frac{A - TV}{A}$, where A = % of dose of I-131 in systemic arterial blood and TV = % of dose of I-131 in thyroid venous blood.

Then 1/D represents the number of mg. of blood which would have to pass through the gland in order for one mg. to be cleared completely of I-131, and

$$P = \frac{U}{Bx\ (t_2 - t_1)} \times \frac{1}{D} \times \frac{1}{w}$$

In experiment 5, circulation of the thyroid was calculated using the data of 5 and 30 minutes to determine thyroid I-131 accumulation and to calculate the average blood levels of radioactivity. A brief preliminary experiment had shown that arterial blood levels of radioactivity were falling so rapidly in the period about an hour after intravenous injection of I-131 that a variation of a minute or two in the time of collection of the thyroid venous and the systemic arterial blood might have meant that arterial blood levels had already fallen below the thyroid venous levels. For this reason thyroidal arteriovenous I-131 differences were determined between 58 and 133 minutes after I-131 injection.

For these rats thyroid circulation can be calculated using the following data:

U = 0.693% of dose, graph 4

B = 0.000514% of dose/mg. of blood, graph 5

w = 24.1 mg.

D = 0.10, table 2

$(t_{30} - t_6)$ = 24 minutes

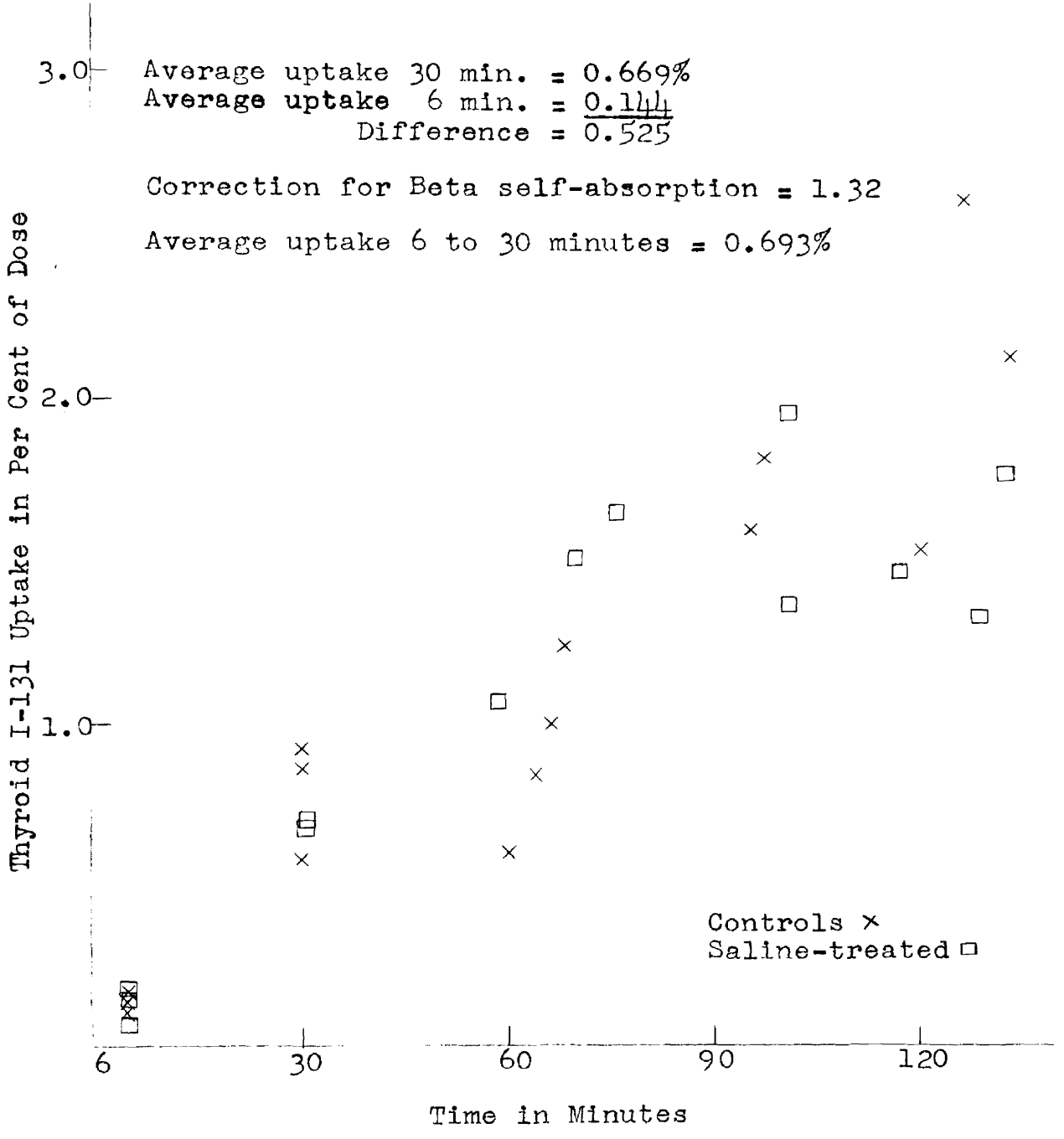
$$P = \frac{0.693}{0.000514 \times 24} \times \frac{1}{0.10} \times \frac{1}{24.1}$$

P = 23 mg. of blood/mg. of gland/minute

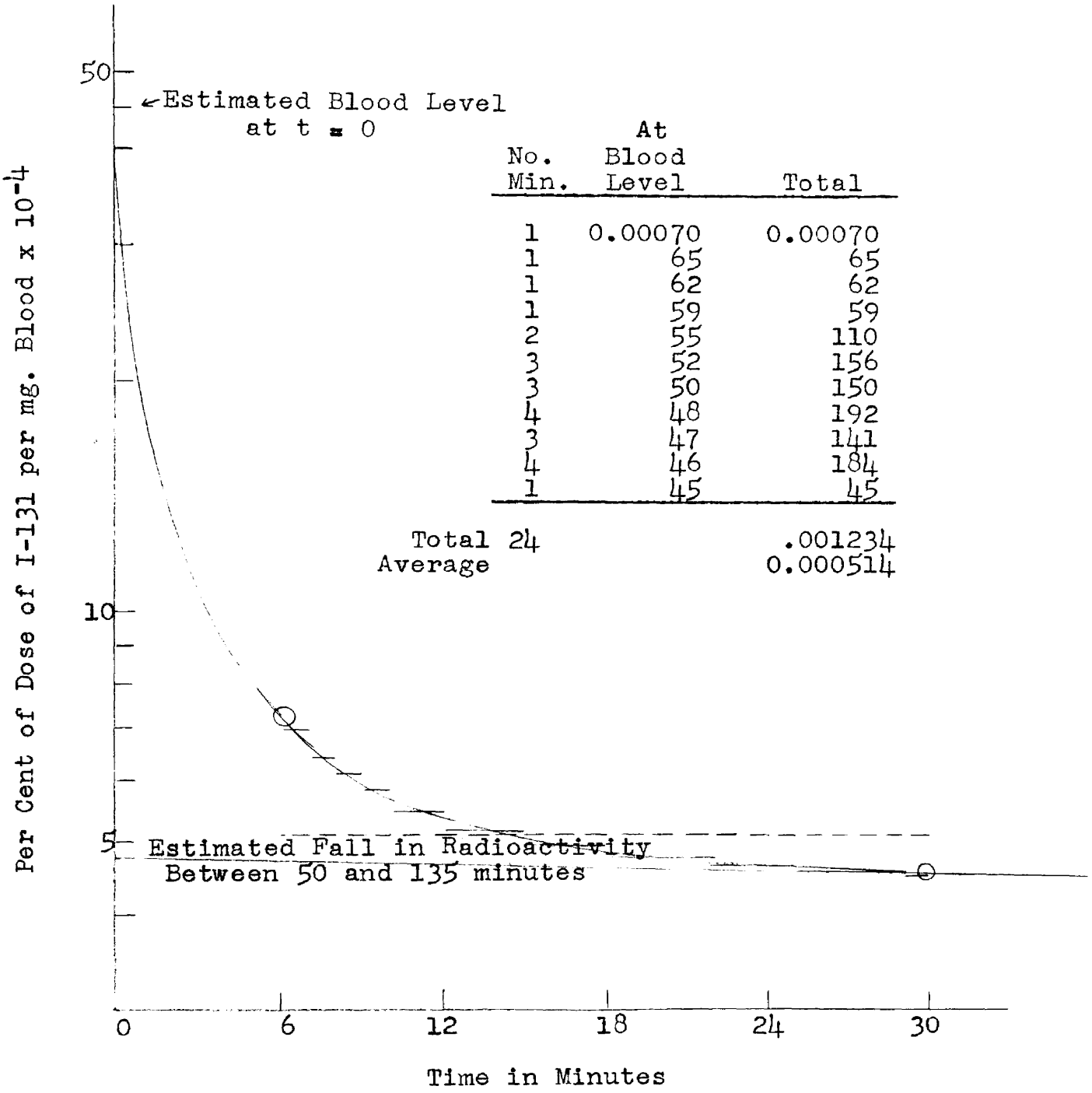
As far as the author knows, this is the first time that arterio-venous differences in I-131 blood level have been measured and hence is also the first time that an absolute figure for the blood circulation of the thyroid gland has been calculated.

