THE INTERRELATIONSHIP OF ENDOCRINE ORGANS WITH SPECIAL REFERENCE TO THEIR RELATION TO CALCIUM AND PHOSPHORUS METABOLISM AND TO BASAL METABOLIC RATE.

by

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INTRODUCTION.

In 1859 Claude Bernard (1) propounded his theory that certain of the glandular structures, whose functions were then unknown, contributed the products of their activities to the liquor sanguinis, which was essential to health and in many cases to life itself. He gave such products of glandular activity the name of "Internal Secretion".

A few years later J.L. & R. Reverdin (2) and Th. Kocher in 1883 described the peculiar syndrome that resulted from surgical extirpation of the thyroid gland. This was termed "Cachexia Strumipriva" by Kocher and "Operative Myxoedema" by the Reverdins.

These observations of Bernard and subsequently by the Reverdins and of Kocher may therefore be regarded as the starting point of the science of endocrinology, a branch of physiology, the importance and knowledge of which has increased steadily in the hands of physiologists, chemists and clinicians for the last fifty years. The thyroid gland therefore, is considered to be the first endocrine organ to establish/

establish a definite evidence as to the existence and importance of the doctrine of internal secretion.

Endocrinology has attained an outstanding and prominent place in physiology, clinical medicine, and therapeutics. Our knowledge of the functions of some endocrine organs has advanced considerably, but notwithstanding the increasing recognition of the importance of these organs, and in spite of the vast amount of literature on the subject that has accumulated within the last few years, our knowledge of endocrinology and particularly of the interrelationships of these organs is still meagre and fragmentary. Even the part known about this interrelationship is still, in many respects, confused and contradictory.

The endocrine system of the animal body is coming to be regarded as a system controlling the quality and quantity of the metabolic processes occurring in different organs and in the body as a whole. It is to be expected, therefore, that measurements of gross metabolism - of oxygen consumption, nitrogen and mineral balance and so forth - will provide a sensitive index of disturbances in the endocrine system induced experimentally.

The particular interest attaching at present to the functions, known and suspected, of the thyroid gland and hypophyseal body both in physiology and clinical/

clinical medicine, led to their selection as the principle bases of the investigation reported in this thesis in which an endeavour has been made to discover correlations between the activities of these organs using amongst other methods the measurement of metabolic exchanges.

"Nothing about a science is more interesting than the progress of that science" Laennec.

THE ANIMALS USED AND THE TECHNIQUE OF THEIR MAINTENANCE.

The animals used in the biochemical, histological and biophysical part of this work were exclusively fully grown albino rats. White mice had also been used for Reid Hunt's test for biologically detecting any increased activity of the thyroid glands.

Seventy six animals were used in the present investigation. Sixty of these were albino rats and sixteen were white mice. Of the sixty rats, twenty four were females and the rest were males. The mice were all males for reasons to be mentioned later.

The age of these animals ranged between nine months for two batches to eight and seven month in other/

other two batches while that of the mice was five months.

The rats were grouped into sets each consisting of three rats of as near as possible the same weight. Each group was kept in a Schafer's (3) metabolism cage the weight of which was ascertained beforehand. Such cages were used throughout this investigation.

The cages now in use, however, differ a little from those described by Schafer in 1912. By means of a modification which Schafer recently introduced, it is insured that the animals are never manipulated and the whole cage can be lifted out of the faeces-collecting funnel without the animals being touched by hand.

The funnel now in use is made of porcelain specially made for the purpose with a fine perforated plate of the same material fixed across its largest diameter. The wire netting of the cage fits outside the funnel. The bottom of the wire cage is fixed by three clips soldered to the wire netting. When fitted on the porcelain funnel, the floor of the cage stands at a height of one and a half inches above the procelain plate allowing the faeces to pass through the meshes of the bottom of the cage but be retained by the porcelain plate. The urine, however, trickles right through and collects into a beaker placed below the funnel outlet. The whole apparatus rests on a tripod.

A/

A hole large enough to admit an adult rat's head is made in the lower part of the middle third of the wire cage. This hole is circumscribed by a smooth metal ring soldered to the edges, so as to avoid any injury to the animal while feeding. To the edge is fitted a feeder made of tin with a movable hinged lid from which the food is introduced or removed as the case may be.

As a result of this device the animals take their food with a minimum chance of scattering it, and any handling or manipulation of the animals during feeding or cleaning of the cages or funnels is completely avoided.

Water was supplied by means of two inverted bulbs introduced through the roof of the cage. Two such bulbs were used to insure that a supply would be constantly present. The bulbs have a narrow neck and the water is kept therein by atmospheric pressure. The rat obtains the water by licking the opening of the stem.

The room where the animals were kept was strictly maintained at a constant temperature of 68°F. throughout the whole duration of the experiments because rodents are particularly responsive to small variations in temperature and require the most careful attention in this respect.

METHODS OF FEEDING AND OF COLLECTING THE EXCRETA.

The food consisted of the following ingredients:-

Indian corn	74.00 grams.
White flour	20.00 "
Cheese	5.00 "
Sodium chloride	1.00 "
Fresh milk	50 cc.
Cod liver oil	2 cc.

The different constituents of the food were bought in large quantities, therefore avoiding any fluctuations in composition from sample to sample. Cheese was also bought in a large amount and allowed to dry partially and then grated very finely.

The dry ingredients were carefully weighed, thoroughly mixed together and then the fresh milk pipetted out and together with cod liver oil, the whole constituents mixed thoroughly.

The amount of milk was found out by experiment to be sufficient to convert the dry ingredients into a paste. This was aimed at to minimise the chance of scattering the food. When scattering did happen, however, the food being in the form of paste was quite easily picked up from the faeces and added to the food left over every day.

The food, as appears from the ingredients mentioned above was rich in vitamines, particularly those/

those concerned with growth. The amount of food given to each group of animals was kept constant throughout, and it consisted of half the amount above mentioned, i.e. 50 gms. of the dry ingredients, 25 cc. of milk and 1 cc. of cod liver oil.

When it was time to add the thyroid extract or the extract of anterior lobe of the hypophysis to the diet, the food was divided into two halves, to one half the extract was added and mixed and this part of the food was placed topmost to make certain that the extract given was all consumed.

Feeding was always done at a fixed hour (4 P.M.) and the animals were trained to eat as much of their ration as they wanted in the afternoon and early hours of the evening for reasons to appear later.

The feeding containers every day just before the feeding hour, were emptied of the food remaining from the previous day, which was then accurately weighed. The containers were then washed and dried and made ready for feeding.

For collecting the urine a beaker was put under the funnel of the metabolism cage. Into this beaker was placed 10 cc. of 2.5% of nitrogen-free sulphuric acid which served the purpose of fixing the nitrogen of the urine and prevented its loss in the form of ammonia.

The/

The urine was collected daily. The beaker after being emptied into a glass stoppered bottle bearing the corresponding number of the group, 10 cc. of sulphuric acid solution were placed in the beaker to rinse it and to wash away any drops of urine that may have clung to its wall, and this was also emptied into the urine bottle. 10 cc. of sulphuric acid were next placed in the beaker to receive the next urine.

At the end of the week the beaker was rinsed with sulphuric acid and the funnel was also rinsed with a known amount of sulphuric acid and all added to the urine bottle.

The faeces also was collected daily, the cage was lifted out, and the funnel was first of all cleaned as much as it was practicable from all the hair, then the faecal matter collected on a piece of cardboard bearing the number of the cage. Such faecal matter was left spread on the card board until the next collection of faeces when it was found to be sufficiently dry to be bottled into wide mouthed glass stoppered jars. The pieces of food that did happen to be found amongst the faecal matter were picked up and weighed with the food left over. The funnels were made clean to receive the cages.

