A STUDY OF CALCIUM METABOLISM WITH SOME OBSERVATIONS OF THE EFFECTS OF THE ADMINISTRATION OF LARGE AMOUNTS OF IRRADIATED ERGOSTEROL.

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THE FATE OF INTRAVENIOUSLY INJECTED CALCIUM CHLORIDE

INTRODUCTION

Our knowledge of the metabolism of the inorganic salts has been developed along with the general field of animal nutrition. The modern era of the science of nutrition may be said to begin with the epochal findings of Lavosier (1780) who introduced the concept of combustion (metabolism) in the animal body by the utilization of oxygen. In 1866, Pettenkofer and Voit published their great work on energy metabolism in animals using the respiration calorimeter as a means of measurement. This was soon followed by a legion of investigations on metabolism which has continued down until the present day.

The study of the inorganic salts in nutrition has shared in this general enthusiasm for metabolic studies. While, to be sure, there were some early observations on the inorganic constituents of the animal body; Berzelius (1807), Weber (1850); it was Sidney Ringer (1880-82) whose investigations on the influence of ions of various inorganic salts on the heart beat turned the full force of scientific interest on their importance in the animal body.

The development of our knowledge of calcium metabolism is of particular interest in the present investigation. Woiske (1871, 72 and 74) was one of the earliest investigators to call attention to the importance of calcium for

growth and maintenance. This was confirmed by Forster (1873 and 76) in his study of the importance of the various constituents in the diet and by Voit (1880) in his careful study of the importance of calcium to the animal body. One of the earliest controlled experiments on the excretion of calcium was carried out by Tereg and Arnold (1883). These investigators attempted to determine the urinary output of calcium before and after the administration of acid calcium phosphate by mouth and by subcutaneous injection. Their results indicate a small increase in excretion. Further evidence that calcium is normally excreted in the urine and feces was soon furnished by Rudel (1894), Rey (1895), Renvall (1904) and Goitein (1906). The work of both Rudel and of Goitein (cf. above) indicate there is some retention of injected calcium. Mendel and Benedict (1909-10) was one of the first to attempt to determine the relative importance of the kidneys and intestines in the excretion of calcium. They believe that injected calcium is excreted principally by the kidneys although they report considerable retention. This is further supported by the more recent investigations of Telfer (1922). Husband, Godden and Richards (1923) and Grosser (1920) however, believe the large intestine is the principal organ for the excretion of calcium. While both the large intestine and kidneys

may play a role in the excretion of calcium the relative importance of each is still uncertain. This will be discussed more fully in a later section. The purpose of the present section is to trace briefly the development of our knowledge of calcium metabolism; particularly those phases which have direct bearing on the present investigation.

Of the total calcium in the body, about 2 per cent is believed to be distributed throughout the soft tissues, the remainder being incorporated in the skelton. That this small amount in the soft tissues is of great physiological importance was early shown by the work of Ringer (cf. above) by Locke (1894), Loeb (1903) and many others.

The calcium content of the body fluids and tissues and the factors which may cause variations in these is of great interest to biologists at the present time. There is a large and growing literature on the effect of the parathyroid glands and of vitamin D on the metabolism of calcium in the animal body. Yet progress of a fundamental nature seems to depend upon a more adequate knowledge of the normal physiology of this ion. Rapid strides have been made since the recent development of micro-methods of analysis for calcium.

Early reports dealing with the calcium content of the blood give quite wide variations in the normal con-

tent. Due to the unreliability of the methods used, they must be regarded with suspicion. Most recent procedures are based on the method developed by Kramer and Tisdall (1921) which consists in the precipitation of the calcium with ammonium oxalate and titration with potassium permanganate. Clark and Collip's modification of this method is the one generally used to day for the determination of serum calcium.

The calcium of whole blood is reported to be 5 to 9 mgm. per cent (Lyman 1917, Kramer and Tisdall 1921, Jones 1921, Alport 1924) most if not all of which is found in the plasma (Abderhalden 1911, Leiboff 1930, Greville 1931, and others). The normal variation of serum calcium is 9 to 12 mgm. per cent the value usually being nearer 10 mgm. per cent (Lyman 1917, Matz 1925, Kramer and Tisdall 1921, Kramer and Howland 1920, Watchorn 1924-25, Esau and Stoland 1930, and many others). There is still considerable uncertainty as to the forms of calcium held in the blood stream. While that is not within the scope of the present discussion, it would seem that only a small portion of the total is ionized (Cushny 1920, Cameron and Moorhouse 1925, Richter-Quittner 1921, Brüll 1930, and Benjamin and Hess 1933).

A great deal of interest has been manifest in the fate of injected calcium salts and the restoration of

the calcium balance of the body. Voorhoeve (1911) appears to have been one of the first to study the tissue calcium after the administration of calcium salts. He reports the administration of calcium (as the chloride or lactate, several grams per day) increased the calcium stock of the tissues and blood, even in well-fed adults. Kost (1918) who treated growing young rabbits with calcium chloride for a week, found insignificant increases in the blood calcium and in the liver, somewhat more in the spleen, kidney and stomach, clearly in the bone, muscle and intestine. Heubner (1918) found the only significant increase after calcium injection in the kidneys. Hetenyi (1924) could account for only about one half of the injected calcium in the blood and excretions at the end of three hours. After lethal doses he found the calcium content of the bones increased by 2 per cent. Taylor and Fine (1930) could find no significant increase in the urinary excretion after calcium injection in cats nor could they recover any significant amount by washing out loops of the intestine. That injected calcium rapidly leave the blood stream has been shown by Heubner (1918), Hetenyi (1924), Salveson, Hastings and McIntosh (1924), Rothlin (1929, Taylor and Fine (1930), Gerschmann (1930), and Greville (1931).

While it is generally agreed that injected calcium rapidly leaves the blood stream, there is no general agreement regarding its fate. It occurred to the author that more knowledge of the mechanism for disposing of an excess of calcium, produced by the intravenous injection of one of its soluble salts, might give some information regarding the normal metabolism of this ion. Much of the early work reported was carried out before adequate methods of calcium analysis had been devised. The purpose of the present investigation was to study again carefully the fate of intravenously injected calcium chloride, making use of recent advances in the technique of calcium analysis.

I. THE INTRAVENOUS INJECTION OF CALCIUM CHLORIDE IN NORMAL ADULT DOGS.

Method.

Adult dogs kept on the ordinary laboratory diet consisting of whole wheat bread, milk and hamburger, were used in these experiments. The animals were under ether anesthesia throughout the experimental period (90 to 180 minutes), the ether being administered from an ether bottle through a tracheal cannula. One carotid artery was cannulated to draw blood for analysis and the femoral vein exposed for injections. Calcium chloride ($CaCl_2$, 5 per cent solution) was injected in all cases.

Serum calcium was determined by the method of Clark and Collip (1925), a modification of the earlier method of Kramer and Tisdall (1921). The calcium is precipitated directly from clear blood serum by means of ammonium oxalate. The calcium oxalate precipitated is taken up in sulphuric acid and the oxalate radical oxidized with a standard solution of potassium permanganate (n/100). The amount of permanganate required to complete the oxidation of the oxalate carried down with the calcium enables the computation of the calcium present, one cubic centimeter of n/100 potassium permanganate being equivalent to 0.2 mgm. of calcium.

At the present time this method is widely used for the determination of blood calcium and with ordinary care has been found to give quite accurate results. Greville (1931) made a careful investigation of this method. Calcium salts were added to horse serum and run in quadruplicate with a maximum error of less than 2 per cent. Recoveries of 98.4 and 97.6 per cent are reported with 0.576 mgm. and 0.289 mgm. of added calcium respectively. The serum calcium of rabbits after the injection of calcium salts were determined both by Clark and Collip technique and the wet combustion method of Widmark and Vahlquist (1931). From this evidence it may be concluded that the method as modified by Clark and Collip is adequate and convenient for the determination of serum calcium.

RESULTS.

The following is a typical protocol obtained following the intravenous injection of calcium chloride (5 per cent).

Protocol I

Dog # 5. Male weight 7.7 kilograms. Ether anesthesia by tracheal cannula.

10:34 Normal blood # 1 drawn from the carotid artery.

10:38-40 Injected 9.2 cc. of calcium chloride into the juglar vein.

10:45 Blood # 2 drawn.

10:55 " # 3 "

11:10 " # 4 "

11:40 " # 5 "

12:10 " # 6 "

12:40 " # 7 "

1:10 " # 8 "

1:40 " # 9 "

Animal killed by opening the thorax.

Analysis of the Blood for Calcium and Inorganic Phosphorus'.

	Serum Calcium	Inorganic Phosp.
Normal	11.6 mg per cent.	5.7 mg per cent.
5 min.	20.8 " "	6.0 " "
15 min.	18.3 " "	6.4 " "
30 min.	16.7 " "	7.0 " "
60 min.	14.3 " "	6.6 " "
90 min.	13.3 " "	-
120 min.	13.3 " "	-
150 min.	13.2 " "	-
180 min.	12.3 " "	7.1 " "

' Inorganic phosphorus determined by the method of Youngberg.

TABLE I

THE RATE OF DISAPPEARANCE OF INTRAVENUOUSLY INJECTED CALCIUM SALTS

Time in Minutes	Serum Calcium mg per cent.	Reported by Hetenyi'	Reported by Gerschmann''
Normal	11.2 mg.	—	11.2 mg.
5 min.	21.9 "	15.0 mg.	19.5 " (2 min.)
15 min.	18.8 "	14.8 "	15.6 "
30 min.	16.4 "	14.5 "	14.3 "
60 min.	14.7 "	13.5 "	13.5 "
90 min.	13.8 "	12.7 "	12.7 "
120 min.	13.5 "	12.6 "	12.1 "
150 min.	13.4 "	—	11.9 "

' Calciven 0.43 gms.

'' Calcium chloride 60 mg per kilogram body weight.

Protocol II

Dog # 56. Male weight 10.8 kilograms. To determine the effect of repeated injections of calcium chloride on the level of serum calcium in a normal animal. Bleeding and injections without anesthesia until last of experiment (see below).

2:10 Blood # 1 drawn. Serum calcium 12.2 mgm. per cent.

2:15 Injected 8 cc. 5 per cent calcium chloride intravenously.

2:30 Blood # 2 drawn. Serum calcium 14.4 mgm. per cent.

2:45 Injected 8 cc. 5 per cent calcium chloride intravenously.

3:00 Blood # 3 drawn. Serum calcium 13.5 mgm. per cent.

3:15 Blood # 4 drawn. Serum calcium 13.3 mgm. per cent.

3:16 Injected 8 cc. 5 per cent calcium chloride intravenously.

3:45 Blood # 5 drawn.

3:50 Injected 8 cc. 5 per cent calcium chloride intravenously.

4:35 Blood # 6 drawn. Serum calcium 16.6 mgm. per cent.

Dog anesthetized with ether for the remainder of the experiment.

4:40 Injected 8 cc. 5 per cent calcium chloride intravenously.