Discussion of Results.

Under the conditions of experiments 3 and 4, intraperitoneal physiological saline produced an increased one and one-half and three hour thyroid I-131 uptake although at the same time there was a decreased circulating level of radioactivity in the blood plasma. There was probably also an increased partition of I-131 to the urine by three hours. In the same experiments, DCA produced an increased uptake of I-131 by the thyroid at three hours. No change in plasma I-131 level was found after DCA and there was no indication of a change in urinary I-131 output. It is necessary



Graph 4: Thyroid I-131 uptake in control and saline-treated rats. Experiment 5.



No. Min.	At Blood Level	Total
1	0.00070	0.00070
1	65	65
1	62	62
1	59	59
2	55	110
3	52	156
3	50	150
4	48	192
3	47	141
4	46	184
1	45	45
Total 24		.001234
Average		0.000514

Graph 5: Average blood level of radioactivity between 6 and 30 minutes. Experiment 5.

Table 2: Ratios of systemic venous to systemic arterial blood and of thyroid venous to systemic arterial blood in control and saline-treated rats, Experiment 5.

Rat No.	Systemic venous / Systemic arterial	Thyroid venous / Systemic arterial
7	0.91	0.88
8	1.07	1.03
14	--	0.78
15	1.00	1.03
16	0.95	0.92
7	1.05	0.89
8	0.98	0.91
13	--	0.71
14	--	0.85
16	0.99	0.97
Mean	0.993	0.897

therefore to look elsewhere than blood I-131 levels for an explanation of what has happened in the treated rats.

It cannot be inferred from these data that the mechanism of the changed uptake with saline is the same as that with DCA. There is not enough evidence to support or refute such a contention although it is extremely interesting that these agents seem to behave in the same way throughout this investigation.

By what mechanism then could these changes in uptake have come about? The factors which are thought to influence thyroid I-131 uptake have been discussed by several authors, notably Oddie (1949), Riggs (1952) and Wollman (1954). Thyroid uptake as measured in these experiments is actually the difference between the amount of I-131 which has been collected from the blood and the amount which has been returned to it. A very early uptake was measured in these experiments so that it can probably be stated that changes in radioactive hormone output have made a negligible change in measured I-131 uptake. The amount of I-131 collected from the blood as summarized by the foregoing authors is a function of the following factors: (1) the blood level of I-131, (2) the rate of blood flow through the gland, (3) the fraction of the blood iodide transferred to thyroid tissue as blood perfuses the gland, which in turn is the function of (a) the gland:blood ratio of I-131 in inorganic form and (b) the rate of organic binding of I-131.

The third of these parameters has been investigated very extensively. Both the gland:blood ratio of inorganic radioactive and the total thyroid I-131 uptake have been studied under many different conditions. As far as the author is aware, no critical study has been made of the effect changes of blood level of I-131 on thyroid uptake--with the exception of Albert and his co-workers' investigation of the effect of hypophysectomy on renal clearance of I-131 (Albert, Tenney and Lorenz, 1952a)--although either changes in urinary output of I-131 or changes in volume of dilution of iodide have been suggested as explanation for the differences in thyroid uptake seen after cortisone (Albert, Tenney and Ford, 1952). Likewise no study has been made of the effect of the rate of thyroid blood flow on thyroid uptake although it is obvious from gross examination that the hyperplastic, hypertrophied gland must have an increased blood supply.

These experiments were planned to examine the effect of blood radioactivity levels on thyroid I-131 uptake. It was hypothesized that the saline-treated animals would show decreased circulating I-131 levels and this expectation was realized. Along with the saline-treated animals was studied a group which had been treated acutely with DCA. Boatman et al. (1952) had found that chronic treatment with DCA increased thyroid uptake, expressed as per cent of administered activity found in the organ divided by the organ's per cent of body weight, with an accompanying increase in circulating I-131 levels.

Changes in thyroid uptake can arise either indirectly through changes in the anterior pituitary secretion of TSH or directly through an effect on the thyroid gland itself. Keating et al. (1945) found in chicks that no elevation in four hour thyroid uptake of I-131 was seen twenty-four hours after a single dose of TSH although mean acinar cell height and thyroid weight were already increased. Stanley and Astwood (1949) found no increase in accumulative gradient six to eight hours although an increased was noted at twenty-four hours after a single dose of TSH in human subjects. No information in rats regarding this point had been found; however, if data from these other species can be extrapolated to the rat, then the effects seen in these experiments occurred too soon after the treatments to have been mediated through the anterior pituitary.

Few studies on thyroid uptake have been made at early times after treatment with any chemical substance. It is believed that this is the first study in which thyroid I-131 uptake has been measured when the treatment with a steroid hormone was given after the radioactivity was administered.

The increased uptake after either saline or DCA cannot be explained in terms of increased blood levels of radioactivity. The saline-treated rats showed decreased blood levels. In the rats which were acutely treated with DCA, unlike the chronically treated ones of Boatman et al. (1952) which showed increased circulating I-131 levels, there was no change in blood levels of radioactivity relative to the controls.

A return to the factors influencing thyroid uptake leads to the question as to whether it might have been one of the other factors discussed which was changed in these rats. No information has been found regarding whether DCA and saline in doses similar to those used have any effect on the circulation in general or on thyroid circulation in particular. If it was not the gland circulation which has been affected, it may have been a change in the inorganic iodine uptake or in the rate of organic binding. The experiments conducted here involved the collateral information believed necessary to determine whether plasma levels of radioactivity influence thyroid uptake. The condition of the groups in respect to other factors can only be conjectured. No data are available on which to base speculation in regard to the mechanism of the change which occurred in the NaCl or DCA treated groups; nevertheless, the experiments were planned in such a fashion that it can be stated that those groups which showed an increased uptake of I-131 had collected a greater amount of iodine in their thyroid glands.

Is the situation which has been set up in these rats of sufficient interest physiologically to merit an extended study to determine the mechanism by which it comes about? The 0.9% NaCl was employed because it was believed that changes of blood I-131 level would be seen with it. It is extremely interesting that such a simple treatment as 10 cc. of physiological saline per 100 gm. body weight would affect thyroid I-131 uptake in experiments in which adrenalectomized

animals were used and stress, acting through the adrenals, was eliminated from consideration; however, whatever the unexplained mechanism of this change may have been, there is no reason at present to conclude that it is important in the normal physiology of the thyroid gland. On the other hand, steroid hormones similar to DCA are normally found in the body. Exhaustive studies on the effect of adrenal hormones of the type involved primarily in the regulation of carbohydrate metabolism, especially cortisone, on the thyroid gland have been made. Cortisone has been found by several authors to decrease thyroid I-131 uptake (Money et al. 1950; Perry, 1951) while having no effect on thyroidal secretion rate of labeled hormone (Albert, Tenney and Lorenz, 1952a). Hormones of the DCA type which influence salt and water metabolism have been little studied. Since the thyroid gland is concerned with concentrating an inorganic ion, iodide, the DCA type hormones could conceivably have a physiologic role in thyroid function. The observation of a decreased thyroid uptake after cortisone and the added information that DCA increased thyroid I-131 uptake when given either chronically or acutely suggest the possibility of a reciprocal effect of DCA and cortisone in the manufacture of thyroid hormone. This is especially true if DCA, like cortisone, affects only thyroid uptake--and there is no information available on this point. It could logically be anticipated that if this were so, DCA might influence the thyroid mechanism for concentrating inorganic iodine and that cortisone could be affecting rate of organic binding. If such a mechanism exists, it could occur in normal animals.

THE METABOLISM OF THYROXINE IN NORMAL AND THYROIDECTOMIZED DOGS

Introduction.

In 1943 Winkler and his associates observed a tolerance to chronic oral dosage with thyroid substance in normal human subjects as compared to patients with myxedema. They found certain non-myxedematous individuals who could tolerate as much as six grains of thyroid substance (U.S.P. dried thyroid) daily for long periods of time with no increase in basal metabolic rate (BMR) or pulse rate. Winkler, Criscuolo and Lavietes (1943) had observed that patients with true myxedema require from 1 to 3 grains daily to maintain normal metabolic rates. Such patients usually become nervous and ill if given more.

Winkler and his associates suggested three possible explanations for the difference in response to thyroid substance found in the two groups:

(1) The normal individual may absorb less of the material from an oral dose than the athyreotic.

(2) The normal individual may be less responsive to the level of circulating hormone. He may require a greater plasma protein-bound iodine level (PBI) to produce a given metabolic increase.

(3) The normal individual may inactivate, store, or destroy greater amounts of hormone.