At the end of the experimental week which was fixed as mid-day of each Saturday, the funnels after being/

being rinsed as mentioned above, were then thoroughly scrubbed and washed with hot water and then dried and made ready for the coming week. This procedure was done to ensure that the excreta collected represented a correct and accurate sample of the week which they represented and to provide the animals with the best possible and practicable hygienic conditions.

The urine was then accurately measured. The faecal matter was dried by being exposed to a steam bath for several hours and then put in an incubator at 45°C. and when thoroughly dry, they were passed through a mincer and then very finely ground in a mortar. Faeces was used for estimations of total nitrogen as well as for foecal calcium and phosphorus.

The estimation of calcium and phosphorus in faeces was however, abandoned early in the work for reasons stated below.

DETAILED DESCRIPTION OF THE EXPERIMENTS.

Experiments were performed at different times of the year between the beginning of September 1931 and the end of February 1933. Some experiments were done from September 1931 till February 1932. Some others were performed between June and September 1932: other experiments again were done between November 1932 and February 1933.

As was previously mentioned, the experiments were/

were conducted in groups. Each group consisted of 3 rats either all males or females. Group No.I was kept for the investigation of thyroid feeding, Group II for feeding of dried extract of the anterior lobe of the hypophysis; Group III for injections of freshly prepared extract of bovine anterior lobe of the hypophysis; and Group IV was used as a control.

Single animals from the male sex with controls were also kept at the same time and run through the whole routine of the experiment only that the time of feeding and injection with extracts was made 3 weeks on one occasion and 4 on another and 8 weeks on a third occasion to ascertain which of these durations was the optimum one to demonstrate whatever effects that appeared to be resulting.

In the groups, however, the feeding and injection were maintained for 6 to 7 weeks. The duration for each experiment was twelve to fourteen weeks, after which the animals were killed and their endocrine organs taken for the histological investigation.

The animals were all bought from the Institute of Animal Genetics of this University and when brought to this department, they were put in the appropriate cages and were kept for 2 to 3 weeks, sometimes more, on the standard diet above mentioned. No analytical estimations of any kind were tried during/

during that period, the object being only to habituate the animals to their new environment of temperature and diet.

Starting from the 3rd or 4th week, estimations of total nitrogen, calcium and phosphorus and recording of the weight of the animals and the basal metabolic rate, by means of Schafer's (13) oxygen consumption metabolism apparatus, which was recorded twice weekly. Schafer's technique regarding the use of the apparatus was adhered to.

The animals were kept for 4 more weeks on the standard diet making the duration of normal feeding a minimum of six to seven weeks. Thereafter dry extract of thyroid (P.D. & Co.) and dry extract of the anterior lobe of the hypophysis of the same laboratory in doses of 0.05 and 0.10 respectively per animal per diem were added to the food in Cages I and II in the manner previously described. Fresh extract of bovine anterior lobe of the hypophysis prepared by myself was administered subcutaneously to animals of group III for the first four weeks and subsequently by intraperitoneal route for the succeeding two or three weeks. Subcutaneous injections were always followed by gentle and firm rotary massage.

No injections were given to animals of group III on the afternoons when they were due for the metabolism/

metabolism apparatus in the following morning, the aim being to avoid disturbing the animals for at least 24 hours before the time for recording their basal metabolic rate which ensures an oxygen consumption rate that was not influenced by any extraneous factors.

THE EXTRACTS ADMINISTERED AND THE TECHNIQUE OF PREPARATION OF THE FRESH EXTRACT OF THE ANTERIOR LOBE OF THE HYPOPHYSIS.

The dry extracts of thyroid and of the anterior lobe of the pituitary body used for feeding were exclusively those prepared by Park Davis and Company.

The fresh extract of the anterior lobe of the hypophysis was, however, prepared by myself from fresh bovine pituitary bodies.

Several methods and techniques are reported in the literature on the subject for preparation of fresh extract from the anterior lobe of the hypophysis.

Schafer (4) used and recommends the use of saline extraction of the gland.

Evans and Long (5) prepared their extract primarily by saline, later they used dilute alkali for such extraction of the gland, their entire aim however, was directed towards isolation of what they called the growth stimulating autacoid of the anterior/

anterior lobe of the hypophysis.

Putnam Teel and Benedict (6) adopted the method of concentrating the globulins and the active principles and salting them out by means of sodium sulphate.

Bugbee, Simond and Grimes (7) prepared their extract by using sodium hydroxide followed by neutralisation with acetic acid to bring the solution to a PH. of 7.2 to 7.6.

Kronchovesky (8) used glycerol for extracting the gland and then diluting it by alkali.

As the main object of this investigation was to find out the interrelation that exist between the anterior lobe of the hypophysis and other endocrine organs, I did the extraction of the gland with sterile saline solution. The technique adopted runsas follows:-

Bovine Pituitary bodies from the slaughter house were used.

These glands were taken from the animals as immediately as practicable after killing. They were then frozen and received in this condition.

The thermos flasks containing the glands were put in the freezer of the Surgery Dept. of this University, the temperature being 25 - 23°F. Pituitary bodies were taken out when needed.

Dissection and separation of the anterior lobes of the hypophyseal bodies was done under strict

sterile conditions, in that all the instruments used were sterilized by boiling beforehand.

The entire gland with its capsule was dipped in surgical spirit for 10 - 15 minutes to render the outside of the gland as sterile as possible. Dissection was then begun and the anterior lobe was freed cleanly from the posterior lobe, pars tuberalis and the solid colloidal deposits of Fenger which lie between the anterior and posterior lobes. The anterior lobe was then weighed under sterile precautions. The weight ranged in different pituitary bodies between 1.00 and 1.62 grams. The gland was then cut into very fine slices and ground in a sterile mortar and extracted with sterile normal saline solution warmed to 37°C. The amount was calculated that 1 cc. of the extract would contain 100 mgms. of fresh anterior lobe substance. The extract was then allowed to freeze and left in that condition for 96 hours before it was made use of.

Such extract when injected subcutaneously followed by rotary massage for one or two minutes gave rise to no abscess formation and to no ill after-effects of any kind. The dose administered was 1 cc. subcutaneously for four weeks and then increased to 1.5 cc. injected intraperitoneally for the succeeding two or three weeks.

THE EFFECT ON GROWTH OF THYROID ADMINISTRATION.

The effect of thyroid administration on the growth of the white rat was studied by many authors.

In 1912 Schafer (3) was the first to show that administration per os of fresh sheep thyroid to white young growing rats increased their rate of growth.

Fordyce (9) in the same year reported a similar result to that of Schafer.

Hoskin in 1916 (10) by feeding very small doses of thyroid to white rats showed that such administration produced definite hypertrophy of the heart, spleen, liver, kidney and the adrenals.

Herring (11) in 1917 on the other hand, although his investigation confirmed Hoskin's as far as the hypertrophy of the heart, spleen etc., yet he pointed out that thyroid feeding tended towards retardation of growth.

Retardation of growth by thyroid administration was also reported by Cameron and Carmichael (12) who also used thyroxin as well as feeding with thyroid extract. These investigators, however, reported the existence of hypertrophy of the liver, kidney, heart, adrenals and pancreas.

The duration of thyroid administration in the work of some of these investigators was short and the doses used were on the small side.