4:45 Blood # 7 drawn. Serum calcium 23.7 mgm. per cent.

5:00 Blood # 8 drawn. Serum calcium 20.4 mgm. per cent.

5:15 Blood # 9 drawn. Serum calcium 20.0 mgm. per cent.

It is obvious from the above data that relatively large amounts of injected calcium may be disposed of by the normal animal. Only during the latter part of the experiment was there any apparent decrease in the organisms speed of restoring its calcium balance. Even then, it is not apparent during the first five minutes since 64 per cent of the injected calcium, (assuming a blood volume of 61 cc. per 100 gms. body weight) had left the blood stream (controls—69 per cent).

Figures in the first column (from this laboratory) are the averages obtained after the injection of 60 mgm. of calcium chloride per kilogram body weight in three normal dogs.

We see from the above data (TABLE I) first column that immediately after the injection of calcium salts the serum calcium is raised to high values (15 to 22.4 mgm. per cent). Powers, Bowie and Howard (1930) using the congo red technique of Hooper, Smith and Whipple (1920), found the blood volume of adult dogs to be 11.28 cc. per 100 gms. body weight, the plasma volume being 6.18 cc. Assuming this plasma volume, we find the calcium injected should give 45.2 mgm. per cent if all of it were contained in the plasma. However, at the end of 5 minutes we find not 45.2 mgm. but 21.9 mgm. per cent, 57 per cent having disappeared from the plasma during the first five minutes after injection. Similar results were obtained by Heubner (1923) in the cat, by Hetenyi (1925) in humans, Rothlin (1929) in rabbits, Taylor and Fine (1930) in cats, Gerschmann (1930) in dogs and Greville (1931) in rabbits, all of whom report a rapid disappearance of intravenously injected calcium salts.

Where has this calcium gone: is it possible the corpuscles have taken up a part of the injected calcium? Abderhalden (1911) could find no calcium in the

blood corpuscles of human blood. Guillaumin (1930) report varying quantities up to 2 mgm. per 100 cc. of blood. Heubner and Rona (1919) found up to 7 mgm. per cent in the corpuscles following the injection of calcium salts. Greville (1931) found in rabbits, after the injection of calcium salts, the quantity of calcium carried down with or inside the corpuscles on centrifuging to be negligible. Results of determination made in this laboratory on whole blood, using the rapid combustion method of Bolliger (1932) compared with serum calcium determinations made by the method of Clark and Collip (1925) are in agreement with the findings of Greville. A large portion of the injected calcium has left the blood stream.

TABLE II

ANALYSIS OF WHOLE BLOOD FOR CALCIUM. RAPID COMBUSTION METHOD OF BOLLIGER.

Serum Ca. by method of Clark and Collip. Mgm. per cent.	Analysis of Whole Blood. Calcium in mgm. per cent assuming the total in the plasma.
10.2	9.8
17.4	17.7
22.8	24.0
23.2	24.0
22.9	21.5

II. THE EXCRETION OF INJECTED CALCIUM CHLORIDE.

Is the injected calcium excreted through the intestine and kidneys sufficiently rapid to account for its disappearance from the blood stream? That calcium is normally excreted by the kidneys and the epithelium of the large intestine has been well established by Rey (1894-95), Patterson (1908), Cushny (1918), Nelson (1918), Husband, Godden and Richards (1923) to mention only a few. Bergeim (1926) has definitely shown that in the rat there is an absorption of calcium along the small intestine and an excretion by the epithelium of the large intestine. Hetenyi (1924) believes calcium is normally excreted, for the most part, by the large intestine, the ratio of large intestine to kidney excretion being 10 or 20 to 1. They state further this ratio may be influenced in favor of the kidneys by many factors. Stewart and Percival (1927) found the calcium excretion by the large intestine of the cat to be 0.33 to 0.9 mgm. in a three hour period. During the same period, the kidneys excreted 0.14 to 0.25 mgm. Walsh and Ivy (1927) studied calcium excretion in dogs with pouches (Thiry-Vella). Daily washings of the upper jejunum gave 0.32 to 1.2 mgm. of calcium, lower ileum 2.0 to 8.5 mgm., the entire colon 1.8 to 15 mgm. daily.

It is evident, there are many difficulties to an

accurate study of normal calcium excretion. The fecal calcium represents unabsorbed calcium as well as excreted calcium. Bergeim (1926), as mentioned above, has shown both absorption and excretion take place in the intestine. There is little or no agreement as to the relative importance of these two processes in normal animals.

Similarly there are many factors which complicate the study of urinary excretion. Brull (1930) found the excretion of calcium in the urine to be proportional to the rate of urine secretion, the excreted calcium acting like a "no-threshold" substance. Not only kidney activity but the composition of the diet may alter urinary calcium. Bogert and Kirkpatrick (1922) found base-forming diets caused a decrease in the urinary calcium and an increase in the fecal excretion, while the reverse was found true of acid-forming diets. Stehle (1917) found an increased excretion of calcium in the urine of dogs after the ingestion of hydrochloric acid.

But what of the excretion following the injection of calcium salts? Rey (1894-95) stated that after intravenous injection of calcium salts 53 per cent could be recovered from the colon in three days. Hetenyi (1924) found after intravenous injection of calcium salts slightly more fecal calcium than in control animals. There was

little or no effect on urinary output of calcium. Salveson, Hastings and MacIntosh (1924) found fecal excretion of calcium during the six days following a large injection of calcium chloride lower than that before the injection. Taylor and Fine (1930) studied calcium excretion in the cat. They observed that injected calcium chloride disappeared rapidly from the blood stream but did not find any change in the excretion through the kidneys or intestine. They state further that evisceration or tying off the kidneys had no effect on the rate of disappearance of calcium salts. In view of the rapid disappearance of injected calcium salts immediately after injection (60 to 70 per cent during the first five minutes) and the general lack of agreement regarding the excretion of calcium salts the author considered it worth while to reinvestigate the possibility of excretion playing a role in this rapid disappearance from the blood stream.

A. The Rate of Disappearance of Intravenously Injected Calcium in Nephrectomized Dogs.

In a series of six dogs, the kidneys were removed. All experiments were performed under condition previously described (page 5). Calcium chloride (60 mg. per kilogram body weight) was injected intravenously. Samples of blood were drawn at regular intervals and

the serum analyzed for calcium.

TABLE III

RATE OF DISAPPEARANCE OF INTRAVENOUSLY INJECTED CALCIUM SALTS.

Time in Minutes.	Serum Calcium	
	Control Dogs.	Nephrectomized* Dogs.
N.	11.2 mg.	11.1 mg.
5.	21.9 "	19.9 "
15.	18.8 "	17.2 "
30.	16.4 "	15.4 "
60.	14.7 "	14.5 "
90.	13.8 "	13.7 "
120.	13.5 "	13.3 "
150.	13.4 "	12.2 "
180.	12.8 " (12.3-13.2)	12.6 " (10.8-12.5)

* Average of Dogs 15, 19, 26, 27, 29 and 37.

The data in TABLE III strongly suggests that the kidneys play a minor role, if any, in the removal of the injected calcium within the experimental period (three hours). The possibility of a compensatory increase in intestinal excretion after removal of the kidneys will be considered in another place. Hetenyi (1924) recovered 9 to 18 per cent of the injected calcium from the urine after three hours. Brull (1930) found no increased output of calcium salts in

the urine formed by a heart-lung-kidney preparation. He believes the urinary output of calcium is dependent upon the rate of urine formation and not upon its concentration in the blood stream. Taylor and Fine (1930) injected calcium intravenously in cats. They could not account for its disappearance through excretion by the kidneys. These observations justify the conclusion that the kidneys play no important role in the rapid disappearance of intravenously injected calcium salts.

B. The Rate of Disappearance of Intravenously Injected Calcium After Removal of the Kidneys and Large Intestine.

In three dogs (numbers 9, 10 and 11) the large intestine as well as the kidneys were removed. The averages of the serum calcium determinations are shown in the table below. (TABLE IV).

TABLE IV

THE RATE OF DISAPPEARANCE OF INTRAVENOUSLY INJECTED CALCIUM CHLORIDE AFTER REMOVAL OF THE KIDNEYS AND LARGE INTESTINES.

Time in Minutes.	Serum Calcium Control Dogs.	Kidneys and Large Intestine Removed.
N	11.2 mgm. (10.3-11.7)	10.3 mgm. (9.5-11.0)
5	21.9 " (19.0-25.8)	23.0 " (19.6-26.4)

TABLE IV (cont)

Time in Minutes.	Serum Calcium Control Dogs.	Kidneys and Large Intestine Removed.
15	18.8 mgm. (16.4-21.3)	19.8 mgm. (18.5-22.0)
30	16.4 " (13.8-18.7)	18.3 " (16.1-21.8)
60	14.7 " (13.0-16.8)	18.2 " (15.9-21.8)
90	13.8 " (12.7-15.5)	17.4 " (14.8-21.0)
120	13.5 " (12.3-14.8)	16.2 " (14.8-17.6)
150	13.4 " (13.2-12.5)	—
180	12.8 " (12.3-13.2)	15.4 " (14.2-16.5)

(Data on the removal of the large intestine and kidneys represents an average of dogs 9, 10 and 11).

Examination of the above data (TABLE IV) shows a consistently slower rate of disappearance in animals with the large intestine and kidneys removed, this difference becoming more marked the longer the time after injection. As in groups previously reported, there is a rapid disappearance during the first few minutes following injection of calcium chloride. However the slower process, the restoration of the calcium balance, is the portion most influenced.

In some manner, the removal of the large intestine hinders the return of serum calcium to the normal level. This will be discussed later. There is necessarily some loss of blood in the removal of a part of the intestinal tract. In these experiments that was not considerable. To determine the effect of this on the rate of disappearance of injected calcium the following experiment was run:-

Dog # 22. Female Wt. 12.7 kilograms.

2:55 Completed bleeding. Bled 19.7 cc. per kilogram body weight.

2:55 Injected $CaCl_2$ (60 mgm. per kilogram body weight) intravenously.

3:00 Blood # 2 drawn. (Approximately 10 cc. of blood was taken for each analysis).

3:25 Blood # 3 drawn.

3:45 Blood # 4 drawn.

3:55 Blood # 5 drawn.

4:25 Blood # 6 drawn.

4:55 Blood # 7 drawn.

5:25 Blood # 8 drawn.

5:55 Blood # 9 drawn.

Analysis of the Blood for Calcium:-

Time in Minutes following injection	Serum Ca. Mgm. per cent.
N	11.3
5	23.4

Time in Minutes.	Serum Ca. Mgm. per cent.
15	——
30	17.5
50	16.1
60	15.9
90	15.0
120	14.5
150	13.7
180	12.7

Using the values for blood volume of the dog found by Powers, Bowie and Howard (1930), 250 cc. in this animal represents 17.8 per cent of the circulating volume. Inspection of the above data shows the disappearance of calcium to be normal. It is unlikely that in any evisceration experiments here reported there was a loss of blood as great as that reported here. While it is impossible to say if impaired circulation may not have been in part responsible for the slower disappearance from the blood stream, yet it may be pointed out that the exposure and manipulation of the viscera in removing the kidneys did not alter the rate of disappearance.