The first of these explanations is probably eliminated by the observation of Winkler et al., in the first publication

mentioned, that normal individuals, when compared with myxedematous ones, show a quantitatively diminished response in terms of increased BMR to intravenous thyroxine. Riggs, Man and Winkler (1945) found that normal and athyreotic subjects showed the same relationship between PBI and BMR; however, when they studied the amount of thyroxine necessary to produce a given increase in PBI, they found that the euthyroid individuals required considerably more thyroxine to produce a given increase in PBI than did the athyreotic. Thus, it appears that the third hypothesis offered by Winkler et al. is the most likely explanation of the difference in response to thyroid substance of normal and myxedematous human beings.

Danowski, Man and Winkler (1946) attempted to confirm the foregoing observation in dogs and to determine the mechanism of the tolerance in normal animals. They were unable to find any difference in the response of control and thyroidectomized dogs to oral desiccated thyroid or intravenous thyroxine. They found no difference in the BMR or PBI of the two groups after 0.77 gm. daily of U.S.P. desiccated thyroid <u>per os</u> for a period of five to sixteen weeks or in PBI after 2 mg. of thyroxine daily for 13 to 37 days.

Borgman (1949) saw distinct differences in the response of intact and thyroidectomized dogs to considerably smaller doses of exogenous thyroid substance or thyroprotein (protamone). One mg. of protamone per kg. per day was needed to restore the metabolic rate of thyroidectomized dogs to normal. Intact dogs tolerated doses of 2, 4, and 8 mg. per kg.

per day for 30 days without rise in basal metabolic rate. Normal dogs were similarly tolerant to desiccated thyroid.

These experiments were planned to determine whether a greater storage, excretion, or destruction of thyroxine would be found in intact dogs than in thyroidectomized ones. A study of the metabolism of I-131-labeled thyroxine was proposed in an attempt to determine whether there is a difference in its behavior in these two groups. It was decided to set up the experiments in such a fashion that the thyroidectomized dogs would have been pretreated with daily doses of non-radioactive thyroxine for a long enough period so that their basal metabolic rates should be normal and to compare these animals with normal ones which had been pretreated in the same fashion and also with normal ones which had not been pretreated. Since the difference between the two kinds of animal seemed to be revealed when large doses of exogenous thyroidal material were given, it was decided to pretreat the animals with a dose which was equal to the estimated daily thyroid secretion rate and then to determine the fate of the radioactivity after a dose of I-131-labeled thyroxine equivalent to ten times the pretreatment dose.

Materials and Methods.

Male mongrel dogs obtained through the usual channels were used in these experiments. The descriptions, weights, and estimated ages of these animals are given in table 7 of the appendix. Thanks is extended to Dr. Robert G. Schirmer for his judgment regarding the last observation. All of the

dogs, as far as could be determined, were in good health at the time of the experiment.

Diet: The dogs were fed on Borden's "Chunx" dog biscuit supplemented by Rival canned dog food.

Thyroidectomy: Thyroidectomies were performed at least six weeks before the experiments by the technique described by Borgman (1949). When the thyroids were removed, at least two parathyroids were carefully teased from the glands and left in place. Their circulation was kept intact whenever possible. If this was not possible, the parathyroid was inserted into a neck muscle. Some of the dogs showed tetany after the operation and were maintained temporarily with calcium gluconate in sufficient dose to control symptoms. None of the dogs showed any signs of tetany at the time of the experiments.

As evidence of the completeness of thyroidectomy, no accumulation of radioactivity was found in the neck region of any of the thyroidectomized dogs post mortem.

Pretreatment with Thyroxine: All of the animals, except one of the groups in experiment 1, were pretreated with L-thyroxine subcutaneously in a daily dose of 6 ugm. per kg. body weight per day. The solution was prepared in 0.9% NaCl with sufficient NaOH added so that the thyroxine was in solution. The dose was calculated from the data of Borgman (1949) as the minimum amount of thyroxine required to maintain a normal metabolic rate in thyroidectomized dogs. In experiment 1, the dogs were pretreated for five days; in experiment 2, for ten days.

Injection of Radioactive Thyroxine: Radioactive L-thyroxine, purchased from Abbott Laboratories, was prepared with carrier so that each cc. of solution contained 60 ugm. of L-thyroxine and 12 uc. of I-131 (experiment 1) or 15 or 20 uc. of I-131 (experiment 3). Sufficient NaOH had been added to put the thyroxine into solution.

One cc. per kg. body weight was injected intravenously into the cephalic vein. The injection vein was not used for bleeding purposes for at least twenty-four hours.

Blood Samples: At fifteen minutes, thirty minutes, one hour, and steadily increasing intervals until the conclusion of the experiment, blood samples were removed from a cephalic vein of the front leg or a saphenous vein of the hind leg. Each sample totaled approximately four cc. and was drawn into a syringe moistened with heparin.

Urine and Feces Samples: During the first four to six hours of the experiment the dogs were under light nembutal anesthesia. Urine was collected by means of a polyethylene tube which had been inserted into the bladder through the urethra. A urine sample, which consisted of all the urine which had flowed from the catheter during the period, was obtained at the time of each blood collection.

The animals were placed in metabolism cages at the end of four to six hours. From this time on urine was collected for each period from a container underneath the cage. The feces, whenever available, were collected from the wire screen in the bottom of the cage.

The data on total urinary and fecal output result from the following procedures. In the case of the urine, an aliquot of known weight was taken from the whole sample for each time period. These aliquots averaged about 500 mg. and were measured on the gramatic balance into the dishes used for counting. The weight of the whole sample was measured on a triple-beam balance. The procedure with the fecal samples was the same except that, prior to the removal of the aliquot, the feces were placed in a Waring Blendor and mixed thoroughly with a minimum amount of water. The weight of this mixture was determined by difference on a triple-beam balance. The weight of fecal samples taken for counting also averaged about 500 mg. Total weights of the urine samples from which aliquots were taken ranged from 4 to 500 gm.; of fecal samples, from 150 to 750 gm. In the experiment as a whole, ratios of total sample to aliquot ranged from 4 to 750.

Certain of the dogs had well formed feces and in addition tended to be neat housekeepers of their cages. Others either had feces which were less well formed or tended to step on them indiscriminately. Data on urine and feces for only those dogs in which separation was judged to be good have been reported here. Dog No. 16 escaped from his cage the first night of the experiment. His data are not reported.

External Thyroid Counts: In experiment 2 external thyroid counts were performed using a count rate meter and an end window G-M tube covered with an aluminum shield. In order to quiet the dogs they were anesthetized with the

ultra-short acting barbiturate, sodium pentothal, given symptomatically in intravenous dose of approximately twelve mg. per kg. The rate recorded was the highest which could be obtained when the tube was just touching the neck. A count over the thigh of the animal was used as background rate.

Statistics: The curves for the plasma I-131 levels at different times after radioactive L-thyroxine were analysed by a method which divided them into the sum of several exponential functions. Points which visually seemed to be in a straight line on a semi-logarithmic scatter diagram of plasma level against time were selected in a manner described later. The equation of such a line is:

$$\log y = \log a + bt$$

where y = % of dose of I-131/gm. plasma

t = time in hours

and a and b are constants.

The constants log a and b can be found by formulas from statistics to be:

$$b = \frac{E}{A}$$

$$\log a = \frac{(\Sigma \log y)}{N} - \frac{b(\Sigma t)}{N}$$

where N = the number of points

$$E = \Sigma(t \times \log y) - \frac{(\Sigma t)(\Sigma \log y)}{N}$$

$$A = \Sigma t^2 - \frac{(\Sigma t)^2}{N}$$

$$D = \Sigma \log y^2 - \frac{(\Sigma \log y)^2}{N}$$

The error in the whole line is σ_e. The probability is 0.68 that all the points lie within $\pm \sigma_e$ of the line log y = log a + bt.

$$\sigma_e = \sqrt{\frac{D - bE}{N - 2}}$$

The error in the slope of the line is σ_b. The probability is 0.68 that the slope falls within $b \pm \sigma_b$.

$$\sigma_b = \frac{\sigma_e}{\sqrt{A}}$$

The data in tables 5 and 6, in which these regression lines are reported, are in terms of $\log_{10}$, which was used in their calculation. Properly these results should be expressed in terms of $\log_e$. However, since the statistics are equally valid whichever base is used (Baten, 1953), $\log_{10}$ was employed for ease in calculation. The means have been converted to $\log_e$ and the formula for each line expressed at the bottom of the tables in the exponential form.

Experimental and Results.

Experiment 1: This was a preliminary experiment in which three groups of animals were studied: two intact dogs, two thyroxine pretreated intact dogs, and three thyroxine pretreated thyroidectomized dogs. Thyroxine pretreated animals were given a daily subcutaneous dose of 6 ugm. of L-thyroxine per kg. Blood plasma levels and urinary output of radioactivity were studied.

A semi-logarithmic plot of per cent of dose of I-131 per mg. of plasma against time, graph 3, appendix, suggests

that in this experiment the blood levels of activity were lower in the normal animals than in the thyroidectomized ones. The urinary output data on these animals, table 6 of the appendix, show that there was probably no difference in output among the groups up to two days. In the four animals which were studied up to seven days, there was greater urinary output in the thyroidectomized than in the normal dogs. Counts of the thyroid uptake in the intact animals had not been made in this experiment, and it is possible that this difference is represented by uptake of radioactivity by the thyroid gland.