In/

In the present investigation administration of thyroid extract was maintained for a minimum of 6 to 7 weeks in the groups: and to 8 weeks in single animals, the dose being 0.05 gram. per rat per diem. As was previously mentioned short durations were also tried on single animals so as to compare the results of short durations with those of prolonged administration.

As appears from the attached tables and graphs, an accelerated growth indicated by increase in weight, in the animals of either sex was never obtained with thyroid administration. In all the experiments performed on animals born at different seasons and conducted at different times of the year retardation of growth and considerable loss of weight was always a constant feature of thyroid administration.

The weight of the animals reported here represents the average weight for the group every week: This was obtained by weighing the animals at the beginning of the week and again at the end of the week; the mean of the two weights was taken as the weight of the animals for the corresponding experimental week.

The loss of weight cannot be attributed to decreased consumption of food or to loss of appetite, as the animals were given more than they could eat and/

TABLE IV. MALES.

No.of weeks	No.of animals in Group	Wt.of Group in Grams	O_2 Consumption.	Amount of food consumed per week.	Total Nitrogen of food consumed	Total Nitrogen of urine	Total Nitrogen of foeces.	Tot Nitr of U + fo
1	3	789.5	1100	301	4.244	1.417	1.054	2.97
2	3	802.0	1245	349	4.930	2.572	1.215	3.78
3	3	805.5	1080	338	4.762	1.942	1.274	3.21
4	3	812.0	1030	321.5	4.538	1.800	1.176	2.97
5	3	792.0	1160	325	4.580	1.660	1.201	2.861
6	3	798	1165	357	5.028	1.580	1.519	3.099
7	3	815.5	1120	368	5.182	1.779	1.417	3.197
8	3	810	1030	370	5.224	2.296	1.352	3.648
9	3	801	1185	330	4.664	1.913	1.176	3.089
10	3	806	1140	346	4.888	2.047	1.646	3.693

TABLE IV. (continued).

Nitrogen Balance	N.balance per kilo of body weight.	Amount of Urine voided per week.	Calcium output in mgms. per 100 cc. urine.	Total Calcium excreted	Phosph.output in mgms. per 100 cc. urine.
1.273	1.612	191	4.5	8.6	99.3
1.142	1.424	192	4.0	7.7	106.4
1.557	1.926	200	4.2	8.4	106.4
1.562	1.922	200	4.1	8.2	106.4
1.719	2.165	190	4.5	8.5	99.3
1.929	2.415	190	4.1	7.8	100.4
1.985	2.422	195	4.0	7.8	106.4
1.576	1.942	198	4.5	8.9	99.3
1.575	1.963	204	4.3	8.7	111.0
1.195	1.482	195	4.6	8.9	92.5

TABLE I. MALES.

No.of weeks	No.of animals in Group	Wt.of Group in Grams	O_2 Consumption.	Amount of food consumed per week.	Total Nitrogen of food consumed	Total Nitrogen of urine	Total Nitrogen of foeces	T... Ni... of ... + f...	Ni... ge... Ba...
1	3	782	960	357.0	5.488	2.118	1.808	3.9...	1.0
2	3	796.5	900	350.0	4.914	2.578	0.855	3.43...	1.4
3	3	807	970	388.5	5.446	2.647	1.183	3.8...	1.(
4	3	820	950	350.0	4.914	1.474	1.001	2.4...	2.
5	3	798	1685	345.5	4.830	2.646	1.062	3.7...	1.
6	3	749.5	1575	357.5	5.012	2.278	1.555	3.83...	1.
7	3	667.5	2000	395.0	5.530	2.571	1.712	4.28...	1.
8	3	672.5	1740	438.5	6.146	3.065	1.791	4.85...	1.
9	3	660.0	1720	398.5	5.586	2.881	0.978	4.85...	0.
10	3	651.0	1880	407.0	6.125	3.112	1.941	5.0...	-0.

TABLE I. (continued)

Nitrogen Balance	N.balance per kilo of body weight.	Amount of Urine voided per week	Calcium output in mgms. per 100 cc. urine.	Total Calcium excreted	Phosph.output in mgms. per 100 cc. urine.	Total Phosphorus output.
1.086	1.375	193	4.0	7.72	128.0	247
1.481	1.861	198	4.0	7.92	107.5	213
1.661	2.059	195	3.5	6.82	113.4	221
2.971	3.620	190	4.2	7.95	128.2	243
1.118	1.410	210	5.0	10.5	138.8	289
1.179	1.571	226	6.7	17.2	134.4	302
1.147	1.718	208	8.7	17.9	151.0	314
1.290	1.921	238	8.0	19.06	165.0	392
0.727	1.101	210	8.1	17.01	151.0	317
-0.645	-0.991	205	9.2	18.8	158.0	324

TABLE VIII. FEMALES.

No.of weeks	No.of animals in Group	Wt.of Group in Grams	O_2 Consumption	Amount of food consumed per week.	Total Nitrogen of food consumed	Total Nitrogen of urine	Total Nitrogen of foeces	Total Nitrogen of Urine + foeces
1	3	624	920	350	4.900	2.044	1.340	3.384
2	3	620	1020	355	4.970	1.884	1.360	3.244
3	3	632	1080	350	4.900	1.526	1.218	2.74
4	3	622	955	390	5.460	2.341	1.184	3.52
5	3	621	1085	357	4.998	1.998	1.197	3.19
6	3	627	1060	380	5.324	2.016	1.978	3.994
7	3	623	1180	374.5	5.250	2.685	1.270	3.95
8	3	630	970	380	5.320	2.700	1.155	3.85
9	3	622	1055	355	4.970	1.953	1.985	3.93
10	3	640	980	370	5.180	2.085	1.459	3.544

TABLE VIII.(continued)

	Nitrogen Balance	N.balance per kilo of body weight	Amount of Urine voided per week	Calcium output in mgms. per 100 cc. urine.	Total Calcium excreted	Phosph.output in mgms. per 100 cc. urine.	Total Phosphorus output
	1.516	2.43	200	4.1	8.2	99.3	198.6
	1.726	2.78	170	4.0	6.8	120	204.0
	2.162	3.425	182	4.3	7.8	111	202.0
	1.935	3.15	163	3.8	6.2	92.5	151.0
	1.802	2.90	155	4.5	7.0	100.4	155.6
	1.330	2.12	165	4.3	7.1	120	198.0
	1.290	2.069	163	4.5	7.3	99.3	169.6
	1.470	2.32	170	4.0	6.8	106.4	180.8
	1.042	1.674	172	4.1	7.1	92.5	159.0
	1.636	2.55	176	4.2	7.4	98.1	187.2

TABLE V. FEMALES.

No.of weeks	No.of animals in Group	Wt.of Group in Grams	O_2 Consumption.	Amount of food consumed per week.	Total Nitrogen of food consumed	Total Nitrogen of urine	Total Nitrogen of foeces	Total Nitrogen of Urine + foeces	Nitrogen Bala
1	3	571	820	269	3.766	1.470	.918	2.388	1.378
2	3	569.75	852	270	3.780	1.475	.803	2.278	1.502
3	3	575.5	875	273	3.822	1.085	1.058	2.143	1.679
4	3	575.5	860	377	5.278	2.229	1.184	3.413	1.865
5	3	555	1045	262	3.682	1.292	0.994	2.286	1.39
6	3	531	990	275	3.864	1.822	0.945	2.767	1.09
7	3	512	1210	368	5.166	1.865	1.218	3.803	1.36
8	3	508	1380	415	5.824	2.962	1.847	4.709	1.01
9	3	512	1345	470	5.594	2.491	2.456	4.947	0.64
10	3	491	1360	471	6.608	3.580	2.702	6.282	0.32

TABLE V. (continued).