C. The Rate of Disappearance of Intravenously Injected Calcium After Removal of the Kidneys, Large and Small Intestines.

Is the difference in the rate of disappearance a general result of the loss of tissue or a specific effect from removal of the large intestine? Would the removal of the small intestine as well as the large intestine alter this rate of disappearance further? To give some information regarding this possibility the small intestine was removed (up to the tail of the pancreas) as well as the kidneys and large intestines. The averages of serum calcium after the injection of calcium chloride are given in the table below. (TABLE V).

TABLE V

THE DISAPPEARANCE OF INTRAVENOUSLY INJECTED CALCIUM IN DOGS BEFORE AND AFTER THE REMOVAL OF THE KIDNEYS AND INTESTINES.

(Injected calcium chloride 60 mg. per kilogram body weight).

Time in Minutes After Injection	Normal Average Mg. %	Ca. Leaving Blood Stream In % Injected	After Removal Of Kidneys & Intestines. Average Mg.%	Ca. Leaving Blood Stream in % Inj.
N	11.2	—	10.7 (9.8-11.7)	—
5	21.9	69	23.9 (22.0-26.3)	62
15	18.8	77	20.7 (19.0-21.8)	71
30	16.4	85	18.9 (18.0-20.5)	76
60	14.7	90	18.3 (17.2-19.5)	78
90	13.8	92	17.9 (16.5-19.0)	79

TABLE V (cont)

Time in Minutes After Injection	Normal Average Mg. %	Ca. Leaving Blood Stream In % Injected	After Removal Of Kidneys & Intestines. Average Mg. %	Ca. Leaving Blood Stream in % Inj.
120	13.5	93	17.4 (16.5-19.0)	80
150	13.4	93	16.5 (15.1-17.7)	83
180	12.8	95	16.1 (15.2-17.0)	84

Injected approximately 35 mg. ca. per kilogram body weight.

The data for the above table is from animals 3, 7, 14, 16, 17, 20 and 21.

The rate of disappearance of injected calcium here is slightly slower than that reported in ~~TABLE~~ Column 3 ~~IV~~. Considering the mass of tissue removed with the removal of the small intestine, it would seem there is a great difference in the affinity of tissues for calcium if the difference observed is due to some tissue "binding" of calcium. There are three rather obvious possibilities to be considered:-

1. That the intestines excrete a portion of the calcium excess and that this excretion is considerable immediately after injection.
2. That these tissues take up and hold a portion of the calcium excess.
3. That both 1 and 2 occur.

We will consider the first of these possibilities in the next series of experiments to be reported.

D. The Excretion of Intravenously Injected Calcium by the Epithelium of the Large Intestine. Recovery Experiments.

As we see from an earlier review of the literature on calcium excretion by the intestine, increases in the rate of excretion of calcium following the injection of one of its soluble salts was reported by Rey (1894-95) and by Hetenyi (1924) more recently.

Stewart and Percival (1929) studied the normal calcium excretion in cats by isolation of segments of the intestine and washing out the excreted material at intervals. Similar studies were made by Walsh and Ivy (1927-28) in the dog using Thiry-Vella pouches. Both report a small normal excretion. Taylor and Fine (cf. above) using the technique of Stewart and Percival, studied the excretion of calcium in cats after the intravenous injection of calcium chloride. They state, "The disappearance cannot be accounted for by excretion through the bowels or kidneys." They believe the injected calcium does not leave the body. Since the removal of the intestines does alter the rate of disappearance of injected calcium salts, it is imperative that the possibility of their excretion be studied.

The method of study used here is essentially the same as that used by Taylor and Fine. The kidneys were removed and the large intestine cannulated, carefully cleaned of all contents and washed with warm physiological saline until absolutely clear. A last portion of the washings was saved and analyzed for calcium. After three hours from the time of calcium injection (60 mgm. $CaCl_2$ per kilogram body weight was injected in all cases) the large intestine was again washed out with 500 cc. of warm physiological saline, the saline being run through the intestine three times. Ten cubic centimeter portions of the clear washings were taken for analysis. To this was added 2 cc. of 4 per cent ammonium oxalate and after standing 24 hours in the ice box centrifuged and titrated according to the technique of Clark and Collip (previously described).

RESULTS.

The results from all dogs in which calcium excretion was studied are shown in tabular form below.

(TABLE VII).

TABLE VI

THE EXCRETION OF INTRAVENOUSLY INJECTED CALCIUM BY THE EPITHELIUM OF THE LARGE INTESTINE. RECOVERY EXPERIMENTS.

Number of Experiment	Serum Calcium Before Inj. Calcium	5 Min. After Inj. Calcium	180 Min. After Inj. Ca.	Calcium Excreted Large Intestine in 180 Minutes.
27	11.8 mgm.	21.4 mgm.	13.3 mgm.	7.6 mgm.'
29	12.6 "	21.5 "	14.8 "	1.4 "
33	10.3 "	23.0 "	—	2.0 "
37	10.4 "	18.9 "	11.8 "	1.43 "
38	11.2 "	16.3 "	14.3 "	2.8 "
44	Control			Trace

(' Walsh and Ivy (1927-28) have shown that repeated washing increases the calcium excreted into the large intestine. In the experiments here reported, the calcium excreted was calculated by subtracting the amount in the last portion of the first washing from the amount obtained after three hours, on the assumption that this represents the amount excreted due to the washing out).

While these values are a little higher than those of Taylor and Fine (1930) who reports 0.77 mgm. in one experiment on a normal dog (studied excretion in the

cat) yet they are in agreement in that they show only a small amount of the injected calcium has been excreted in the experimental period. Examina tion of TABLE IV shows a difference in serum calcium between normal dogs and those in which the kidneys and large intestine had been removed of 2.6 mgm. per cent. In the case of a dog of average size (12 kilograms) there should be about 741 cc. of plasma which would contain, after injection, a total of 19.2 mgm. of calcium more than the normal amount. This represents the loss attributable to the large intestine. We see that the greatest recovery reported here is 7.6 mgm. with 3.06 mgm. as an average value. If the intestine excretes as much as 19.2 mgm. of calcium it was not recovered from the washings in our experiments. The small amount excreted in our experiments are wholly inadequate to explain the rapid disappearance that occurs during the first five minutes after injection. Since the kidneys were removed in these experiments, we must conclude a large portion of the injected calcium is retained in the body.

Since the calcium leaves the blood stream and since it is retained in the body we have the possibility of diffusion:-

1. Into the soft tissues.

2. Deposition in the bones.

3. Both diffusion into the soft tissues and deposition in the bones.

Greville (1931) suggested that both processes do occur. He believes that there is first a rapid diffusion into the tissue spaces until there is some form of equilibrium between the calcium in these spaces and the raised blood calcium. The calcium then disappears from the tissue spaces and blood stream, the rate of disappearance at any given instant being proportional to the excess present.

If this be correct, the first part of the disappearance curve would take place in the complete absence of the skeletal system. The amount of calcium leaving the blood stream would be proportional to the amount of calcium injected and the amount of soft tissues taking part in the reaction. The next series of experiments will consider this possibility.

III. THE RATE OF DISAPPEARANCE OF INTRAVENOUSLY INJECTED CALCIUM CHLORIDE IN PREPARATIONS CONTAINING ONLY SOFT TISSUES.

A. After tying Off the Blood Vessels to Skeletal Parts.

METHOD.

In all dogs used in these experiments the thorax was opened under ether anesthesia and the animal kept alive by artificial respiration. The blood vessels to the extremities, all vessels to the head except the internal carotids and all vessels to the dorsal body wall were tied off. The intercostals were ligated from the opened thorax. The vessels to dorsal abdominal wall were exposed by an incision into the abdominal cavity through the dorsal wall. These were then dissected from other tissue near their origin from the abdominal aorta and ligated. This procedure was found to disturb the abdominal viscera the least.

Calcium chloride in a 5 per cent solution was injected into the jugular or subclavian vein near the heart. Blood was drawn at regular intervals thereafter and analyzed for calcium as previously described.

RESULTS.

The following is a typical protocol from these experiments:

Protocol Dog # 41 Wt. 9.8 kilograms.

Blood vessels to trunk and limbs were tied off. Right internal carotid left open.

11:20 Normal blood # 1 drawn.

11:22-23 Injected 10 cc. 5% $CaCl_2$ intravenously.

11:27 Blood # 2 drawn. 5 minutes.

11:40 Blood # 3 drawn. 18 minutes.

12:12 Blood # 4 drawn. 45 minutes.

12:34 Blood pressure 35 mm. Hg. Heart beat weak.
Injected 2 cc. adrenaline 1:10,000.

12:37 Blood pressure 80 mm. Hg. and staying up well.

12:57 Blood # 5 drawn. 90 minutes.

2:00 Blood # 6 drawn. 153 minutes.

2:27 Blood # 7 drawn. 180 minutes.

Animal killed.

Analysis of the Blood for Calcium:-

N. 10.8 mgm. per cent.

5 min. 27.8 mgm. per cent.

18 " 27.2 " "

45 " 24.2 " "

90 " 24.3 " "

153 " —

180 " 21.0 " "

The amount of calcium injected in the above experiment should give a total value of 57.8 mgm. per cent in the plasma. However at the end of five minutes

we find only 27.8 mgm. 64 per cent left the blood. This compares very favorably with control animals in which there was an average disappearance of injected calcium during the first five minutes of 69 per cent. TABLE VIII gives the results of seven experiments in which the above technique was employed. It is obvious from the examination of this data that there is a rapid disappearance of injected calcium from the blood stream during the first five minutes after

TABLE VII

THE DISAPPEARANCE OF INTRAVENOUSLY INJECTED CALCIUM CHLORIDE AFTER TYING OFF THE BLOOD VESSELS TO THE SKELETON (INCLUDING SOME SKELETAL MUSCLE).

Number of Experiment.	Ca. Inj. per 100. cc. plasma. Mgm.	Ca. in Mg. Per Cent 5 Min. after Injection.	Calcium Leaving Blood in Per Cent Injected. 5 min.	30 min.	60 min.	180 min.
36	66	25.4	77	78		
40	55	19.0	83			87
41	47	27.8	64			78
43	50	31.8	57	72		76 (145 min.)
45	38	28.2	55		58	
49	45	30.5	55	69		
50	50	38.0	44	48		

injection and that the bony tissues play no role in this. In the past it was generally believed calcium salts decreased the permeability of the capillary wall. That this is not the case has been shown by Yanagawa (1916), Bayliss (1918) and Smith and Mendel (1920). That calcium does penetrate the wall of the capillaries has been shown by Arnold and Mendel who demonstrated its appearance in oedema fluid and serous transudates. Thus, there is little doubt but that there is a rapid diffusion out into the tissues spaces of a large amount of the injected calcium. It would seem that during the first few minutes some equilibrium is reached between the blood and tissues after which only slow changes in the concentration toward normal occur. The presence of calcium in the tissues will be dealt with more fully at another place in this paper. Certainly the nature of these processes whereby calcium is taken out of circulation and held by the soft tissues invites more study. This study is only a beginning in the study of tissue calcium. Future work must deal with the problems of the normal content of various tissues and the tissue spaces, the forms in which it is held in the tissues and the role it plays in normal cell metabolism.