This study revealed the necessity for carrying out an experiment in which data were collected until seven days, 168 hours, after injection of thyroxine. It also demonstrated that, if any interpretations of the dynamics of the circulatory fall in radioactivity were desired, it would be necessary to have several values between 90 and 172 hours. It seemed that sufficient observations had been made at early times.

The preliminary data also revealed the need to determine thyroid uptake at the conclusion of the experiment.

It also suggested the advisability of developing a satisfactory method for counting fecal output so that it too could be measured.

Examination of blood curves and urinary output data showed no apparent difference between the control animals which had been pretreated with thyroxine and those which had

not. It was decided to include only two groups in the next experiment: intact dogs and thyroidectomized dogs, each of which had received pretreatment with thyroxine. Since radioactive thyroxine by then was being made on a regular schedule by the Abbott Laboratories and could be obtained within a known length of time, it was decided to pretreat the animals with thyroxine for a slightly longer time which finally resulted in a ten day pretreatment period in place of the five days used in experiment 1.

Experiment 2: This experiment was performed in order to evaluate the need for considering thyroidal recirculation of I-131 in the normal dogs. Three dogs were given approximately 100 microcuries of carrier-free I-131 subcutaneously and counts over the thyroid gland were begun on the third day. Albert (1951) used a similar method for determining the biological decay of thyroidal radioiodine in rats. The mobility of the skin and musculature of the dog's neck seems to make external counting more variable in dogs than it is in other species such as the rat. These data did not show a linear trend in a semi-logarithmic plot until the seventh day after I-131. Counts were then followed without further treatment of the dogs up until the fourteenth day; on the fourteenth day an intravenous dose of 60 ugm. of L-thyroxine per kilogram was given to the dogs and counts were continued until the twenty-second day, at which time the experiment had to be abandoned because the counts were too low to be measured. A semi-logarithmic plot of counts per second against time is

recorded in graph 4 of the appendix. These counts have been corrected for physical decay and have been adjusted so that the count of each dog on the seventh day is recorded as 100 per cent. These dogs have an estimated half time for thyroidal radioactivity of eight days. It was hoped that the thyroid secretion would have been completely suppressed by the 60 microgram dose of L-thyroxine for the period of seven days during which the experiments were performed. This was not unreasonable since the dose represented ten estimated daily secretion rates. The individual observations were too variable to permit an estimate of the length of time that thyroid secretion was suppressed in each dog individually but a plot of the mean values of all three dogs--these are average logarithms--suggests that secretion was inhibited for at least three days.

Experiment 3: Two groups of animals were studied here: six normal dogs and six thyroidectomized dogs. Both groups were pretreated for ten days with a daily subcutaneous dose of 6 ugm. of L-thyroxine per kg. Blood levels, urinary output and fecal output of radioactivity were studied for a period of approximately 172 hours. Thyroid uptake was measured in the intact dogs at the conclusion of the experiment.

The total output of radioactivity in feces and urine is recorded in tables 3 and 4. Thyroid uptake is also included for the intact dogs. These data are only for those animals in which urine and feces were judged to be well separated.

Table 3: Total urinary and fecal output and thyroid uptake of I-131 in normal dogs after radioactive L-thyroxine. Experiment 3.

Dog No.	Urinary I-131 Output % of Dose	Fecal I-131 Output % of Dose	Thyroid I-131 Uptake % of Dose	Total Accountable I-131 % of Dose
3	43.1	59.7	3.6	105.9
10	34.5	62.8	8.6	105.9
12	37.6	34.3	6.1	78.0
23	--	--	--	--
13	56.3	42.8	4.2	103.3
20	--	--	--	--
Average	42.9	49.9	5.6	98.3

Table 4: Total urinary and fecal output of I-131 in thyroidectomized dogs after radioactive L-thyroxine. Experiment 3.

Dog No.	Urinary I-131 Output % of Dose	Fecal I-131 Output % of Dose	Total Accountable I-131 % of Dose
2	--	--	--
4	46.5	36.0	82.5
16	--	--	--
17	31.1	56.4	87.5
1	58.5	54.4	112.9
15	--	--	--
Average	45.4	48.9	94.3

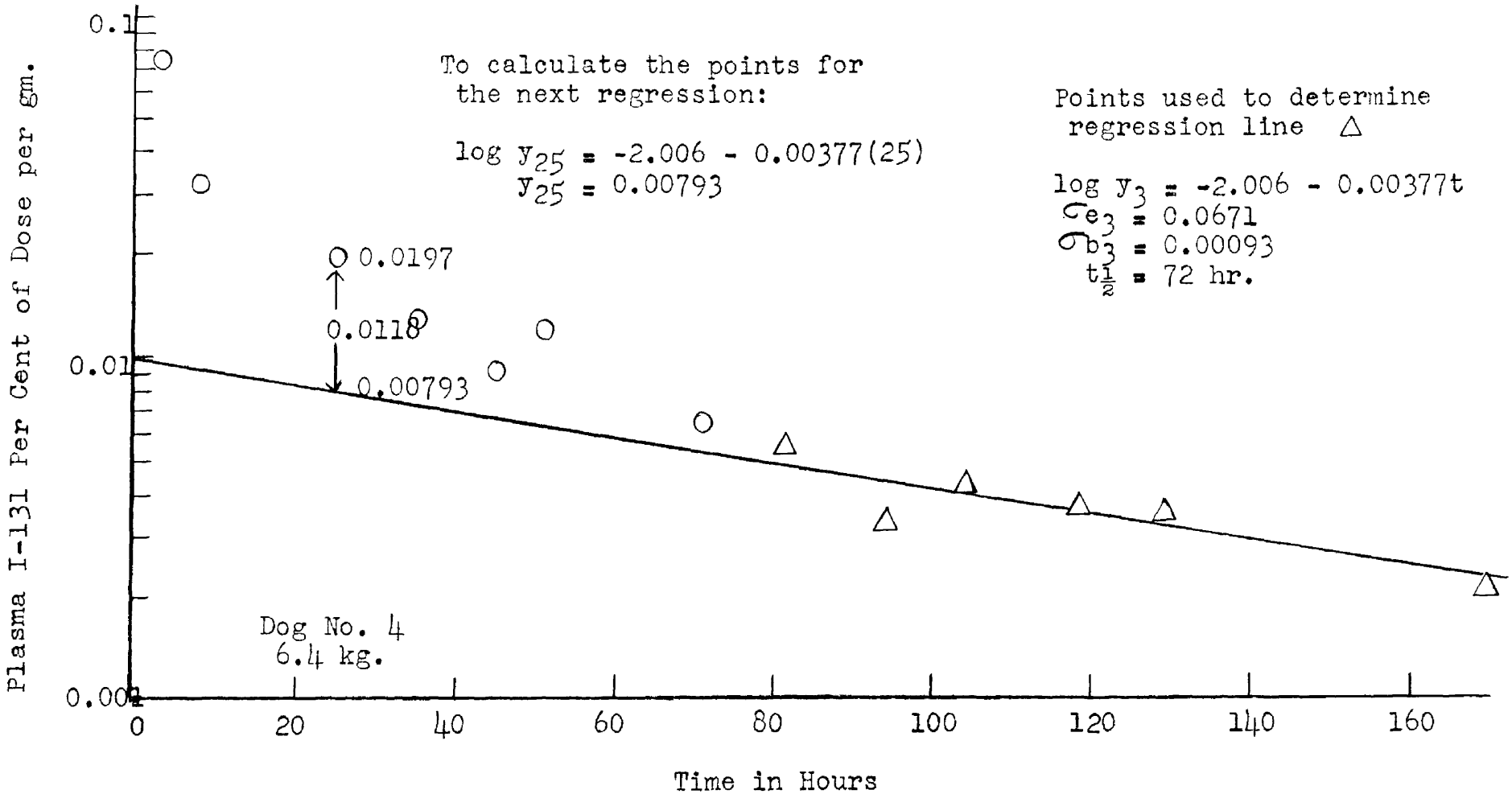
The method of analysis of the blood curves in experiment 3 is outlined in graphs 6 and 7 and the results obtained in tables 5 and 6. The third exponential function was the first to be determined. A semi-logarithmic plot of the plasma I-131 levels against time was examined for each dog. As many points at the end of the curve as appeared to be in a straight line were selected by visual inspection. The best semi-logarithmic straight line through them was determined by the method of least squares. A similar procedure was used to obtain the other two regressions. Graphs 6 and 7 indicate how this process was carried out. The method of analysis used is a standard one for this kind of data well described by Jones (1950).

Tables 3 and 4 indicate that there was no difference seen between total fecal output of radioactivity in these dogs. Likewise the sum of the thyroid uptake and urinary output of the intact dogs was equivalent to the urinary output of the thyroidectomized dogs.