Nitrogen Balance	N.balance per kilo of body weight.	Amount of Urine voided per week	Calcium output in mgms. per 100 cc. urine.	Total Calcium excreted	Phosph.output in mgms. per 100 cc. urine.	Total Phosphorus output
1.378	2.415	150	3.9	5.9	92.5	138.8
1.502	2.635	155	4.5	7.0	92.5	143.4
1.679	2.895	155	3.5	5.4	98.1	152.4
1.865	3.240	150	4.0	6.0	85.5	128.0
1.396	2.515	145	4.4	6.4	100.4	145.6
1.096	2.065	167	5.4	9.0	111	185.4
1.363	2.660	222	5.8	12.8	110.2	244.2
1.015	1.993	226	6.0	13.5	118.8	268.5
0.647	1.264	201	7.4	14.9	134.4	270.0
0.326	0.668	213	7.2	15.3	130.1	277.0

and this fixed amount was kept up throughout the experiment, and on the other hand they always had a very sharp appetite and consumed practically all that they were able to eat for the 24 hours during the first four or five hours that followed their feeding hour. Such a sharp appetite with thyroid feeding was also noticed in the case of white mice. In both the white rat and the white mouse a sharp appetite was maintained until the animals were killed.

The water intake was also high and as it will be shown later, the water output was also remarkably high.

It was thus concluded from the results obtained that thyroid extract (P. D. & Co.) decreased the size of the body as a whole and caused loss of weight.

(See tables I & V.)

The endocrine organs as well as the liver and spleen however, as regards thyroid administration, exhibited a different state of affairs. These organs were removed from the body of the animal, after it was killed, by careful dissection and then carefully weighed.

From the attached tables it is clearly shown that the thyroid extract influenced different organs differently. Some of the endocrine organs responded with hypertrophy and others with atrophy and others again although they did not show any histological evidence of atrophy yet showed some decrease in weight.

Of the organs that showed a very marked atrophy and decreased weight, the testicle exhibited these effects most.

The spleen and ovary showed a decrease in weight without any gross histological manifestations of degeneration in the spleen but with decrease in the connective tissue stroma in the case of the ovary. The testes however, showed a very marked degeneration as is described in the histological investigation of this work.

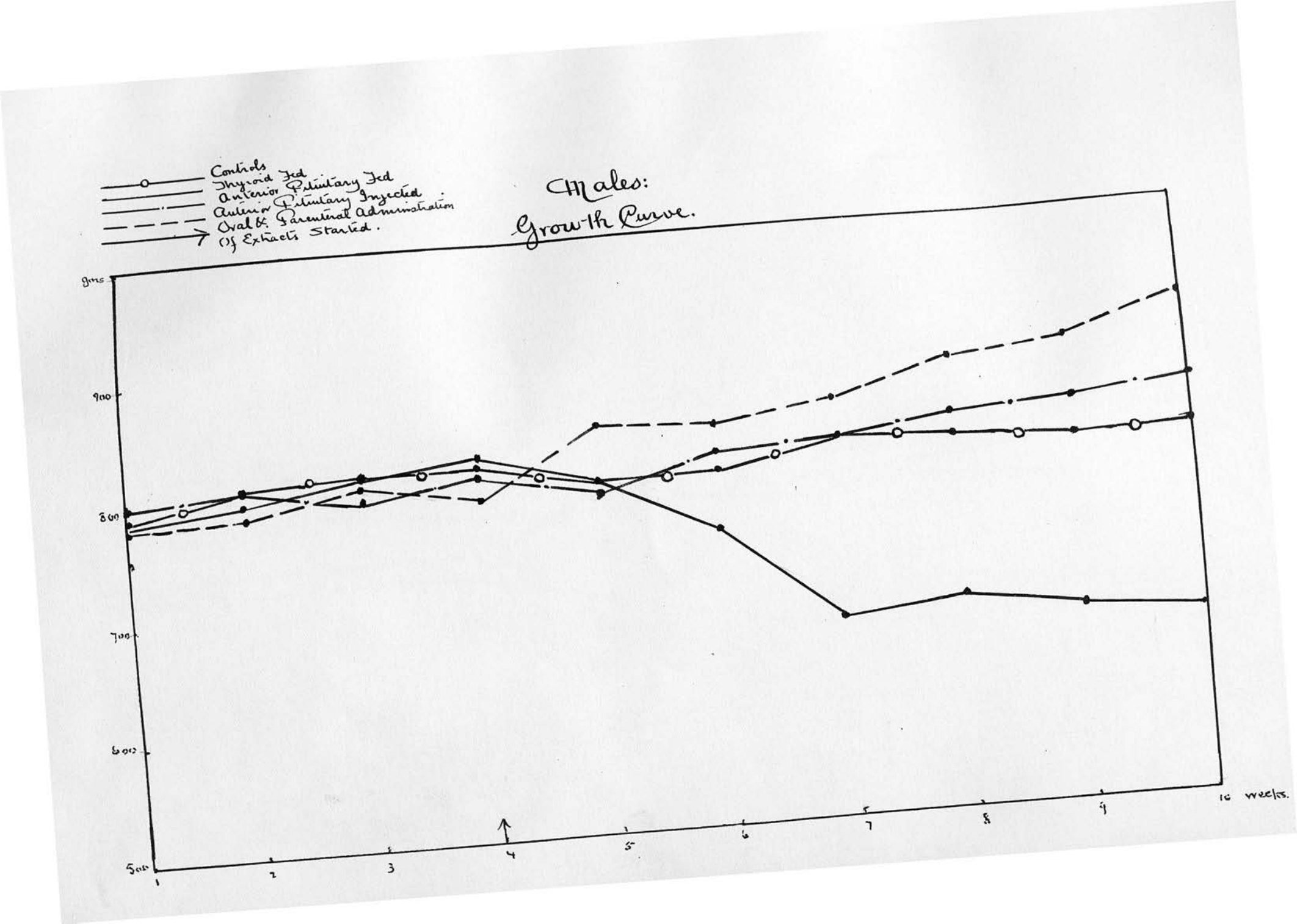
The thyroid gland of the animals fed on thyroid showed a marked decrease in weight accompanied by a histological picture showing partial degeneration in some parts.

The pancreas, liver and adrenals, however, showed a marked hypertrophy and increase in weight. The general body fat was practically absent.

No attempt had been made to investigate such organs as the heart etc. as the present investigation dealt only with endocrine organs.

THE INFLUENCE ON THE BASAL METABOLIC RATE OF THYROID ADMINISTRATION.