B. The Disappearance of Calcium Added in the Form of Calcium Chloride to the Starling Heart-Lung Preparation.

Having shown that there was a rapid disappearance of injected calcium in preparations containing only soft tissues, it would be interesting to see if a similar thing would occur in preparations containing only a few organs. The heart-lung of Starling offers an ideal opportunity to do just that. The data from three satisfactory preparations is given in the table below. (TABLE VIII)

TABLE VIII

THE DISAPPEARANCE OF CALCIUM ADDED AS CALCIUM CHLORIDE TO A STARLING HEART-LUNG PREPARATION.

Number Exper.	Ca. Inj. per 100 cc. Plasma.	Normal Serum Ca. in Mgm. %.	Serum Ca. In Mgm. % 5 Min. After Inj.	Per cent Inj. Ca. Leaving Blood First 5 Minutes.	At Other Periods. Ca. in Mgm. %
28	43 mgm.	7.8 mgm.	33.8 mgm.	39.5	After 45 min. 28 mgm.
32	28.8 "	8.4 "	23.0 "	49.3	After 75 min. 20.0
53	42.0 "	8.0 "	27.0 "	54.7	After 53 min. 18.2

A comparison of the disappearance curves obtained in normal dogs with those in which the skelton had been tied off from the circulation and with the heart-lung preparations shows in general the same contour,

particularly during the first few minutes after-injection. There is first a rapid fall immediately after the injection of calcium chloride and then a very slow decrease. It will be seen from an examination of both TABLES VII and VIII that there is still some further loss of blood calcium after the first few minutes, although this takes place more slowly. In experiment 53 (heart-lung) there was a loss of 8.8 mgm. between the 5th and 53rd minutes and in number 43 (soft tissue preparation) there was a loss of 3.8 mgm. between the 5th and 145th minutes. While it has been shown that the bone trabeculae stores calcium on a high calcium diet (Bauer, Aub and Albright 1929) this process may be much slower than the rate of disappearance here observed. These experiments show that there is a rapid loss of injected calcium salts from the blood stream, this taking place in the complete absence of bone tissue.

The data here reported is in general agreement with the hypothesis of Greville (1931) who suggested, on the basis of the normal disappearance curve, that there is a rapid diffusion into the tissue spaces until some kind of an equilibrium is reached between these and the blood stream. It may be further stated,

that there is a rapid diffusion into the tissue spaces until some kind of an equilibrium is reached between these and the blood stream. It may be further stated, that there is a rapid diffusion into the tissue spaces immediately after injecting a hypertonic calcium chloride solution followed by some slower process which is capable of further reducing the blood calcium below the level resulting from the rapid diffusion into the tissue spaces. Just what this process is, it is not possible at the present time to say. It does not involve the skeleton, although the bones may be necessary before calcium equilibrium can be regained.

IV. THE CALCIUM CONTENT OF SOME SOFT TISSUES FOLLOWING THE INTRAVENOUS INJECTION OF CALCIUM CHLORIDE.

Our knowledge of the calcium content of the soft tissues is all too meager. That it is very variable was first shown by Parhon, Dumitresco and Nissipexco (1909) and since confirmed by Rona and Heubner (1918). The calcium content of the tissues was reported increased following high calcium intake by Voorhoeve (1911-13). Similar findings are reported by Kost (1918) for some organs (blood, liver, spleen, kidney and intestine). Heubner and Rona (1923) made a careful analysis of the tissues of cats for calcium after chronic and acute poisoning with calcium chloride. They could find no significant increase in their calcium content.

However, since their animals were not killed for analysis until 2 to 18 hours after the last calcium injection, it is possible that the calcium equilibrium of the tissues had been restored to or near normal. Hecht (1924) and Jungmann and Samter (1924) report similar findings after calcium chloride, acetate or hexose phosphate. Yet, examination of their data shows increases in the skin in some instances, large amounts of calcium in the small intestine, large intestines, contents of the large intestine, and the kidneys, particularly after the injection of calcium hexose phosphate. Hetenyi (1924) believes he can account for 78 per cent of the injected calcium in the bones at the end of three hours. There is no general agreement regarding normal calcium content of the tissues or the effect of injection of calcium on that content. Having demonstrated the rapid disappearance of the calcium excess into the soft tissues from the blood stream, a study was made of the calcium content of some of these in an attempt to detect this increase which must have occurred.

METHOD.

Various methods of tissue analysis for calcium have been published. A few of the more recent ones are; Jansen (1918), Barral and Barral (1928) and Widmark and Vahlquist (1931). Most of the earlier methods required incineration to destroy the organic material. Widmark

and Vahlquist (cf. above) however, devised a technique of analysis in which the destruction of the organic ma terial was carried out in solution by the addition of sulphuric and nitric acids. Calcium was determined by the ordinary oxalate-permanganate method after the complete oxidation of the organic material. The method used in this laboratory makes use of the Bolliger (1932) technique for the rapid digestion of biological materials. The material (usually 4 to 7 gms) is oxidized with perchloric and nitric acids until practically colorless, neutralized to methyl red with ammonium hydroxide and made up to 25 cc. volume. To a 10 cc. portion in a conical centrifuge tube, 2 cc. of 4 per cent ammonium oxalate is added, mixed and allowed to stand over night. This is then centrufuged for 15 minutes at about 2000 revolutions per minute. After washing (cf. the method of Clark and Collip, 1925) the calcium oxalate is dissolved in 3 cc. of approximately normal sulphuric acid and titrated with n/100 potassium permanganate solution, each cubic centimeter of this being equivalent to 0.2 mgm. of calcium.

RESULTS.

To determine the reliability of our method of digestion and titration, the following controls were performed;

1. Blood serum was analyzed by both the Clark and Collip method and after digestion according to the technique of Bolliger (cf. above).
2. Duplicate titrations were made of digested material.

The results of these is shown below.

TABLE IX

THE ACCURACY OF THE ANALYTICAL PROCEDURE

Serum Calcium Determinations

Clark and Collip Mgm. per cent.	After Digestion According to the Technique of Bolliger. Mgm. per cent.	
12.0	11.35	
12.6	13.0	
19.7	20.0	Average. Clark and Collip 13.6 mgm. per cent.
20.5	20.5	
8.0	7.5	Average. After Digestion According to Bolliger's Technique.
9.8	9.4	13.9 mgm. per cent.
13.0	15.5	Average Difference- 5.2 per cent.
		Maximum Error 19%

Duplicate Titrations.

Organ	Calcium in Mgm. Per Cent.	
	O	D
Kidney	22.3	24.0
	18.5	22.0
	8.23	7.2

TABLE IX (cont)

Heart (Vent.)	13.0	15.0
	28.0	29.0
	13.8	13.3
Liver	12.1	10.7
	3.6	3.5
Large Int.	22.1	23.0
	22.6	20.0
	44.2	44.2
	8.4	9.0
Small Int.	16.6	16.0
	11.7	11.6
	9.3	9.9
Lung	34.3	34.8

Average Difference- 0.3 mgm.

Maximum Difference- 3.5 mgm. per cent.

Per Cent Difference- 1.6 per cent.

Per Cent Maximum Difference- 19 per cent.

The above table suggests an average error for the procedure of 5.8 per cent with a maximum error of 38 per cent.

TABLE X

THE CALCIUM CONTENT OF SOME ORGANS FOLLOWING THE INJECTION OF CALCIUM CHLORIDE.

(Ca. in mgm. per cent).

Organ	Controls (4 exper.)	Intact Animal (1 exper.)	Soft Tissue Preparation (2 exper.)	Heart-Lung (1 exper.
Heart	8.6 (4.3-13.8)	12.4	25 (14.0-36)	24.7
Liver		7.0	7.1 (3.6-10.7)	
Kidney	14.1 (4.0-29.0)	15.3	22.0 (20.0-24.0)	
Stomach	10.5 (6.2-15.3)		7.3 (4.5-10.2)	
Large Int.	21.4 (9.0-31.4)	22.1	32.0 (20.0-44.0)	
Small Int. U.	7.0 (2.1-16.6)		6.1	
M.	5.4 (2.1-11.7)	Trace	37.0	
L.	13.5 (9.3-1717)		25.6	
Lung	16.8 (5.6-30.7)	21.9	29.4 (24.9-34.0)	29.0

That data which makes up this table (TABLE X) is from some preliminary studies in a series planned to investigate the normal calcium content of the tissues and some of the factors which influence it. Examination of the above data shows the normal con-

tent of calcium in the tissues is subject to wide variations which is in agreement with all previous studies reported. Values which exceed the maximum error for our procedure were found in the heart and small intestine of preparations containing only soft tissues, following the injection of large amounts of calcium chloride. Values are also high in the large intestines and lungs and in the heart and lungs of the heart-lung preparation analyzed. While no conclusion can be drawn from this meager data, it suggests that there is an increase in the tissue calcium of some organs after the injection of calcium chloride. This is in general agreement with the previous data reported.

Examination of TABLE I Column 3 shows the removal of the alimentary tract below the pancreas greatly interferes with the return of the serum calcium to normal levels although there appears to be a rapid diffusion from the blood stream during the first few minutes following injection. (62 per cent of the injected calcium being removed during the first few minutes following injection which compares favorably with controls in which 69 per cent was found to have left the blood stream during the same period). Whereas, in the intact animal 95 per cent had left during the first three hours, in those without kidneys or alimentary tract below the pancreas

only 84 per cent had been removed from the circulation. Analysis of the large and small intestines for calcium in the experiments here reported suggest the calcium content of these may be significantly higher thirty minutes after injection (when the experiments were terminated). While more study is required on this point, study now in progress, yet there is a suggestion from this and previous data given that these organs are capable of "binding" a portion of the excess injected and thus holding it until it can be stored in the bones or excreted. It would thus be rendered biologically inactive (non-ionized and non-diffusible). It is not improbable that this exists in some equilibrium with the blood stream so that its elimination by storage in the bones or excretion will gradually restore the calcium content of the soft tissues to normal.