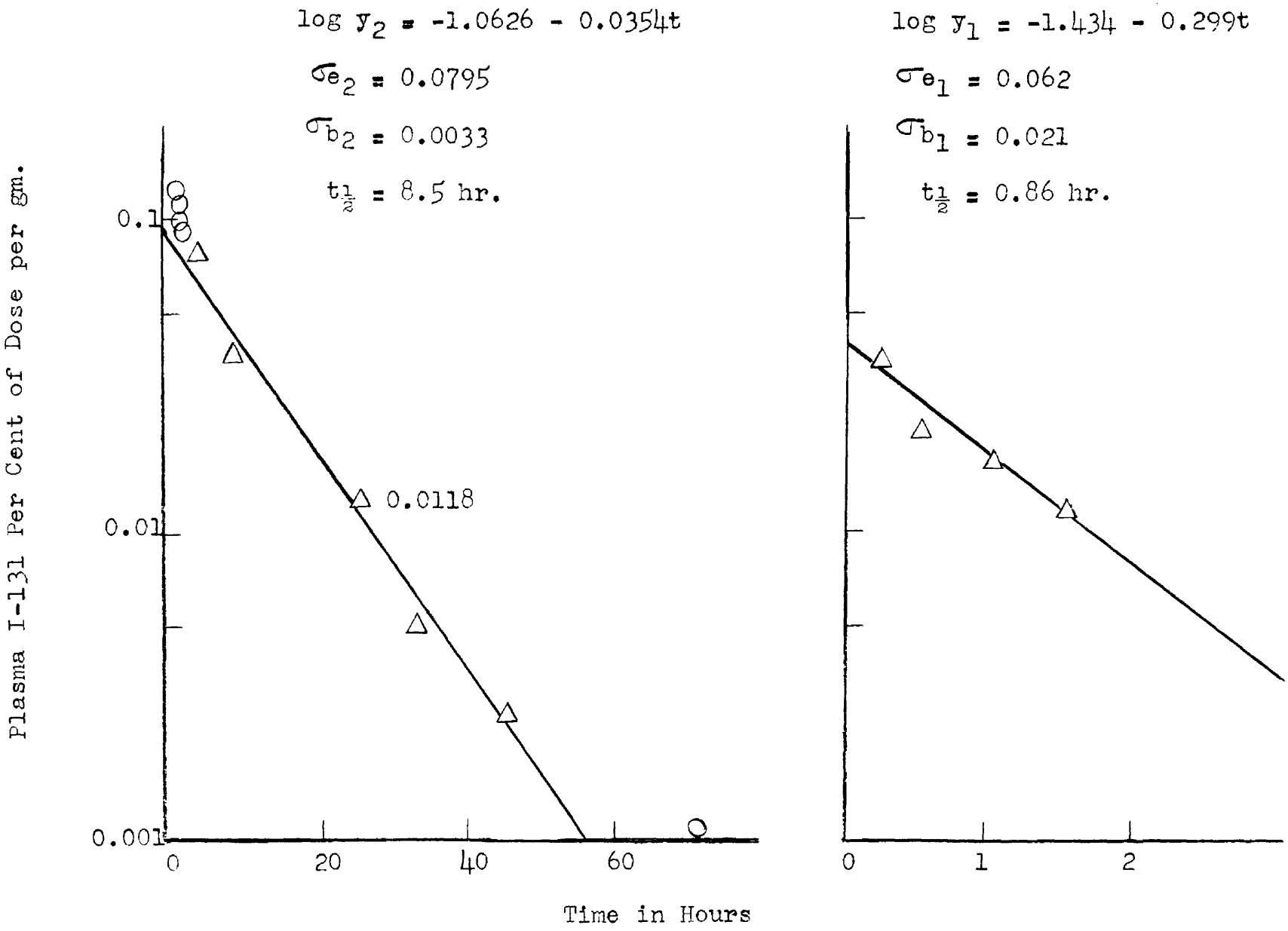
Statistical analysis by means of a "t" test of the data regarding blood levels of radioactivity showed no difference between the groups in log a_1, b_1, log a_3, or b_3. In intact dogs the mean of log a_2 was significantly less than thyroidectomized dogs, and in the intact dogs the mean of b_2 was significantly more than in the thyroidectomized.

Discussion of Results.

To interpret the results in experiment 3, it is necessary to consider some of the things which are known about the metabolism of thyroxine.



Graph 6: Example of method used to obtain points for calculating third regression line. Experiment 3. A few points, such as the value for 61 hours here, have been omitted because they were obviously in error.



Graph 7: Example of method used to obtain first two regression lines. Experiment 3. First regression line is obtained from second in the same manner that second was obtained from third.

Table 5: Analysis of blood levels of radioactivity of normal dogs. Experiment 3.

Dog No.	N	log a	b	σ_e	σ_b	$t_{\frac{1}{2}}$ hr.
First Exponential						
3	4	-1.294	-0.299	0.070	0.052	1.0
10	3	-1.328	-0.508	0.025	0.046	0.59
12	3	-1.471	-1.124	0.136	0.251	0.27
23	4	-1.512	-0.136	0.262	0.195	2.2
13	4	-1.431	-0.458	0.017	0.013	0.66
20	4	-1.107	-0.328	0.0057	0.0014	0.92
Mean		-1.357	-0.476			0.94
Second Exponential						
3	4	-1.2497	-0.0311	0.0840	0.0034	9.7
10	3	-1.1193	-0.0557	0.00866	0.00058	5.4
12	5	-1.2430	-0.03556	0.0201	0.00057	8.5
23	3	-1.2217	-0.0468	0.0325	0.0019	6.4
13	6	-1.4147	-0.0250	0.0568	0.0011	12.0
20	4	-1.1363	-0.0302	0.0193	0.00063	10.0
Mean		-1.2308	-0.0374			8.7
Third Exponential						
3	7	-1.9689	-0.00453	0.0575	0.00063	66
10	8	-1.8520	-0.00470	0.0311	0.00028	64
12	7	-2.1063	-0.00463	0.0228	0.00027	62
23	10	-1.7402	-0.00397	0.0324	0.00008	76
13	5	-2.3066	-0.00393	0.0356	0.00053	67
20	5	-2.2221	-0.003172	0.0310	0.00047	77
Mean		-2.0327	-0.004155			68.7

$$y = 0.044\, e^{-1.10t} + 0.059\, e^{-0.086t} + 0.0093\, e^{-0.0096t}$$

Table 6: Analysis of blood levels of radioactivity of thyroidectomized dogs. Experiment 3.

Dog No.	N	log a	b	σ_e	σ_b	$t_{\frac{1}{2}}$ hr.
First Exponential						
2	4	-1.337	-0.4741	0.053	0.039	0.64
4	4	-1.434	-0.352	0.062	0.021	0.86
16	5	-1.488	-0.226	0.075	0.032	1.3
17	4	-1.288	-0.313	0.031	0.024	0.96
1	4	-1.593	-0.299	0.241	0.180	1.0
15	4	-1.301	-0.347	0.019	0.045	0.87
Mean		-1.407	-0.335			0.94
Second Exponential						
2	5	-1.3027	-0.0333	0.0686	0.0019	9.0
4	4	-1.0626	-0.0354	0.0795	0.0033	8.5
16	3	-1.3918	-0.0311	0.0559	0.00070	9.7
17	3	-1.3817	-0.0282	0.0333	0.0013	10.2
1	4	-1.5034	-0.0269	0.0206	0.00075	11.2
15	4	-1.3647	-0.0392	0.0318	0.0014	7.7
Mean		-1.3345	-0.0323			9.4
Third Exponential						
2	7	-1.8902	-0.00541	0.0302	0.00031	56
4	6	-2.0065	-0.00377	0.0671	0.00093	72
16	8	-2.2003	-0.00512	0.0356	0.00037	59
17	8	-1.9007	-0.00324	0.0324	0.00033	93
1	7	-2.2032	-0.00554	0.0356	0.00037	54
15	9	-1.9212	-0.006780	0.0269	0.00022	44
Mean		-2.0203	-0.004976			63.0

$$y = 0.039\ e^{-0.77t} + 0.046\ e^{-0.074t} + 0.0095\ e^{-0.0116t}$$

Gross and Leblond (1950) were the first to study the metabolism of physiological doses of thyroxine. They studied doses of 0.007 ugm. of I-131 labeled L-thyroxine, 0.07 ugm. and 20 ugm. in 80 to 120 gm. rats. At 24 and 72 hours they found most of the injected dose in the urine and feces. They showed that with increasing dose of thyroxine, the per cent of dose excreted in the feces increased.

Urinary excretion of radioactivity after L-thyroxine is chiefly inorganic in form although small amounts of organically bound iodine may be present. After administration of carrier-free iodide to myxedematous humans maintained at euthyroid level, Albert and Keating (1949) found 80 per cent of the dose in the urine and only 0.8 per cent in feces.

By butanol extraction Myant and Pochin (1950) found an average of 9 per cent (5.7 - 23.8) of the urine of five human subjects, after radioactive L-thyroxine, to be due to thyroxine-like material.

The fecal excretion of radioactivity after injection of labeled L-thyroxine is largely organically bound. After carrier-free I-131 Albert and Keating (1949) found 0.8 per cent in feces at 72 hours, after L-thyroxine, 23 per cent. Taurog, Briggs and Chaikoff (1951, 1952) found two organic forms of iodine in bile, thyroxine itself and a thyroxine containing compound which they suggested is a glucuronide of thyroxine. Radioactive L-thyroxine is absorbed from the gastro-intestinal tract but not completely so (Albert et al. 1952b).