In describing the details of the general points of the experiments reported in the present work, measurement of the basal metabolism in terms of oxygen/


Controls
Thyroid Fed
Anterior Pituitary Fed
Anterior Pituitary Injected
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Of Extracts Started.
Males:
Growth Curve.
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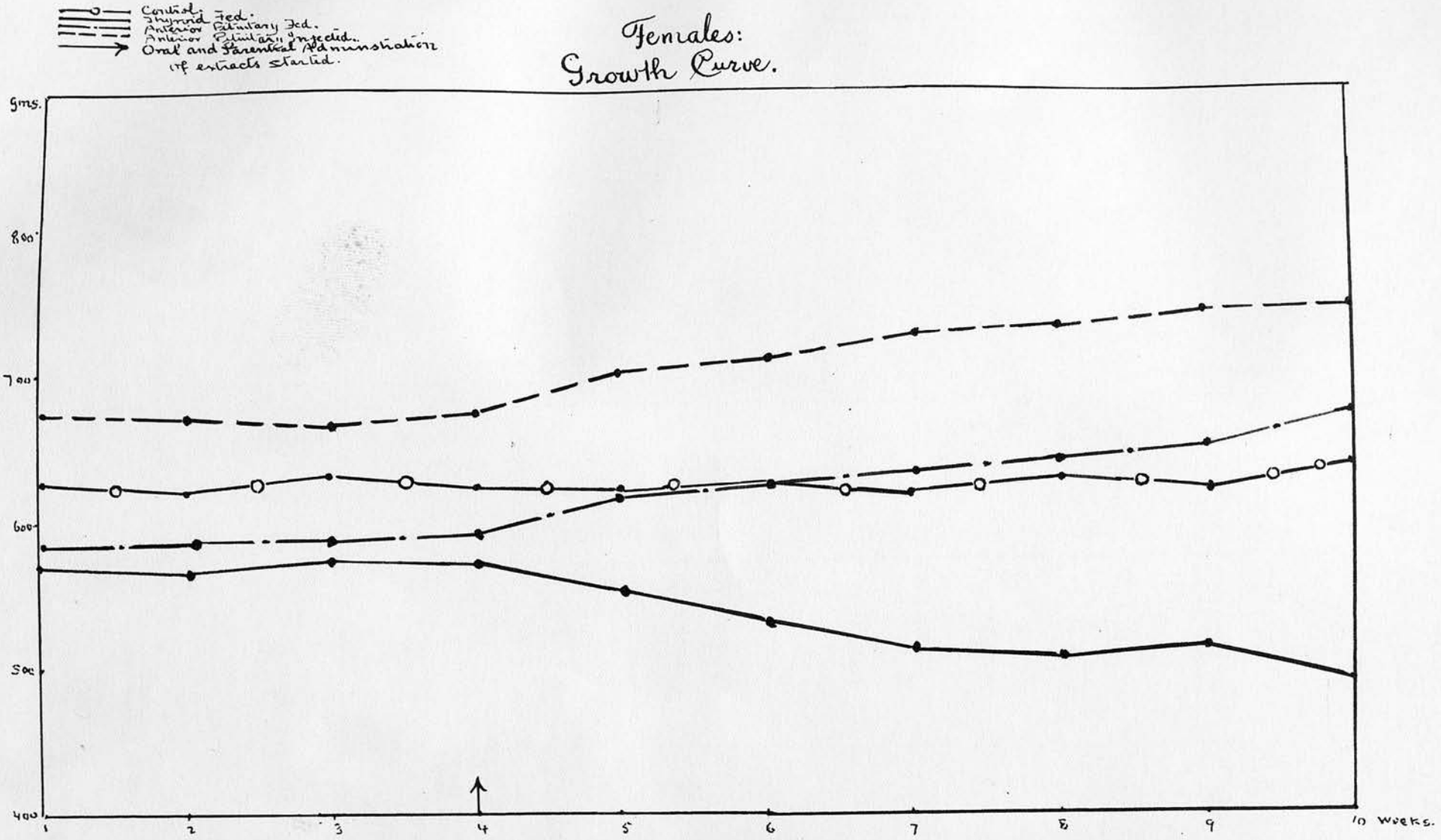
Control.
Thyroid Fed.
Anterior Pituitary Fed.
Anterior Pituitary Injected.
Oral and Parenteral Administration of extracts started.
Females:
Growth Curve.
Gms.
800
700
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500
400
1
2
3
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5
6
7
8
9
10 weeks.

TABLE A.

Weight of Endocrine Organs of Normal Control Animals.

Wt of Animal	Sex	Wt. of Thyroids	Wt. of Adrenals	Wt. of Pancreas	Wt. of Ovaries	Wt. of Testicles	Wt. of Liver	Wt. of Spleen	Duration of administration of Extract.
265	M.	20	43	0.889		2.22	8.80	0.961	
269	M.	22	40	0.841		2.00	8.73	0.902	six weeks.
272	M.	28	38	0.813		2.61	8.44	0.892	
210	M.	26	37	0.621		2.18	6.54	0.806	
220	M.	23	35	0.590		2.01	6.84	0.811	seven weeks.
218	M.	21	32	0.613		2.11	7.68	0.841	
Average 2445		23.3	37.5	0.726		2.19	7.84	0.869	
280	M.	28.5	44	0.850		2.06	8.67	0.901	three weeks.
300	M.	28	41	0.823		2.46	9.12	0.912	four weeks.
305	M.	25	53	1.170		2.19	9.01	1.102	eight weeks.
210	F	23	48	0.765	31		7.60	0.833	
213	F	22	50	0.800	42		8.01	8.28	six weeks
217	F	25	42	0.711	40		7.96	0.870	
180	F	21	49	0.600	35		5.95	0.611	
181	F	20	57	0.792	34		7.07	0.750	seven weeks.
190	F	21	53	0.895	34		7.19	0.820	
Average 198.5		22	49.8	0.761	36		7.29	0.785	

TABLE B.

Weight of Endocrine Organs of Thyroid Fed Amimals.

Wt. of Animal	Sex	Wt. of Thyroids	Wt. of Adrenals	Wt. of Pancreas	Wt. of Ovaries	Wt. of Testicles	Wt. of Liver	Wt. of Spleen	Duration of administration of Extract.
211	M.	21	54	1.275		1.910	9.875	0.740	
230	M.	21.5	48	1.182		1.980	10.510	0.710	six weeks.
210	M.	20	45	1.261		1.557	10.720	0.623	
185	M.	21	47	0.971		1.257	9.913	0.612	
180	M.	17	52	1.101		1.572	8.230	0.721	seven weeks
175	M.	15.5	41	1.520		1.250	9.751	0.700	
198.6		19.30	47.5	1.22		1.587	9.833	0.684	
238	M.	28	50	0.998		2.13	9.680	0.851	three weeks
240	M.	22	59.5	1.025		1.95	10.571	0.777	four weeks
215	M.	14	61	1.705		0.907	11.00	0.705	eight weeks
157	F.	16	51	0.775	27 mg.		9.023	0.614	
165	F.	19	88	1.102	31		8.806	0.689	
170	F.	22	64	1.431	30		9.728	0.700	six weeks.
155	F.	18	58	1.201	25		8.250	0.582	
160	F.	17	73	1.275	28		8.230	0.701	
171	F.	21	60	1.625	30		9.612	0.714	
162		18.84	66	1.234	28.5		8.94	0.6651	