IV. DISCUSSION AND SUMMARY.

Benjamin and Hess (1933) in a recently published article suggest there are four forms of calcium in the blood stream, two diffusible and two non-diffusible. Of the diffusible calcium, about two thirds is in the form of an adsorable calcium-phosphorus complex; the remainder contains the calcium ion. Of the non-diffusible calcium, about one quarter is adsorable and the remainder is probably bound to the plasma proteins. In hypercalcemia induced by the subcutaneous injection of calcium lactate, there was an increase in the serum calcium from a normal of 11.0-11.6 mgm. per cent to 15.3 mgm. per cent. The protein bound fraction was not significantly altered (normal 4.3 mgm. per cent was increased to 4.6 mgm. per cent) but there was an increase in the adsorable complex from a normal of 3.9-4.9 mgm. per cent to 7.2 mgm. per cent. The supposedly ionized portion was increased from a normal value of 2.9 to 3.5 mgm. per cent. Thus we see there is a disproportionately large formation of some non-ionized calcium complex following the injection of a soluble calcium salt. Brull (1930) making use of a vivi-diffusion technique finds a similar normal partition in the blood stream. He finds a large portion of the calcium added as calcium chloride rapidly becomes

non-diffusible. He further shows that the addition of an ionized salt of calcium to the heart-lung preparation causes an immediate reduction of systolic and diastolic volume and an increase in the duration of the contractions, results which would be expected from many experiments which have been performed on the heart of lower animals. However, this effect rapidly disappears and normal heart action is restored. (We have noticed a similar response in our heart-lung experiments). Since no such accomodation as this is known to occur with the isolated heart, it suggests that the injected calcium is rapidly deionized by the organism. Miss Benjamin (cf. above) has found such a form and has shown that it is increased after the injection of calcium lactate, increased more than other forms of calcium found in the blood stream.

Thus we are led to construct the following picture of the events which occur following the injection of calcium chloride:- There is a rapid diffusion of the injected calcium into the tissue spaces accompanied by the deionization of the injected calcium, possibly part of the deionized portion being represented by the calcium complex of Benjamin and Hess. Excretion plays little or no role in the rapid disappearance observed and it would seem from the work of others (Rey 1894-95, Hetenyi

1924, Salveson, Hastings and MacIntosh 1924, Taylor and Fine 1930, and others) that a portion may be retained for several days after injection. Brull (1930) suggests there is an excretable form of calcium (not ionized). Excretion would then depend upon the production of this form rather than the presence of an excess calcium in the body fluids. After this rapid diffusion and the resultant equilibrium the calcium content of the blood and tissue spaces is considerably higher than normal. Reactions of a slower nature then become apparent; reactions which are capable of disposing of this excess. That bone, particularly cancellous bone, does take up and hold a portion of the excess calcium absorbed on a high calcium diet has been demonstrated in a series of clever experiments carried out by Bauer, Aub and Albright (1929). This suggests itself as the logical site for the storage of the excess calcium removed from the blood and tissues, although a small amount may be excreted. Since calcium disappearance curves obtained from animals containing only soft tissues demonstrate both the rapid diffusion and the slower decrease in calcium which becomes apparent after this rapid diffusion, the deposition in the bones would appear to be the terminal phase of a complex reaction in which the tissues

probably play an important role.

SUMMARY.

1. Intravenously injected calcium chloride rapidly leaves the blood stream. Following the intravenous injection of 60 mgm. of this salt per kilogram body weight as a 5 per cent solution, 69 per cent was found to have left the blood stream during the first five minutes and 95 per cent had left within 3 hours.

2. This rapid disappearance does not depend upon excretion, since it occurs in the absence of the intestine and kidneys. This is further confirmed by attempts to recover excreted calcium from the large intestine. Only small amounts could be obtained 3 hours after injection.

3. Removal of the large intestine in some way retards the restoration of the normal level of serum calcium after the injection of calcium chloride. The probable significance of this is discussed.

4. The rapid disappearance of injected calcium chloride observed in the intact animal can be observed in preparations containing no bone

tissue. It is concluded that this reaction depends upon the blood and soft tissues alone, although the skeleton may be necessary for the restoration of the normal level of calcium in the blood and tissues.

5. Some preliminary analyses of various organs for calcium following the intravenous injection of calcium chloride lend further support to the concept that a large amount of the calcium excess may have diffused into the tissue spaces.

ADDITIONAL TABLES

BIBLIOGRAPHY

TABLE XI

ANALYSIS OF BLOOD FOR CALCIUM FOLLOWING THE INJECTION OF CALCIUM CHLORIDE INTRAVENOUSLY.

($CaCl_2$, 60 mgm. per kilogram body weight injected in all cases).

CONTROLS.

Time after Injection.	Experiment # 1	Experiment # 5	Experiment # 18	Average
0.	10.3	11.6	11.7	11.2
5.	19.0	20.8	25.8	21.9
15.	16.4	18.3	21.3	18.8
30.	13.8	16.7	18.7	16.4
60.	13.0	14.3	16.8	14.7
90.	12.7	13.3	15.5	13.8
120.	12.3	13.3	14.8	13.5
150.		13.2	13.5	13.4
180.		12.3	13.2	12.8
220.			13.0	

TABLE XI (cont.)

AFTER THE REMOVAL OF THE KIDNEYS

Time after Injection.	Exper. # 15	Exper. # 19	Exper. # 26	Exper. # 27	Exper. # 29	Exper. # 37	Average
N.	10.2	11.7	9.9	11.8	12.6	10.4	11.1
5.	20.0	20.5	18.6	21.4	21.5	18.9	19.9
15.	17.0	16.8	15.8	-	18.7	18.0	17.2
30.	14.5	15.7	15.7	-	17.3	15.2	15.4
60.	13.3	15.0	15.5	-	-	-	14.5
90.	12.6	14.2	-	-	15.9	12.2	13.7
120.	12.6	13.2	14.1	-	-	-	13.3
150.	11.8	12.7	-	-	-	-	12.2
180.	10.8	12.5	13.3	13.3	14.8	11.8	12.6

TABLE XI (cont).

AFTER THE REMOVAL OF THE LARGE INTESTINE AND KIDNEYS

Time after Injection.	Experiment # 9	Experiment # 10	Experiment # 11	Average
N.	9.5	11.0	10.5	10.3
5.	23.0	26.4	19.6	23.0
15.	19.0	22.0	18.5	19.8
30.	17.0	21.8	16.1	18.3
60.	17.0	21.8	15.9	18.2
90.	16.5	21.0	14.8	17.4
120.		17.6	14.8	16.2
150.				
180.		16.5	14.2	15.4

TABLE XI (cont.)

AFTER THE REMOVAL OF ALL INTESTINES AND KIDNEYS.

Time after Injection.	Exper. # 3	Exper. # 7	Exper. # 14	Exper. # 16	Exper. # 17	Exper. # 20	Exper. # 21	Average
N.	11.4	10.8	10.5	9.8	10.4	10.5	11.7	10.7
5.	23.5	23.4	26.3	24.8	23.7	24.0	22.0	23.9
15.	20.0	19.3	21.8	21.3	21.8	21.7	19.0	20.7
30.	19.0	18.3	20.5	19.1	17.7	19.5	18.0	18.9
60.		18.6	19.5	17.2	17.7	18.5	18.0	18.3
90.	19.0	18.5	18.0	16.5	17.0	18.3	18.0	17.9
120.	19.0		17.0	16.5	16.8	17.5	17.7	17.4
150.			16.5	15.1	16.8		17.7	16.5
180.				15.2	17.0			16.1
				14.7				

TABLE XII

THE DISAPPEARANCE OF INTRAVENOUSLY INJECTED CALCIUM CHLORIDE AFTER TYING OFF THE BLOOD VESSELS TO THE SKELETON (INCLUDING SOME OF THE VESSELS TO SKELETAL MUSCLE).

Time After Injection.	Exper. # 36 66 mgm. Ca. per 100 cc. plasma.	Exper. # 40 55 mgm. Ca. per 100 cc. plasma.	Exper. # 41 47 mgm. Ca. per 100 cc. plasma.	Exper. # 43 50 mgm. Ca. per 100 cc. plasma.	Exper. # 45 38 mgm. Ca. per 100 cc. plasma.	Exper. # 49 45 mgm. Ca. per 100 cc. plasma.	Exper. # 50 50 mgm. Ca. per 100 cc. plasma.
N.	10.3	10.0	10.8	10.5	11.2	10.3	10.2
5.	25.4 (6 min.)	19.0	27.8	31.8	28.2	30.5	
15.		17.6	27.2	27.3	27.4 (18 min.)	24.7	38.0 (10 min.)
30.	24.6 (24 min.)	17.2 (50 min.)	24.2 (45 min.)	24.4		24.3	35.7
60.	24.0			24.0			
90.		18.0	24.3		27.1		
120.	22.8 (103 min.)	17.3	21.0				
150.		17.2 (168 min.)		22.6			
180.							

TABLE XIII

THE DISAPPEARANCE OF CALCIUM ADDED AS CALCIUM CHLORIDE TO A STARLING HEART-LUNG PREPARATION.

Time after Injection in Minutes.	Experiment # 28. 43 mgm. Ca. per 100 cc. plasma.	Experiment # 32. 28 mgm. Ca. per 100 cc. plasma.	Experiment # 53. 42 mgm. Ca. per 100 cc. plasma.
N.	7.8	8.4	8.0
5.	33.8	23.0	27.0
15.	32.8	22.5	27.4
30.	28.0 (45 min.)	21.6 (45 min.)	19.6
60.			18.2 (53 min.)
90.	28.0	20.0 (75 min.)	

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SOME OBSERVATIONS OF THE EFFECTS OF LARGE DOSES OF IRRADIATED ERGOSTEROL

INTRODUCTION--(Historical)

In the development of civilizations, particularly of Western Civilization, the rise of urban centers somewhat removed from the sources of raw foods has resulted in the incorporation of large amounts of processed foods and of cereals into the daily dietary. The substitution of these for the "cruder" foods of the primitive and early agricultural stages of development was not without ill effects on the normal functions of the human body. While it is sometimes difficult to reconstruct the food habits of prehistoric peoples, it has been shown by many recent studies on the dietary habits of primitive man (Semple 1911; Hooton 1918; Ekblaw 1921; and others) that excellent health is generally maintained. It is also significant that deficiency diseases are rarely found among animals living away from man. Gradually these diseases came to be recognized and some knowledge of their cause and cure was developed through mass experience. The use of citrus fruits and wine in the prevention of scurvey, of cod-liver oil in the prevention of rickets and in the encouragement of growth, the use of whole cereals in the prevention of beri-beri long antedate the science of nutrition and our knowledge of the vitamins.

The beginnings of modern nutrition may be said to

begin with the studies of Voit (1881) in Germany and of Atwater and associates (1895) in America. Their studies turned attention to the composition of food and the importance of each constituent in animal nutrition. It was soon realized that there were certain normal constituents of the diet not found in purified food mixtures (containing only proteins, carbohydrates, fats, mineral matter and water) that were necessary for growth and well being of the animal organism (cf. Hopkins 1906, 1912; McCollum 1909; Pekelharing 1905). The name "vitamine" was first applied to certain of these accessories by Funk (1912).