The plasma radioactivity after radioactive L-thyroxine has been found to be chiefly organic in form. Myant and Pochin (1950), estimating from known renal iodide-131 clearance, calculated that a mean of less than ten per cent of plasma radioactivity, at 1, 6, and 24 hours after a physiologic dose of radioactive L-thyroxine, was in the form of iodide.

Albert and Keating (1952) studied the metabolism of radioactive L-thyroxine in rats by a method similar to the one used here except that each time period was represented by a group of rats. They gave 0.6 ugm. L-thyroxine to 35-55 gm. rats. They obtained three regression lines by analysing the blood concentration of radioactivity in a manner similar to that used here with half-times of 0.36 hr., 15 hrs., and 62 hrs. They believed that the first one represented equilibration of the radioactivity with a larger volume of dilution almost entirely within the gastro-intestinal tract, that the second represented disappearance of the radio-thyroxine from its initial volume of dilution into the excreta, as well as into tissues, and the third indicates that I-131 is leaving blood by some other route than urine and feces into some larger equilibrium volume within the body.

O'Neal (1953) studied plasma PBI in thyroidectomized dogs after injection of 500 ugm. and 5000 ugm. of thyroxine. By a method of analysis similar to that used here they found two regressions with half-times of 0.97, 2.15, 1.15 and 27.7, 18.3, and 27.7 hours after 500 ugm. They found an increased

rate of disposal after 5000 ugm. so that at 50 hrs. 300 ug. was left as compared to 113 ug. after 500 ug. dose.

A comparison of Albert and Keating's results in rats with the data obtained in this work shows a good agreement in the half times obtained:

$t_{\frac{1}{2}}$ in Hours

Rats	Dogs	
	Normal	Thyroidx
0.36	0.94	0.94
15	8.7	9.4
62	69	63

The first half-time obtained by O'Neal was similar to the one obtained here in dogs. The second was in accord with neither of the other half-times obtained here; it is suggested that this may have been because of their relatively large dose (500 ug.).

Since fecal output after radioactive thyroxine is known to be organic in form and is the only organic output of great quantitative significance, it was believed that if a greater output of fecal activity was found in the intact than in the thyroidectomized animals it could be taken to indicate that the intact animal excretes a greater amount of each dose of thyroxine as such than does the thyroidectomized animal. As can be seen by examination of tables 3 and 4, this hypothesis was not realized. As far as can be told from these data, if a difference between the two groups exists it is not in the output of the radioactive thyroxine as such.

The control data on thyroid output of radioactivity in experiment 2 showed that the recirculation of iodine-131 in the intact dogs was at most a small per cent of dose. The maximum uptake of the thyroid gland in these dogs was ten per cent. If it is assumed that thyroid output was inhibited for three days and then proceeded at a rate such as to have a half-time of eight days, the maximum recirculation of radioactivity would have been 30 per cent of that present or a maximum of three per cent of dose.

Although there is a statistically significant difference between normal and thyroidectomized dogs in respect to log a_2 and b_2, the final levels of radioactivity in the blood of the two groups are the same. Thus it would appear that the over-all result of giving radioactive L-thyroxine to these dogs has been about the same in the two groups.

There is nothing in these data which could account for the approximately two-fold difference in response to thyroid substance seen by Winkler and his co-workers (1943) in man or to thyroid substance and protamone by Borgman (1949) in dogs. Although Winkler et al. (1943) found a lessened response to intravenous thyroxine in euthyroid subjects as compared to athyreotic they state that this effect was not as marked as the one seen after thyroid substance; therefore, it may be that the tolerance of normal individuals is a phenomenon which occurs only after oral thyroid substance or thyroprotein and not after intravenous thyroxine.

CONCLUSIONS

The three hour thyroid uptake of I-131 was increased in adrenalectomized rats after 1.25 mg. of desoxycorticosterone acetate per 100 gm. body weight given intraperitoneally immediately after intravenous injection of 20 uc. of I-131 with 8.5 ugm. of carrier I-127 as NaI. The plasma level and the urinary output of radioactivity were not changed by the treatment.

The one and one-half hour and three hour uptake of I-131 in adrenalectomized rats were increased after 10 cc. per 100 gm. body weight of 0.9% NaCl given under the same conditions. In this case the blood level of radioactivity was decreased, and the urinary output of radioactivity was increased.

The arterio-venous I-131 difference for the thyroid gland of the rat was measured and found to be 10% under the conditions of the experiment.

The estimated absolute thyroid circulation in the rat was calculated to be 23 mg. of blood per mg. of gland per minute.

A comparison was made of the metabolism of 60 ugm. I-131 labeled L-thyroxine in normal and thyroidectomized dogs after ten days pretreatment with 6 ugm. per day of non-radioactive thyroxine. No difference was seen in total fecal output of radioactivity up to seven days after injection of the labeled thyroxine. Combined thyroidal-urinary I-131 output of normal dogs equalled urinary output of thyroidectomized dogs. The plasma levels of radioactivity of both groups were described

by the sum of three semi-logarithmic regression lines having half-times of 0.94, 8.7, and 69 hours in intact dogs and 0.94, 9.4, and 63 hours in thyroidectomized dogs; 8.7 hours was statistically significantly less than 9.4 hours.

Appendix

Table 1: Factors for correction for the physical decay of I-131, based on an 8.00 day half-life.

Day	Hour	Correction		Day	Hour	Correction	
		-t	+t			-t	+t
	4	1.02	0.985		100	1.43	0.697
	8	1.03	0.971		104	1.46	0.687
	12	1.04	0.958		108	1.48	0.677
	16	1.06	0.944		112	1.50	0.668
	20	1.08	0.930		116	1.52	0.658
1	24	1.09	0.917	5	120	1.54	0.649
	28	1.11	0.904		124	1.56	0.639
	32	1.12	0.891		128	1.58	0.631
	36	1.14	0.878		132	1.61	0.621
	40	1.15	0.866		136	1.63	0.612
	44	1.17	0.853		140	1.66	0.603
2	48	1.19	0.841	6	144	1.68	0.594
	52	1.21	0.829		148	1.71	0.586
	56	1.22	0.817		152	1.73	0.578
	60	1.24	0.806		156	1.75	0.570
	64	1.26	0.794		160	1.78	0.561
	68	1.28	0.782		164	1.81	0.553
3	72	1.30	0.771	7	168	1.83	0.545
	76	1.32	0.760		172	1.86	0.538
	80	1.34	0.749		176	1.89	0.530
	84	1.35	0.739		180	1.92	0.521
	88	1.38	0.725		184	1.94	0.515
	92	1.39	0.717		188	1.97	0.507
4	96	1.41	0.707	8	192	2.00	0.500

Appendix

Table 2: Factors for correction for beta self-absorption, based on data using I-131-labeled rat liver.

mg.	mg./cm.^{2}	Correction
20	5.3	1.06
25	6.6	1.08
30	8.0	1.10
35	9.3	1.11
40	10.6	1.13
45	11.9	1.15
50	13.3	1.17
55	14.6	1.19
60	15.9	1.21
65	17.3	1.22
70	17.6	1.24
75	19.9	1.27
80	21.2	1.29
85	22.6	1.31
90	23.9	1.33
95	25.2	1.35
100	26.5	1.37
105	27.9	1.39
110	29.2	1.41
115	30.5	1.44
120	31.9	1.46
130	34.5	1.51
140	37.2	1.56
150	39.8	1.61
160	42.5	1.65
170	45.1	1.70
180	46.2	1.74
190	50.4	1.78
200	53.1	1.82

Appendix

Table 3: Body weights, thyroid weights, and thyroid I-131 uptakes of control rats. Experiment 5.

Rat No.	Time of Sacrifice Minutes	Body Weight gm.	Thyroid Weight mg.	Thyroid Uptake % of Dose
1	6	404	21.5	0.11
2	6	442	25.2	0.17
3	6	516	30.2	0.18
4	30	400	24.4	0.91
5	30	418	23.2	0.58
6	30	468	27.8	0.85
7	60	340	17.6	0.59
8	68	392	25.9	1.25
9	64	442	23.2	0.83
10	66	371	18.2	0.99
11	95	494	23.9	1.60
12	97	416	23.2	1.83
14	133	382	18.5	2.13
15	120	362	21.5	1.55
16	126	474	23.3	2.61

Appendix

Table 4: Body weights, thyroid weights and thyroid I-131 uptakes of saline-treated rats. Experiment 5.

Rat No.	Time of Sacrifice Minutes	Body Weight gm.	Thyroid Weight mg.	Thyroid Uptake % of Dose
1	6	402	22.0	0.18
2	6	452	23.1	0.07
3	6	484	30.7	0.15
4	30	340	19.4	0.46
5	30	418	22.5	0.68
6	30	468	19.4	0.71
7	75	358	23.1	1.65
8	58	404	26.1	1.07
10	69	388	20.3	1.51
12	102	414	18.6	1.98
13	100	428	14.6	1.37
14	132	382	16.8	1.77
15	117	356	19.5	1.47
16	128	486	35.2	1.32

Appendix

Table 5: Arterial, venous and thyroid venous blood levels of radioactivity of control and saline-treated rats. Experiment 5. Control rats have been listed first.