Average

Average

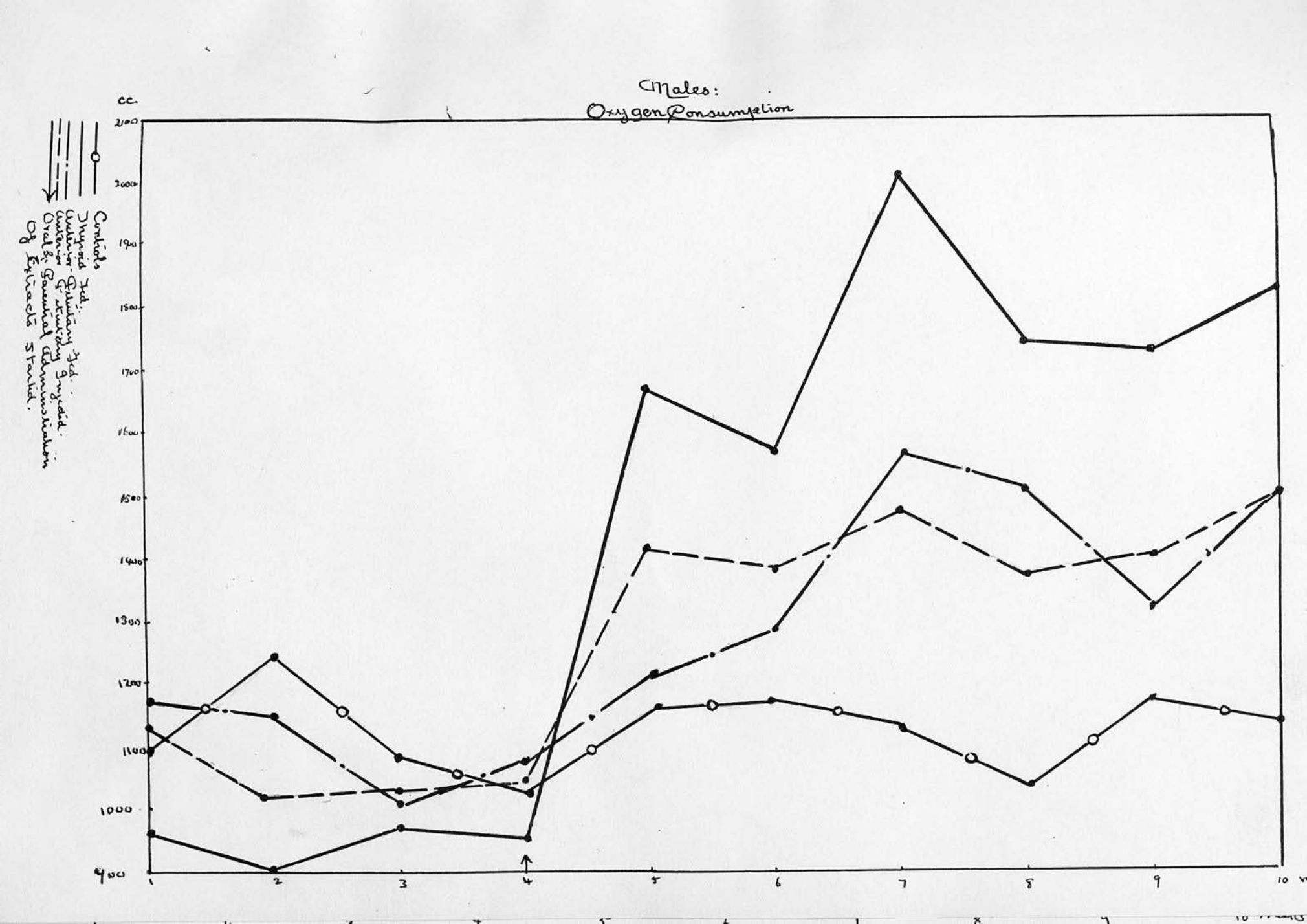
oxygen consumption was alluded to.

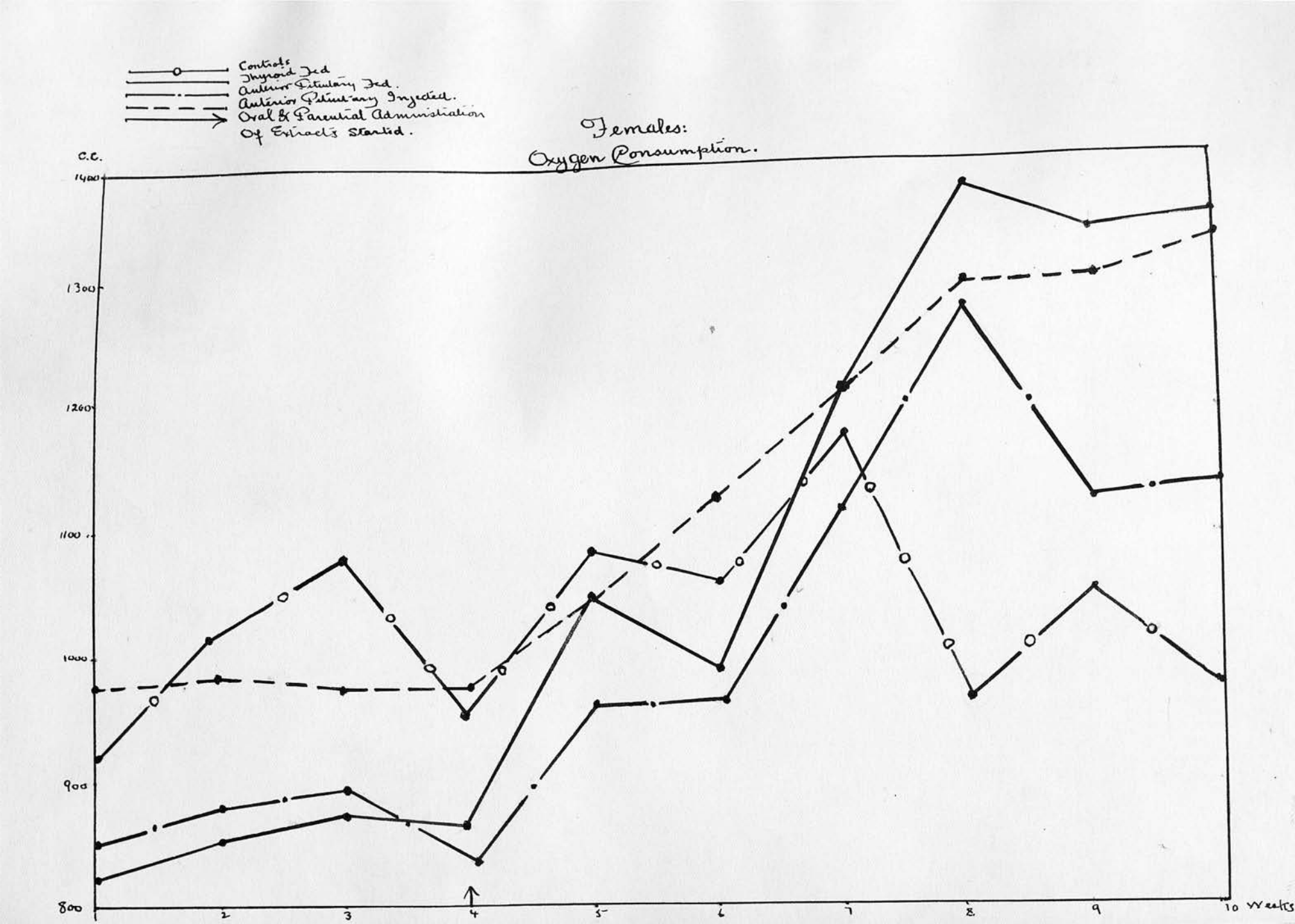
In detail the basal metabolic rate was ascertained by means of Schafer's (13) oxygen consumption apparatus for small animals which was kept in a special room heated to exactly the same temperature as that where the animals were kept. In the same hour of the day and on the same day of each week and twice weekly, the basal metabolism was measured. Each morning before removing the cages containing the animals to be put under the bell jar of the apparatus, the machine driving the oxygen from the spirometer to the bell jar was run for an hour to ensure that the apparatus was in good working order and that there was no loss of oxygen. The rest of the details of using the apparatus described by Schafer were adhered to.

After ascertaining the reading of the spirometer before and after the hour in which the animals had to be kept under the bell jar, the animals were removed, and the entire cage was weighed, the net weight of cage was previously known.