The development of our knowledge of vitamin D is of particular interest to the present study. The preventive and curative value of cod-liver oil in rickets and its general value in a great variety of illnesses has long been known. It seems to have been in common use in 1789 when we first find mention of it in English medicine by Darbey of the Manchester Infirmary. More accurate knowledge of the specific agents in this oil that are of value in medicine has only recently been developed. The English Physiologist Mellanby (1918-1919) is generally credited with the discovery of the specific antirachitic factor in cod-liver oil.

McCollum and his associates (1922) demonstrated the presence of two distinct fat soluble vitamins in cod-liver oil, the easily oxidized A and the more stable D. This important discovery was soon followed by the independent and almost simultaneous discovery by Hess (1924) and Steenbock and Black (1924) that certain substances become antirachitic upon irradiation. Of these, ergosterol, a sterol found abundantly in ergot and yeast, is widely used in the prevention and cure of rickets.

THE RESPONSE OF THE SERUM CALCIUM AND INORGANIC PHOSPHORUS OF THE CAT TO LARGE DOSES OF IRRADIATED ERGOSTEROL.

Rosenheim and Webster (1927) are generally credited with the discovery that irradiated ergosterol is one of the most important sources of the antirachitic agent. Since that date there have been a great many studies carried out to determine the physiological action of this substance. In light of the disturbed calcium and possibly phosphorus metabolism which occurs in rickets and the curative action of irradiated ergosterol, a great deal of interest is attached to the effect of this substance upon the level of these elements in the blood stream.

Hess and Lewis (1928) studying clinical cases of rickets found irradiated ergosterol, cod-liver oil or both raised the serum calcium in all cases to or above normal levels. The inorganic phosphorus was reported raised in three, unchanged in two and lower in one. In twelve normal infants studied the serum calcium was raised in all while the inorganic phosphorus was found to be raised in one, unchanged in six and lowered in one. Hess, Weinstock and Rivken (1929) report in experimental animals that irradiated ergosterol increased both the serum calcium and phosphorus. They found

blood inorganic phosphorus values in dogs as high as 13 to 14 mgm. per cent several days after treatment had begun. Harris, Stewart and Moore (1929) found in rats, on a synthetic diet with a normal calcium-phosphorus ratio, the administration of 0.1 per cent of irradiated ergosterol was followed by a 50 per cent increase in blood inorganic phosphate and a 25 per cent increase in the serum calcium. In rabbits receiving a normal mixed diet, the administration of ten milligrams per diem of irradiated ergosterol per animal was followed, after upwards of a fortnight at the normal level, by a 50 per cent increase in the blood inorganic phosphate. There was no significant rise in serum calcium although abnormal deposits of calcium had already appeared in the body. Jones, Kapoport and Hodes (1930) found the daily administration of 10 mg. of irradiated ergosterol to normal dogs produced a marked hypercalcemia within two weeks. They found no constant effect on the concentration of the inorganic phosphorus of the blood. Ashford (1930) found in rabbits an increase in the blood inorganic phosphorus and calcium. There was no change in the organic acid-soluble fraction. Shohl, Goldblatt and Brown (1930) reported in rats the serum calcium was definitely higher and the inorganic phosphorus were quite variable. They report high values

at certain points but they are not consistently high. While it is generally agreed that irradiated ergosterol causes a rise in serum calcium the response of inorganic phosphorus is more uncertain.

Several investigators have recently demonstrated the toxic effect of large doses of irradiated ergosterol on the growth of the rat and determined the quantity of substance necessary to produce this effect (cf. Rosenheim and Webster 1927; Harris and Moore 1928; Bills and Wirick 1930). There is scant information regarding the level of irradiated ergosterol intake that will cause definite alterations in the serum calcium and possibly the inorganic phosphorus. Kramer, Shear and McKenzie (1929) found the administration to the rat of 100,000 times the curative dose for two weeks followed by 20,000 times the curative dose for another two weeks resulted in an increase in serum calcium of 5.2 mgm. per cent over the controls (control average 10.9 mgm. per cent). The values for the inorganic phosphorus were not reported. Harris, Stewart and Moore (1929), as previously stated, produced rises of serum calcium of 50 per cent when irradiated ergosterol was administered to rats as 0.1 per cent of the diet for a period of 14 days.

In the course of some experiments carried out in

this laboratory on the effect of irradiated ergosterol on the normal cat, a study was made of the serum calcium and inorganic phosphorus. The present section presents the following;

1. The toxicity of irradiated ergosterol to the cat.
2. The response of serum calcium and phosphorus to non-toxic and toxic levels of intake of irradiated ergosterol.

METHODS.

Young cats were confined to small laboratory cages in a room with a fairly constant temperature (about 25° C. with a maximum variation of less than ten degrees Centigrade. They were given a generous diet of whole milk, ground hamburger and whole wheat bread. Irradiated ergosterol was administered in corn oil, the oil being given per os. One cubic centimeter of the following dilutions were administered daily, 25 D, 50 D, 100 D, 150 D, 200 D, 250 D and 1000 D. The duration of the administration of oils was for a period of 21 days in most instances (see table 2 for the exception). Serum calcium was determined by the Clark and Collip (1925) modification of the technique of Kramer and Tisdall (1921). Inorganic phosphorus was determined by the Youngberg (1930) technique.

1. The Toxicity of Irradiated Ergosterol to the Cat.

Examination of table shows that irradiated ergosterol produced no signs of toxicity until after the administration of 1 cc. of irradiated ergosterol (250 D) daily, an amount equal to 1250 rat units per kilogram body weight. There was considerable variation in general nutrition at the border line of toxicity (200 to 250 D). Two animals receiving one cubic centimeter oil (200 D) daily showed serum calcium levels above

normal but with no loss in weight (gained 360 and 145 gm. respectively). The two receiving irradiated ergosterol in a potency of 250 D lost 100 and 500 gms. respectively with a serum calcium level above normal. In general the toxicity was most severe in those with the highest serum calcium values but it did not follow closely the level of serum calcium.

The most cha racteristic finding in cats suffering from toxic ammounts of irradiated ergosterol was the loss of appetite which usually was apparent after 10 to 14 days after the administration of oils was begun. Then followed emaciation, lethargy and general weakness leading up to prostration. Just before death there was a stiffness of the whole body with fine tremors of the muscles of the limbs and back developing frequently. Kreitmair and his co-workers, Moll, Hintzelmann and Wenzel (1928) studied the toxicity of irradiated ergosterol in a great many animals, including the cat. They state the cat was the most sensitive animal of all to calcium deposition in the tissues. While tissue studies were not made here, it is not improbable that such deposits occurred and may have been the cause of the rigor of severe poisoning.

These observations on the toxicity of irradiated ergosterol are in general agreement with other in-

vestigators who have made similar studies. It may be stated that the administration of 1250 or more rat units per day per kilogram body weight is followed by toxic symptoms within ten to fourteen days. This was always accompanied by a rise in serum calcium.

2. The Response of Serum Calcium and Inorganic Phosphorus to Non-toxic and Toxic Levels of Intake of Irradiated Ergosterol.

Examination of data (table 1) shows no significant alteration in the level of serum calcium until an amount of irradiated ergosterol equivalent to 1250 rat units (Bills 1931) per kilogram body weight was given. In general, the increased concentration of calcium in the blood stream followed the increase in irradiated ergosterol given even though the response shows considerable variation from animal to animal (in one experiment no change in calcium was observed). At no level of serum calcium was there any significant change in the concentration of inorganic phosphorus. Massengale and Nussmeier (1930), studying the effect of diet and irradiated ergosterol on the chicken, report that when the calcium/phosphorus ratio of the diet is exceedingly high irradiated ergosterol raises the serum calcium but has little effect on the inorganic

phosphorus. It is improbable that this plays any role in the experiments here reported since the diet given contains a normal ratio of calcium and phosphorus. Irradiated ergosterol produces a rise in the serum calcium of cats without any significant change in the concentration of the inorganic phosphorus.

Table 1.

The Concentration of Calcium and Inorganic Phosphorus of the Blood after the Administration of Irradiated Ergosterol.

Number of Expers.	Treatment	Gain or Loss in Weight	Serum C. Mg. %	Inorg.P. Mg. %
10	Irrad. erg. up to 765 rat units/ kilogram body weight.	Gained 180 gms.	10.6 (10.0-11.1)	6.3 (4.7-7.2)
4	Irrad. erg. from 1250 to 1750 rat units/ kilogram body weight.	Lost 24 gm.	13.8 (12.4-15.6)	6.8 (5.8-7.3)
5	Irrad. erg. from 5200 to 9200 rat units/ kilogram body weight.	Lost 277 gms.	17.8 (10.5'-25.2)	6.5 (6.3-7.0)

(' One animal failed to show a response to treatment. Next lowest value is 13.3 mg. per cent).

In a second series of four adult cats, three were given irradiated ergosterol (1000 D-Bills and others 1931) in corn oil, 9 cc. being given over a period of six days. On the sixth day after beginning the administration of oil the serum calcium was 12.0, 13.7 and 14.5 mg. per cent respectively (see table 2). After 23 days the serum calcium was still above normal in two cases. No significant alterations in the inorganic phosphorus were found.

Table 2.

The Serum Calcium and Inorganic Phosphorus after the Administration of 9 cc. of Irradiated Ergosterol (1000 D).

Days	Control		Exper. 1		Exper. 2		Exper. 3	
	Ca.	P.	Ca.	P.	Ca.	P.	Ca.	P.
1	10.3	5.3	9.8	-	10.1	5.1	10.7	5.9
6	9.8	5.1	9.7	5.1	9.8	5.4	10.7	5.5
	Administration of Irradiated Ergosterol Begun.							
12	9.3	4.7	13.7	4.9	14.5	5.1	12.0	5.9
	Administration of Irradiated Ergosterol Terminated.							
21	10.4	6.0	10.5	5.1	14.0	4.7	15.6	5.3
29	11.8	6.7	—	—	14.4	3.0	12.4	3.0

These observations on the duration of the hypercalcemia induced by irradiated ergosterol are in agreement with those of Hess and others (1929) on infants and of Reed and others (1933) on dogs, both studies indicating the hypercalcemia long outlasts the period of treatment.

SUMMARY.

1. The administration of 1250 or more rat units daily of irradiated ergosterol was found to be toxic to young cats. With the smaller doses, toxic symptoms were manifest within ten to fourteen days. With larger doses, they appeared somewhat sooner and were often quite marked at the end of fourteen to twenty-one days. The symptoms of toxicity are depression, loss of weight and loss of appetite accompanied by an elevation of the serum calcium.

2. Toxic doses of irradiated ergosterol may produce marked elevations of the serum calcium without any significant alterations in the concentration of the inorganic phosphorus of the blood.

3. The hypercalcemia induced long outlasts the period of administration of irradiated ergosterol.

(All irradiated ergosterol used in these experiments was furnished through the courtesy of Mead, Johnson and Co.)

THE SIZE AND STRUCTURE OF THE THYROID GLAND OF THE CAT AFTER IRRADIATION OR THE ADMINISTRATION OF IRRADIATED ERGOSTEROL.