Rat No.	Time of Sacrifice Minutes	Systemic Arterial I-131 %Dose/mg.	Systemic Venous I-131 %Dose/mg.	Thyroid Venous I-131 %Dose/mg.
7	60	0.000598	0.000546	0.000529
8	68	0.000489	0.000521	0.000502
14	133	0.000749		0.000581
15	120	0.000502	0.000500	0.000517
16	126	0.000340	0.000319	0.000317
7	75	0.000371	0.000389	0.000333
8	58	0.000473	0.000464	0.000430
13	100	0.000306		0.000216
14	132	0.000313		0.000264
16	128	0.000216	0.000215	0.000209

Appendix

Table 6: Urinary I-131 output, intact control dogs (4 and 7), intact thyroxine-pretreated dogs (5 and 8) and thyroidectomized thyroxine-pretreated dogs (3, 6, and 9). Experiment 1.

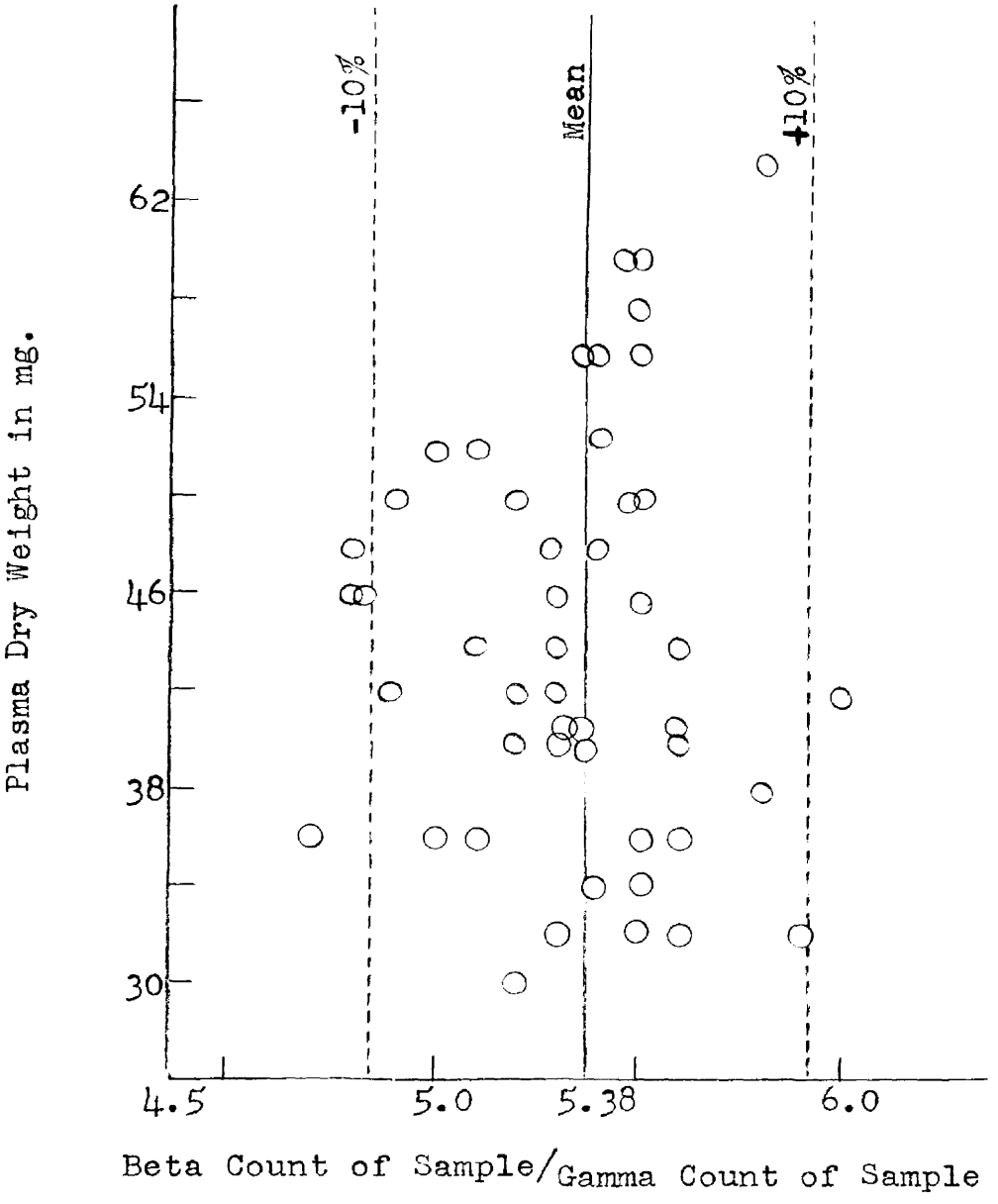
Day	Controls				Thyroidectomized		
	4	7	5	8	3	6	9
0-1	28.6	4.05	21.2	15.6	16.1	16.8	23.5
1-2	7.2	28.1	12.8	16.2	10.0	19.1	18.1
2-3		17.2		6.7		12.6	9.35
3-4		2.19		2.51		6.13	6.31
4-7		0.843		1.76		1.95	3.32
0-2	35.8	32.2	34.0	31.9	26.4	35.8	41.6
0-7		54.1		46.4		60.5	67.2
0-2 (Mean)	34.0		33.0		34.6		

Appendix

Table 7: Weights, descriptions and estimated ages of paired dogs. Experiment 3.

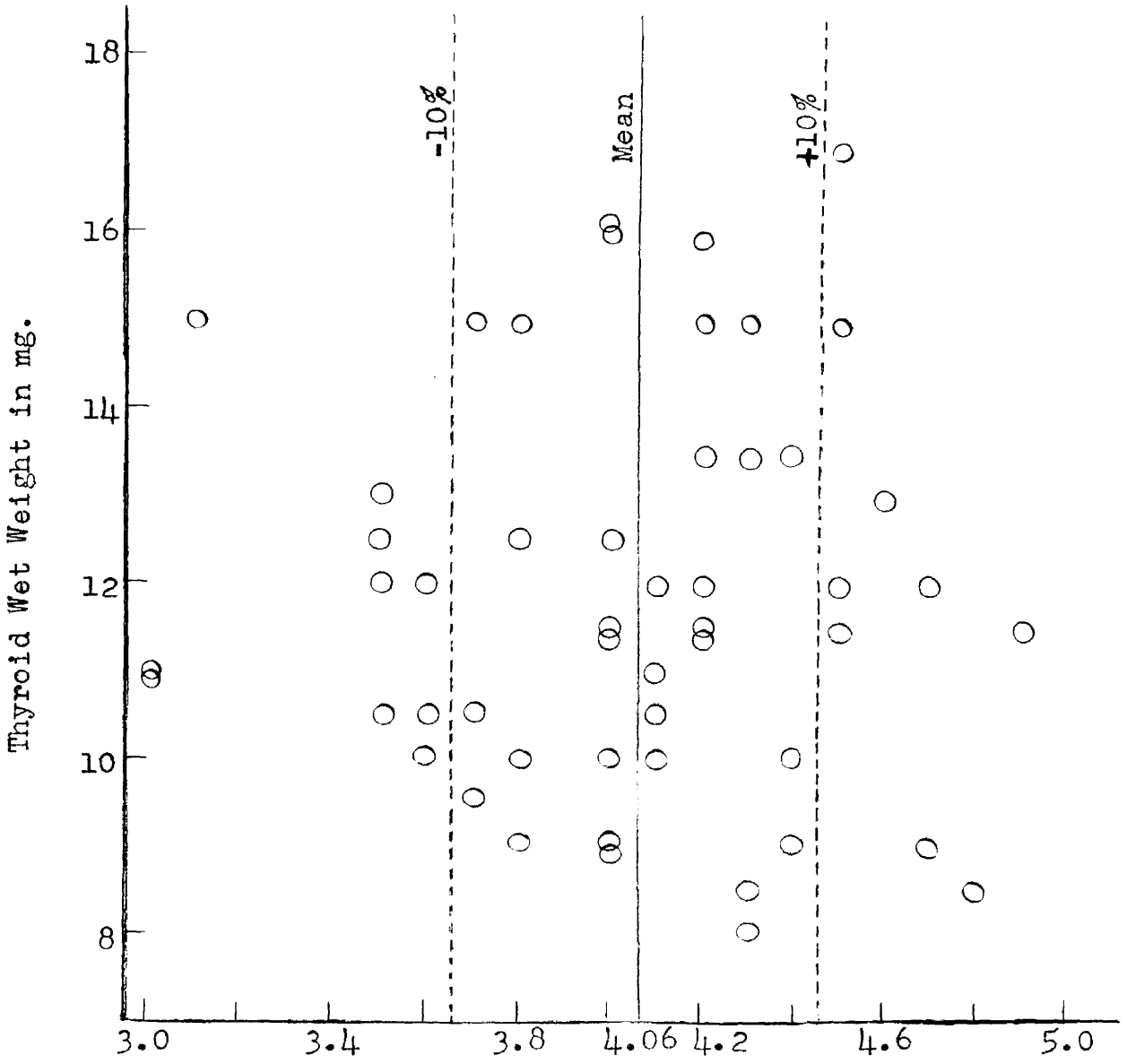
Dog No.		Weight kg.	Description	Age
3	Intact	8.3	Tan and white	1 yr.
2	Thyroidx	9.2	Brown	2 yr.
10	Intact	7.3	Black, tan, white	2 yr.
4	Thyroidx	6.4	Black and tan	4 yr.
12	Intact	9.3	Black	2½ yr.
16	Thyroidx	8.3	Brown and white	7 mo.
23	Intact	8.3	Black and white	1 yr.
17	Thyroidx	9.0	Black	1½ yr.
13	Intact	13.6	Black	1½ yr.
1	Thyroidx	13.9	Black	4-6 yr.
20	Intact	5.9	Tan	8 mo.
15	Thyroidx	8.8	Brown	1 yr.