The mean of the two readings of oxygen consumption represented the average consumption for the corresponding week.

In 1904 Magnus-Levy (14) showed that by administration of desiccated thyroid to cretins and myxoedematous/.


Males:
Oxygen Consumption
cc.
2100
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
1
2
3
4
5
6
7
8
9
10
Controls
Thyroid Fed.
Anterior Pituitary Fed.
Anterior Pituitary Injected.
Oral & Parenteral Administration
of Extracts Started.


Controls
Thyroid Fed
Anterior Pituitary Fed.
Anterior Pituitary Injected.
Oral & Parental Administration
Of Extracts started.
Females:
Oxygen Consumption.
C.C.
1400
1300
1200
1100
1000
900
800
1
2
3
4
5
6
7
8
9
10 Weeks

myxoedematous patients, the basal metabolism rose to 55% above normal.

Salmon (15) in the same year and Styerer (16) in 1907 and Von Bergmann (17) in 1909 showed that there was an increase in basal metabolism rate in hyperthyroidism whether produced pathologically or experimentally.

Asher and Horrisberger (18) in 1921 stated that moderately large doses of thyroid caused greater expiratory exchanges and the basal metabolism was markedly increased whether the carbohydrate storage had been previously depleted or not.

Schafer (19) states that the increase in basal metabolism in hyperthyroidism may be 100% above normal.

In the present work, my results determined definitely that there was a very marked rise in the oxygen consumption rate by thyroid administration, the rise varying between about 66% and 95%.

THE EFFECT ON NITROGEN METABOLISM OF THYROID ADMINISTRATION.

The literature on the subject of the effects of thyroid administration in relation to nitrogen metabolism indicates much disharmony in the results reported by different workers.

Schafer (20) in 1912 by administering fresh sheep/

sheep thyroid to very young growing rats had shown that such administration caused a retention of nitrogen.

Hewitt (20) in 1915 using small amounts of thyroid reported a similar result to that of Schafer.

Kojima (21) in 1917 stated that thyroid administration caused a marked decrease in the output of nitrogen in the urine and a marked nitrogen retention.

Janney and Henderson (22) in 1920 showed that administration of small doses of thyroid caused retention of nitrogen in a child with hypothyroidism.

Courvoisier (23) in 1917 on the other hand found that iodothyrin produced an increase in the output of nitrogen.

Shöndorff (24) results as well as those of many other authors as Ord, White & Roos (25); and Dening(26) and Dinkler (27) etc., indicated that thyroid administration caused an increase in the nitrogen output and an associated diuresis.

In the work reported here, however, with a complete control over diet and the temperature of the room where the animals were kept, and by means of the almost immediate fixation of nitrogen by the sulphuric acid in the beakers receiving the urine together with the corroborative evidence gained by weighing the animals twice weekly, the results obtained/

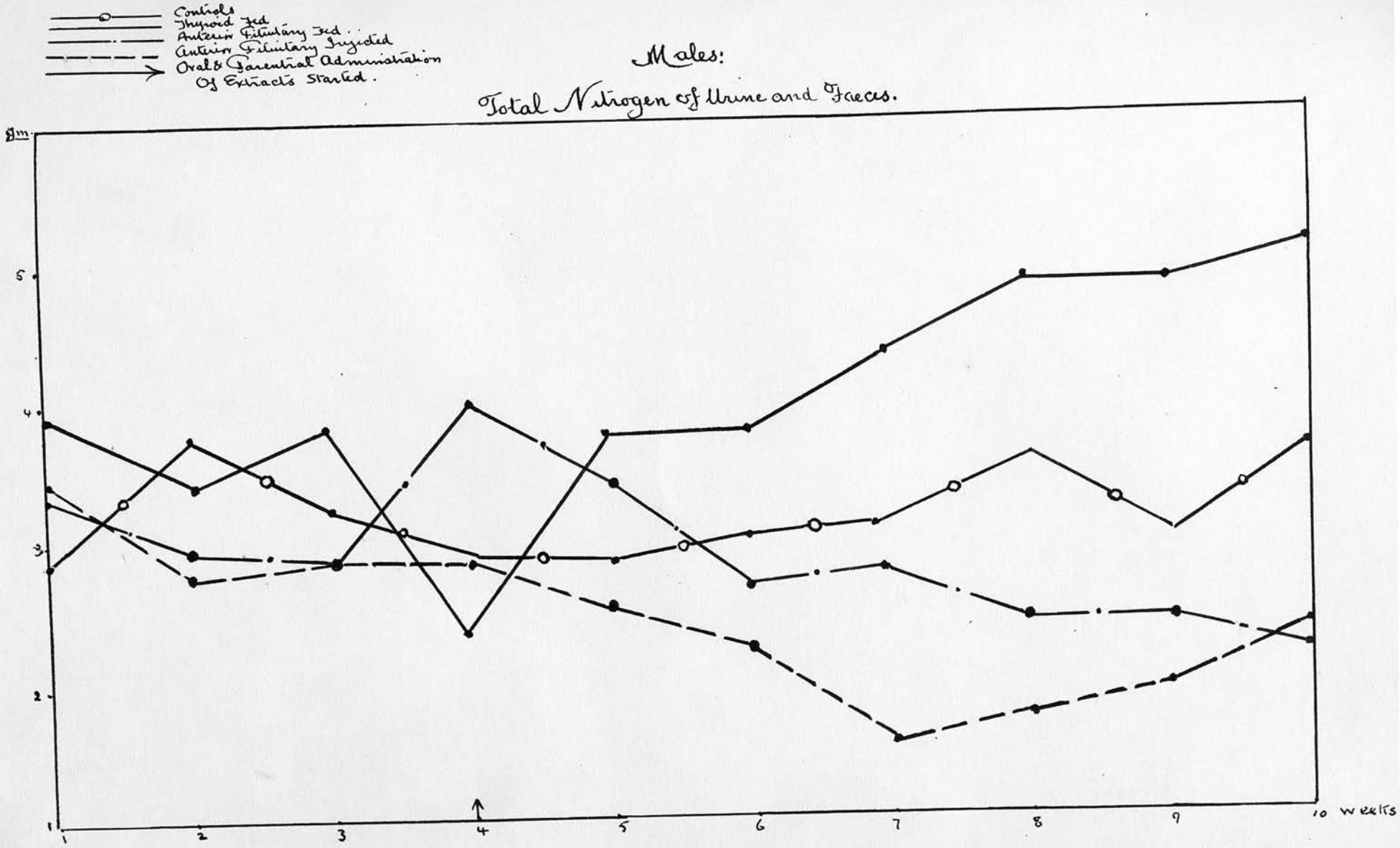
obtained indicated that the nitrogen output was much increased with thyroid administration. The nitrogen balance was a much lower positive than the control animals and also lower positive than that obtained prior to thyroid administration.