Many recent investigations have shown that sunlight, particularly the ultra-violet portion of the spectrum, plays an interesting role in animal physiology. Considerable interest is attached to the extensive experiments carried out by Brown, Pearce and Van Allen (1923-24), Pearce and Van Allen (1926) and Brown (1929) who have definitely demonstrated seasonal changes in organ weights of the rabbit. While there are many factors that may be involved, yet these investigators believe the changes observed are more closely correlated with sunshine than any other seasonal variations. Seasonal changes in the structure of the thyroid gland, a hyperplasia occuring during the winter months, have been frequently reported (Seidell and Fenger 1912; Herzfeld and Klinger 1922; Kendall 1921). That rickets develops in young animals when deprived of sunlight is well known. It is of interest here to note that Murray (1923), Sorour (1923) and Stoltenberg (1929) found hyperplasia of the thyroids of rachitic animals. Sorour (1923) states this hyperplasia is particularly marked in rats kept in darkness.

Bergfeld (1930) found a definite relationship between the function of the thyroid of the rat and light, darkness interferring with proper functioning. Rosenkranz (1931) found that rabbits kept in a dark room or oxen confined to stalls show considerable increase in the parenchyma, with a decrease in the number of follicles and colloid content. Animals kept on the same diet and exposed to sunlight did not show these changes. These findings suggest there is some relationship between the structure of the thyroid gland and sunlight. While temperature and other factors (Cramer 1916; Mills 1918) may play a role in producing these seasonal changes in structure, there is a definite suggestion that the so-called "summer" and "winter" type glands may result from the different amounts of sunlight, particularly the ultra-violet portion found at these seasons. That certain substances become biologically active upon irradiation with ultra-violet is well established. The present study was carried out to determine the effect of one of these, irradiated ergosterol, and of direct irradiation upon the size and structure of the thyroid glands of experimental animals (young cats) under controlled laboratory conditions.

Methods.

Young cats were confined to small laboratory cages. These were kept in a room with a northern exposure, the light coming through two large windows. The temperature was kept fairly constant at about 25°C. (maximum variation less than 10°C.). They were fed a liberal diet of hamburger, raw milk and whole wheat bread. Irradiated ergosterol was diluted to the desired potency with corn oil and administered per os. The following dilutions 25 D, 50 D, 100 D, 150 D, 200 D, 250 D and 1000 D were used, each cat receiving one cubic centimeter of oil per day. The irradiated animals were exposed to a carbon arc (Everready "C" carbons; 8 amp. 45 volts A.C.) for thirty minutes daily at a distance of one meter. The duration of irradiation and the administration of oils was for twenty-one days, after which time the cats were killed, the thyroids removed, weighed and microscopic sections prepared. All experiments here reported were carried out during the months from Oct. 14 to March 21.

Results.

1. The Microscopic Structure of the Thyroid Gland after the Administration of Irradiated Ergosterol

Examination of data (see table) indicates that

there were no significant changes in the structure of the thyroid glands of cats receiving irradiated ergosterol until after the administration daily of one cubic centimeter of oil containing a potency of 250 D or more. A slight increase in the colloid content of those receiving one cubic centimeter of cod-liver oil daily was observed, however the recent observations of Levine, Remington and Von Kolnitz (1933) suggest the high iodine content of this oil rather than a specific effect of the vitamins it contains may have been the cause of the changes observed. Corn oil, the solvent used in making these irradiated ergosterol dilutions contains little or no iodine. The administration of one cubic centimeter of irradiated ergosterol daily of a potency of 250 D or more resulted in a definite change in the microscopic structure of the thyroid gland. Whereas in cats receiving no oil the vesicles were small with many completely collapsed, those receiving irradiated ergosterol 250 D or more showed predominance of moderate and large vesicles and abundant colloid. The height of the lining epithelium varied with the size of the vesicle, being columnar in the very small or collapsed vesicles and flattening out to cuboidal in the larger vesicles.

Marine and Lenhart (1911) called attention to

the extremely sensitive nature of the thyroid, which fact has been amply confirmed by many studies since that date. This advises caution in attributing the observed changes in structure to any one factor. Following the administration of large amounts of irradiated ergosterol there is a hypercalcemia, loss of appetite and depression. These symptoms were always manifest in those animals showing the most marked changes in the microscopic structure of the thyroid. Watson (1907), Missiroli (1910), Douglas (1915) and Jackson (1916) have shown that inanition may cause changes in the structure of the thyroid similar to those found above. It is possible that the inanition resulting from the administration of toxic amounts of irradiated ergosterol may have caused the changes in structure observed here. Mills (1918) found morphine decreased the activity of the thyroid. Hypercalcemia and the resultant depression may exert a simila r action. More study is required before a direct correlation between these changes and irradiated ergosterol can be established. Non-toxic amounts cause no changes in the microscopic structure of the thyroid gland of young cats.

2. Changes in the Microscopic Structure of the Thyroid Gland Following Irradiation.

After irradiation with the "C" carbon arc there was a definite increase in the colloid content of the thyroids in three out of eleven experimental animals. In three others there was a slight increase over the assumed normal (taken for comparison) while five showed no change. In all of these experiments the serum calcium was normal and the cats were in a good nutritive state. In twenty-one non-irradiated animals (6 controls without oil, 4 receiving 1 cc. of corn oil daily, 1 receiving 2 cc. of corn oil daily and 10 receiving non-toxic doses of irradiated ergosterol in corn oil) there was a definite increase in the size of the vesicles and the colloid content in one, a slight increase in four and no change in sixteen. From this data it would seem that irradiation may cause changes in the structure of the thyroid gland toward the resting type although this was not a constant finding. Since the "C" carbon arc emits a large per cent of its total energy between 3200 and 4000 Å. it is possible that other than the "vital" spectrum (3200 to 2900 Å.) could bring about these changes.

3. The Weight of the Thyroid Gland Following Irradiation or the Administration of Irradiated Ergosterol.

Marine and Lenhart (1909) found the stainable colloid of the thyroid varies directly with its iodine

content. Seidell and Fenger (1912) observed that there was, in general, an inverse relation between the size of the gland and its iodine content. With these observations in mind, an attempt

The Weight and Microscopic Structure of the Thyroid Gland of Young Cats Following Irradiation or the Administration of Irradiated Ergosterol.

Number of Expers.	Treatment.	Wt. of the thyroid gland at the end of the experiment in per cent of body wt. at the beginning of the experiment.	Microscopic Appearance.
4	None	0.0125	Vesicles small or collapsed. Cells generally columnar. Moderate amount of colloid in 5. Slightly more colloid in one.
2	None	—	
4	Corn oil 1 cc. per day.	0.0076	Slightly more colloid in 1. No change in 4.
1	Corn oil 2 cc. per day.		
2	Cod-liver oil. 1 cc. per day.	0.0075	Vesicles somewhat larger than in controls. Moderate increase in colloid content of all glands.
1	Cod-liver oil. 2 cc. per day.	0.019	

10	Irrad. Erg. 25 to 250 D 1 cc. per day.	0.0092	No change in 9. Slightly more colloid in one.
2	Irrad. Erg. 250 D. 1 cc. per day.	0.0077	Vesicles moderately enlarged. Considerably more colloid than controls.
5	Irrad. Erg. 1000 D. 1 cc. per day.	0.0151	Vesicles large and well filled with colloid.
6	Irrad. with "C" carbon arc 30 min. daily.	0.0118	Definite inc. in the size of the vesicles and colloid content in 2 animals. Slight in 1. No change in 3.
5	Same as above except backs were shaved before irradiation.	0.0073	Increase in the size of the vesicles and an increase in colloid content in 1. Slight increase in 2. No change in 3.

*(Since there were alterations in body weight, sometimes marked during the experimental period, it would be difficult to get an accurate comparison of the change in gland weight, if any, expressed in terms of the final weight. A more accurate basis of comparison is obtained if one expresses the weight of the gland at the end of the experiment in per cent of the body

weight found at the beginning of the experiment. This would show changes in gland weight expressed in terms of a common unit, that is the normal body weight).

was made to determine if there was any correlation between the weight of the gland and its content of colloid under the conditions of these experiments. The normal weight of the thyroid of the cat was found to be quite variable, there being only a low correlation between body weight and gland weight. There was no correlation found between the weight of the gland and its content of colloid or the experimental procedure in these experiments.

The weight of the gland was found to be more constant than body weight, which agrees well with the findings of Jackson (1916) who reports no change in the weight of the thyroid glands of six rats whose body weight had been reduced 29 to 38 per cent by acute inanition. The weight of the thyroids of the experimentals showing the greatest loss of body weight (1000 D) was still high (0.015 per cent of the body weight found at the beginning of the experiment) and was not significantly different from the control values. Similarly after irradiation with the carbon arc, there was

no constant alteration in the gland weight. It must be concluded that the increase in colloid which occurs in these experiments is not accompanied by any significant change in the weight of the thyroid gland.

Discussion.

The observed seasonal changes in the size and structure of the thyroid gland cannot be produced by the administration of non-toxic amounts of irradiated ergosterol to the cat. However, there is some evidence that direct radiation may cause the production of the so-called "summer" or resting type glands. If the seasonal changes observed result from the greater amount of ultra-violet light in summer sunshine with the resultant greater irradiation of the skin sterols, the action of these is not identical with irradiated ergosterol. Recent observations by Barnes, Brady and James (1930), Hess and Supplee (1929-30), Steenbock, Kletzien and Halpin (1932), and others indicate that antirachitic substances from different sources are not identical. The possibility that other portions of sunlight than the ultra-violet may play role in these changes observed cannot be ignored. This requires more study.

The recent studies of Smith (1932) are of interest here. He finds in districts with a low iodine content

of the soil (silts - mixed mud and sand - from post Tertiary sediments of fluvatile, marine or glacial origin) the incidence of endemic goiter is in fairly close inverse proportion to the radiation index. He states further, the iodine content of potatoes in districts of South Carolina, according to the distance from the sea, appear to vary with solar radiation during the late summer months. These findings suggest irradiation may influence the availability of iodine, its utilization or both, the summer gland resulting from the higher content of iodine in the summer foods or a better utilization of the iodine normally present.

An increase in the colloid content of the thyroid is usually associated with a decrease in weight of the gland (Seidell and Fenger 1912). No correlation was found between the weight of the thyroid and its content of colloid in these experiments. It is possible, due to the wide variations found in normal gland weights, that small differences would not be detected in the small number of experiments here reported.

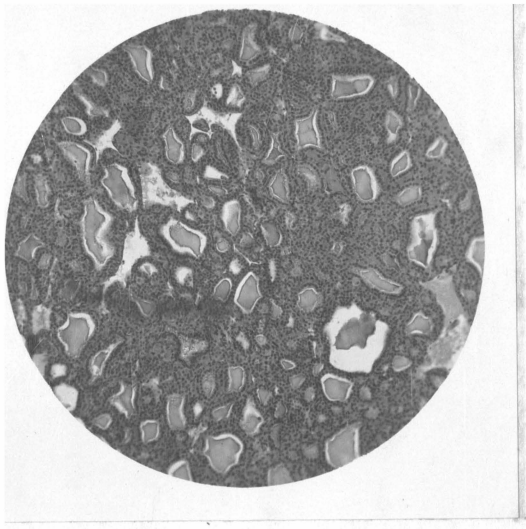
Summary.