Appendix



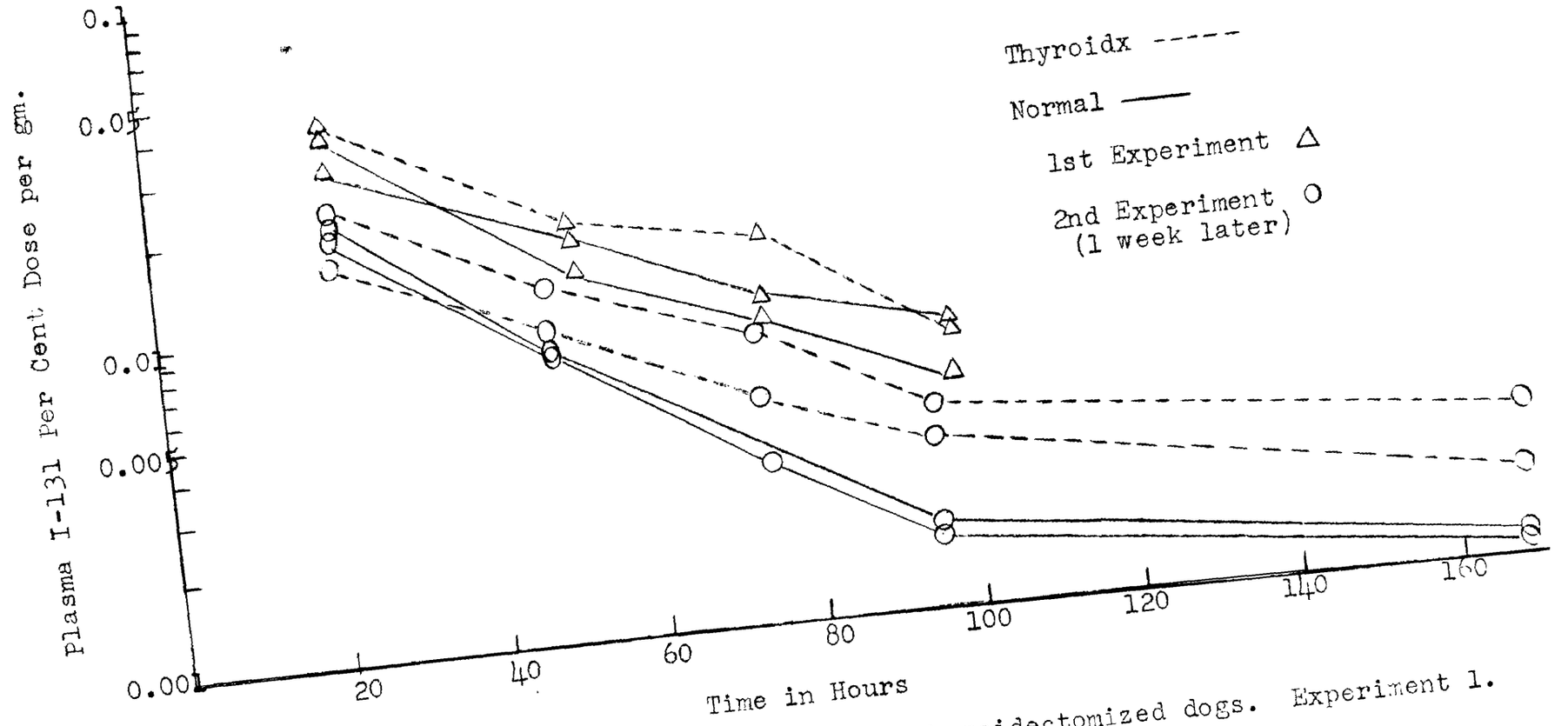
Graph 1: Scatter diagram of beta-gamma counting of rat plasmas labeled with I-131. Beta counts were corrected for self-absorption.

Appendix



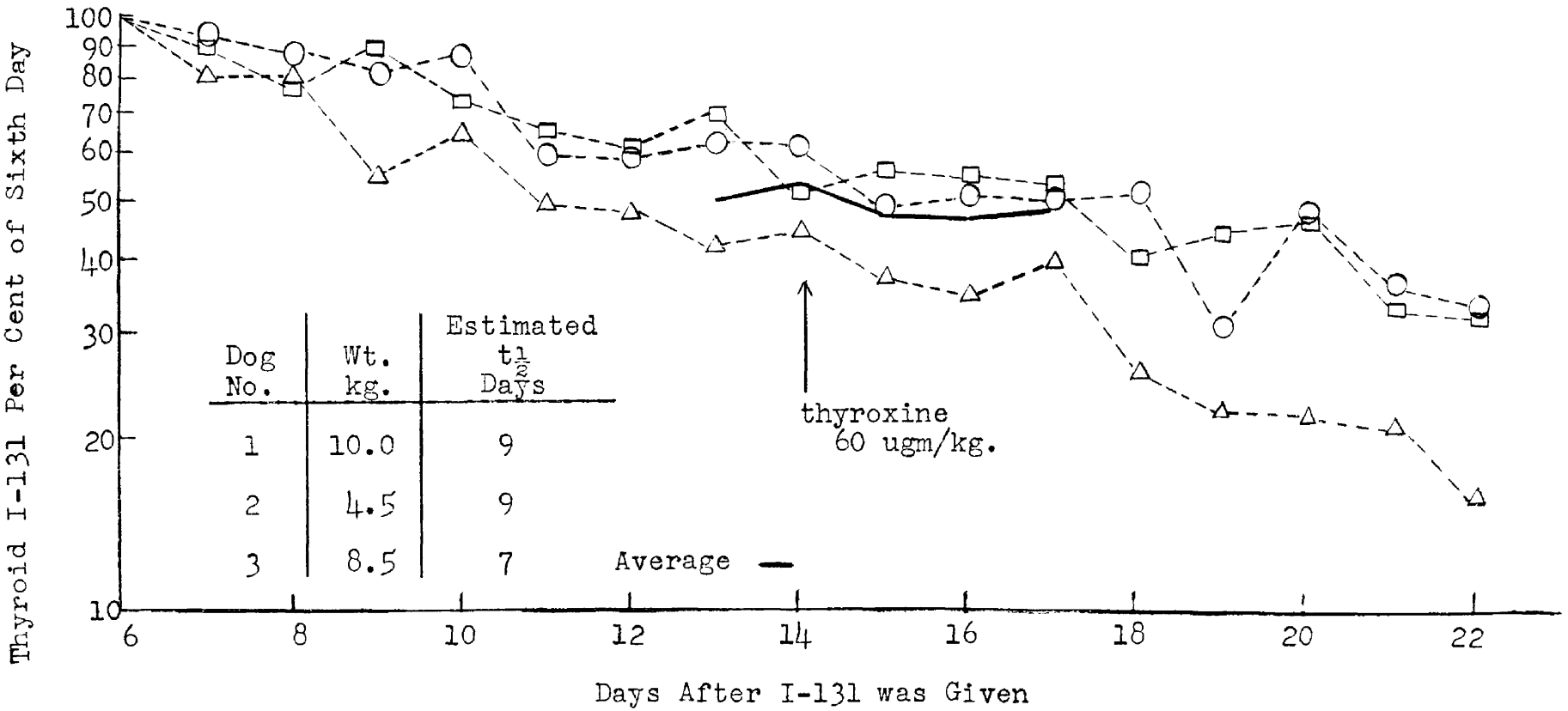
Graph 2: Scatter diagram of beta-gamma counting of dried rat thyroids labeled with I-131. Average self-absorption correction for these thyroids is $\frac{5.38}{4.06} = 1.32$.

Appendix



Graph 3: Plasma I-131 levels in normal and thyroidectomized dogs. Experiment 1.

Appendix



Graph 4: Thyroid output of I-131 in dogs. Experiment 2. I-131 levels in thyroid gland have been adjusted so that amount present on sixth day equals 100 per cent.

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