1. The administration of non-toxic amounts of irradiated ergosterol in corn oil to young cats caused no significant alterations in the normal structure of the thyroid gland.

2. The administration of toxic amounts of this substance resulted in marked changes in structure (large vesicles well filled with colloid). The appearance was similar to the resting type described by Marine and others. Some of the difficulties to attributing this change to a direct action of irradiated ergosterol are pointed out.

3. There was a tendency toward the resting type thyroid after the irradiation of young cats daily for thirty minutes for a period of 21 days with a "C" carbon arc. There were none of the toxic symptoms mentioned above at any time during the period of irradiation. While the response of individual cats to irradiation is some what variable (six out of eleven showing some change) yet the changes observed seem to be more closely correlated with irradiation than any other observed factors.

4. There were no significant changes observed in the weights of the thyroid glands in any of the above experiments.



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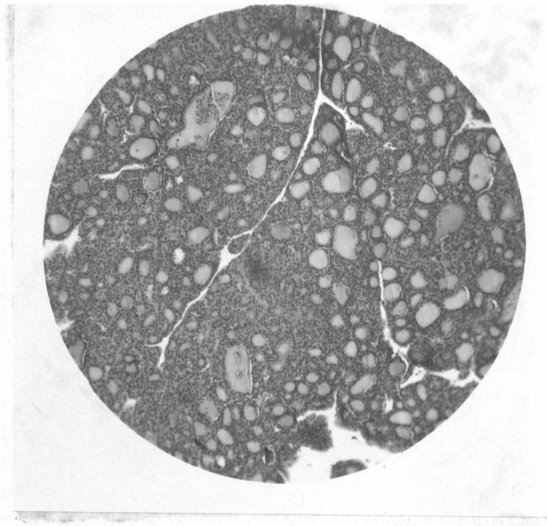
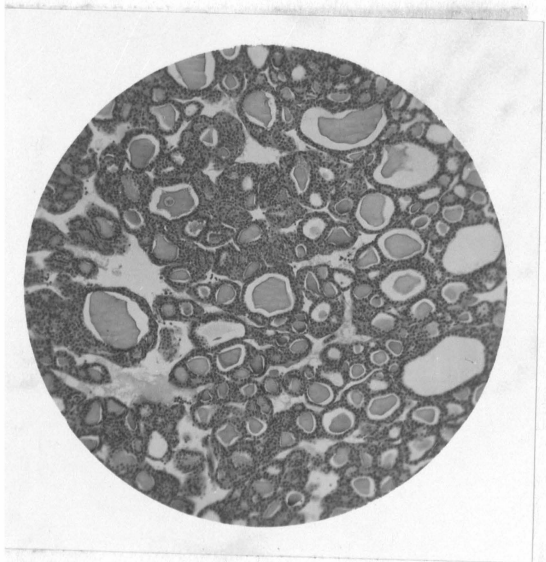
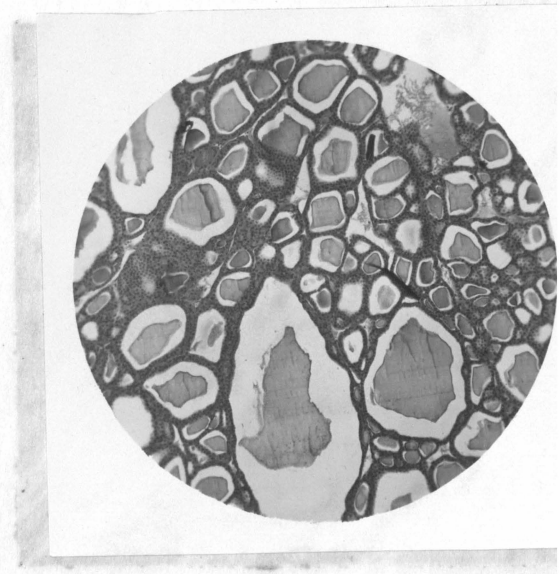


Cod-Liver Oil + +



Irradiated Ergosterol + + 250 D



Irradiated Ergosterol + + + + 1000 D

THE EFFECT OF IRRADIATION AND IRRADIATED ERGOSTEROL.

DATA IN FULL.

THE CONCENTRATION OF CALCIUM AND INORGANIC PHOSPHORUS OF THE BLOOD AFTER THE ADMINISTRATION OF IRRADIATED ERGOSTEROL OR IRRADIATION WITH THE "C" CARBON. ARC.

N. of Exper.	Treatment	Gain or Loss in Weight.	Serum Ca. Mgm. %	Inorg. P. Mgm. %
71	Control	105	10.6	6.7
76	"	190	11.0	
77	"	210	10.0	6.4
79	"	365	10.7	6.5
87	Corn Oil 1 cc. per day.	170	10.5	6.5
111	"	80	10.3	6.8
112	"	230	10.2	4.7
81	" 2 cc. per day.	450	10.3	6.8
91	Cod-liver Oil. 1 cc. per day.	335	10.5	5.5
92	"	595	9.5	5.5
82	" 2 cc. per day.	225	10.0	-
101	Irrad. Erg. 25 D	230	10.2	4.7
102	"	155	10.7	6.6
103	Irrad. Erg. 50 D	280	11.1	6.0
104	"	260	10.0	6.2

N. of Exper.	Treatment	Gain or Loss in Weight.	Serum Ca. Mgm. %	Inorg. P. Mgm. %
105	Irrad. Erg. 100 D	350	11.0	6.6
106	"	200	10.8	6.6
107	Irrad. Erg. 150 D	390	11.0	7.2
108	"	80	10.7	6.1
109	Irrad. Erg. 200 D	370	13.6	7.3
110	"	145	15.6	5.8
89	Irrad. Erg. 250 D	500	13.7	7.0
90	"	100	12.4	7.1
72	Irrad. Erg. 1000 D	296	25.2	6.3
74	"	210	19.0	6.3
83	"	145	10.5	6.7
85	"	280	23.5	-
93	"	60	15.5	6.6
73	Ultra Viol.	119	10.5	6.7
75	"	490	10.0	6.5
78	"	390	10.1	6.4
80	"	300	12.4	7.1
84	"	150	12.1	6.7
86	"	130	9.8	6.5
95	Ultra Viol. (Back Shaved)	125	10.5	7.6

N. of Exper.	Treatment	Gain or Loss in Weight.	Serum Ca. Mgm. %	Inorg. P. Mgm. %
96	Ultra Viol. (Back Shaved)	110	9.8	7.9
97	"	365	9.1	8.5
98	"	110	8.8	7.4
99	"	340	9.7	7.6

IRRADIATED ERGOSTEROL AND IRRADIATION ON THE THYROID GLAND OF THE CAT.

Animal No.	Treatment	Relative increase in the size of the thyroid vesicles and content in colloid.
60	Control	0
64	"	0
71	"	0
76	"	+
77	"	0
79	"	0
63	Corn Oil 1 cc. per day.	0
87	"	+
111	"	+ +
112	"	+ ?
81	Corn Oil 2 cc. per day.	0
91	Cod-liver Oil. 1 cc. per day.	+ ?
92	"	+
65	"	+
82	Cod-liver Oil. 2 cc. per day.	+
101	Irrad. Erg. 1 cc. 25 D daily.	+ ?

Animal No.	Treatment	Relative increase in the size of the thyroid vesicles and content in colloid.
102	Irrad. Erg. 1 cc. 25 D daily.	0
103	Irrad. Erg. 1 cc. 50 D daily.	0
104	Irrad. Erg. 1 cc. 50 D daily.	0
105	Irrad. Erg. 1 cc. 100 D daily.	0
106	"	0
107	Irrad. Erg. 1 cc. 150 D daily.	0
108	"	0
109	Irrad. Erg. 1 cc. 200 D daily.	0
110	"	0
89	Irrad. Erg. 1 cc. 250 D daily.	+ +
90	"	+ +
72	Irrad. Erg. 1 cc. 10000D daily.	+ ?
74	"	+ + + +
83	"	0
85	"	+ + +

Animal No.	Treatment	Relative increase in the size of the thyroid vesicles and content in colloid.
93	Irrad. Erg. 1 cc. 1000 D daily.	+ +
62	"	+ +
67	"	+ + +
73	Irradiated 30 min. daily.	+
75	"	+ ?
78	"	+ +
80	Irradiated 30 min. daily.	C
86	"	0
95	Irradiated 30 min. daily. (Backs Shaved)	0
96	"	+ ?
97	"	+ + +
98	"	0
99	"	-

0- Same as the control. Assumed normal.

\+ Vesicles slightly larger than normal.

\+ + Vesicles moderately larger than normal.

\+ + + Vesicles much larger than normal.

\+ + + + Vesicles markedly enlarged. Abundant colloid.

Number of Experiment	Treatment	Gland Weight at the End of the Exper. in per cent of Body Weight at the Beginning of the Experiment.	
71	Control	0.011	
76	"	0.011	Av. 0.0125
77	"	0.012	
79	"	0.012	
87	Corn Oil	0.008	
111	"	0.007	Av. 0.0076
112	"	0.008	
81	Corn Oil (2 cc.)	0.021	
91	Cod-liver Oil	0.006	Av. 0.0075
92	"	0.009	
82	Cod-liver Oil (2 cc.)	0.019	
101	Irrad. Erg. 25 D	0.010	
102	"	0.010	
103	Irrad. Erg. 50 D	0.0098	25 D to 150 D Inclus. Av. 0.0069
104	"	0.0098	
105	Irrad. Erg. 100 D	0.0082	
106	"	0.0092	
107	Irrad. Erg. 150 D	0.0086	

Number of Experiment	Treatment	Gland Weight at the End of the Exper. in per cent of Body Weight at the Beginning of the Experiment.	
108	Irrad. Erg. 150 D	0.0076	
109	Irrad. Erg. 200 D	0.0103	
110	"	0.0083	200 and 250 D Av. 0.0085
89	Irrad. Erg. 250 D	0.0072	
90	"	0.0082	
72	Irrad. Erg. 1000 D	0.0094	
74	"	0.0131	
83	"	0.0133	Av. 0.0151
85	"	0.0188	
93	"	0.0208	
73	Ultra Viol.	0.0086	
75	"	0.0100	
78	"	0.0152	Av. 0.0118
80	"	0.0101	
84	"	0.0182	
86	"	0.0090	
	Ultra Viol. (Back Shaved)		
95	"	0.0059	
96	"	0.0092	
97	"	0.0072	Av. 0.0073

Number of Experiment	Treatment	Gland Weight at the End of the Exper. in per cent of Body Weight at the Beginning of the Experiment.
98	Ultra Viol. (Back Shaved)	0.0076
99	"	0.0073

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