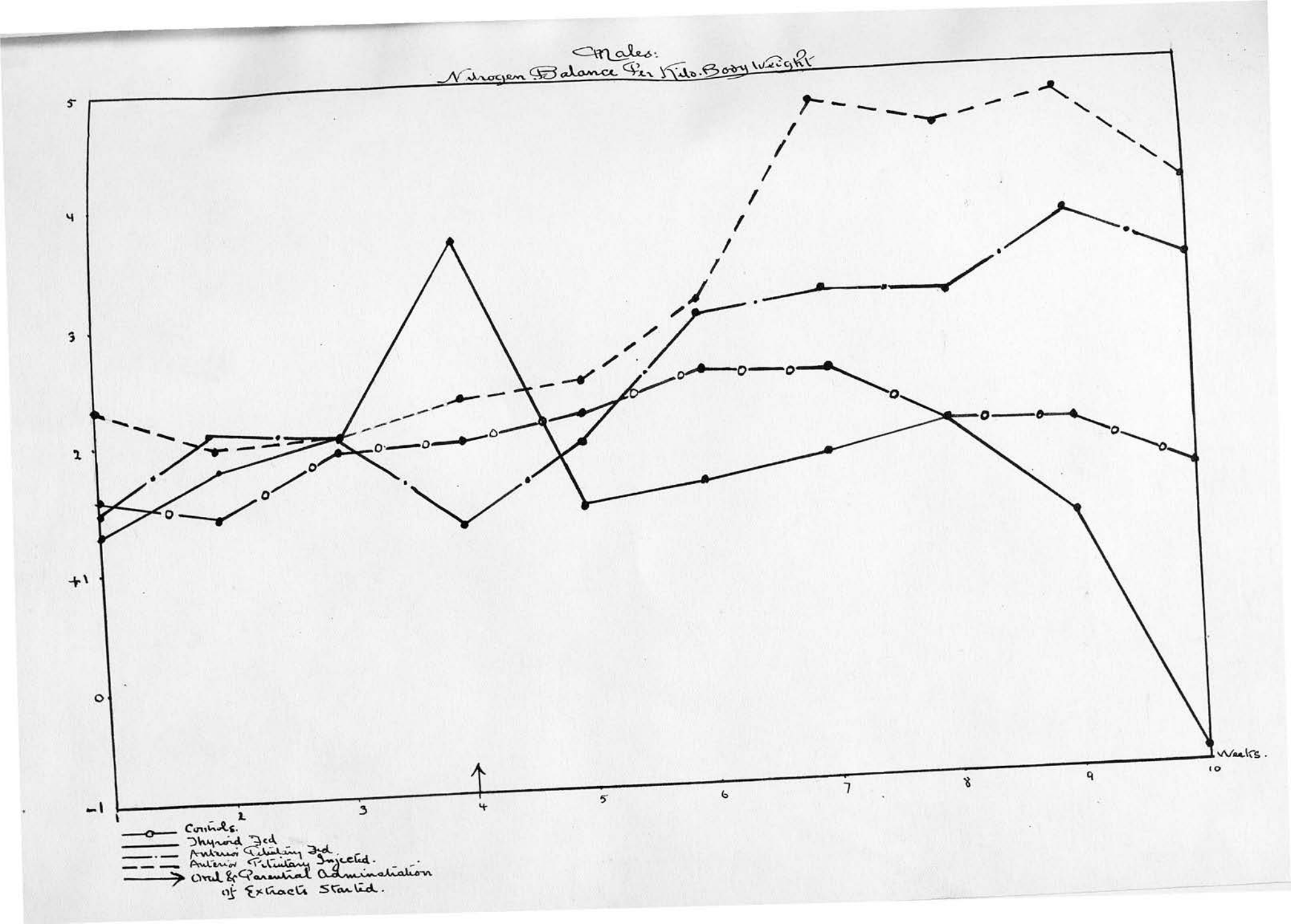
This increase in nitrogen output was not so very marked in the first week of thyroid administration although the basal metabolic rate was appreciably raised as was indicated by the oxygen consumption rate.

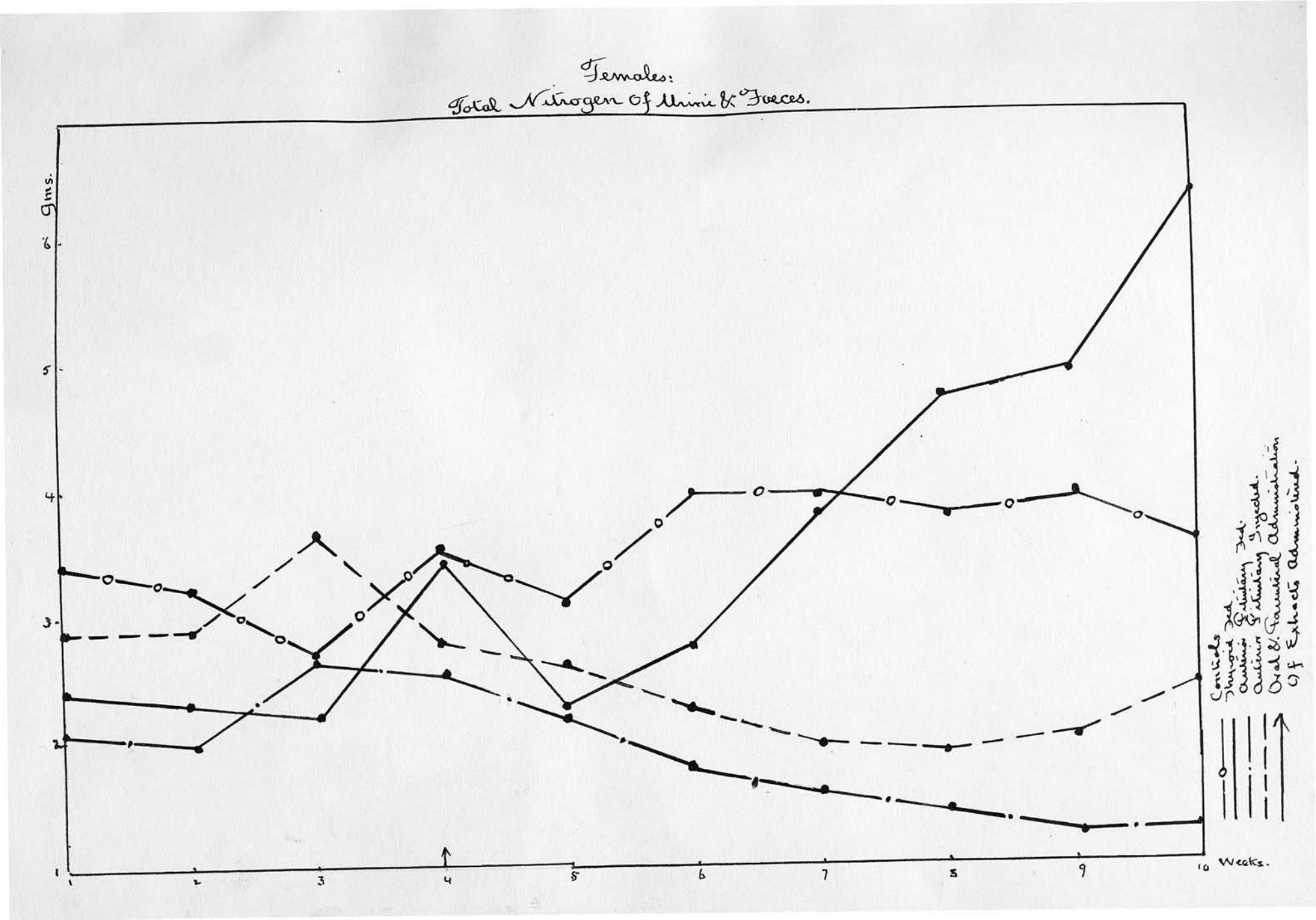
An appreciable increase in nitrogen output was generally attained by the second or third week of administration when it was sustained, with some fluctuations occasionally, for one week in some cases and two or three weeks in other instances. Thereafter this increase in nitrogen output begins to rise and by the end of the sixth or seventh week of administration the output of nitrogen was at its maximum being 47% to 56% higher than the highest value obtained prior to thyroid administration.

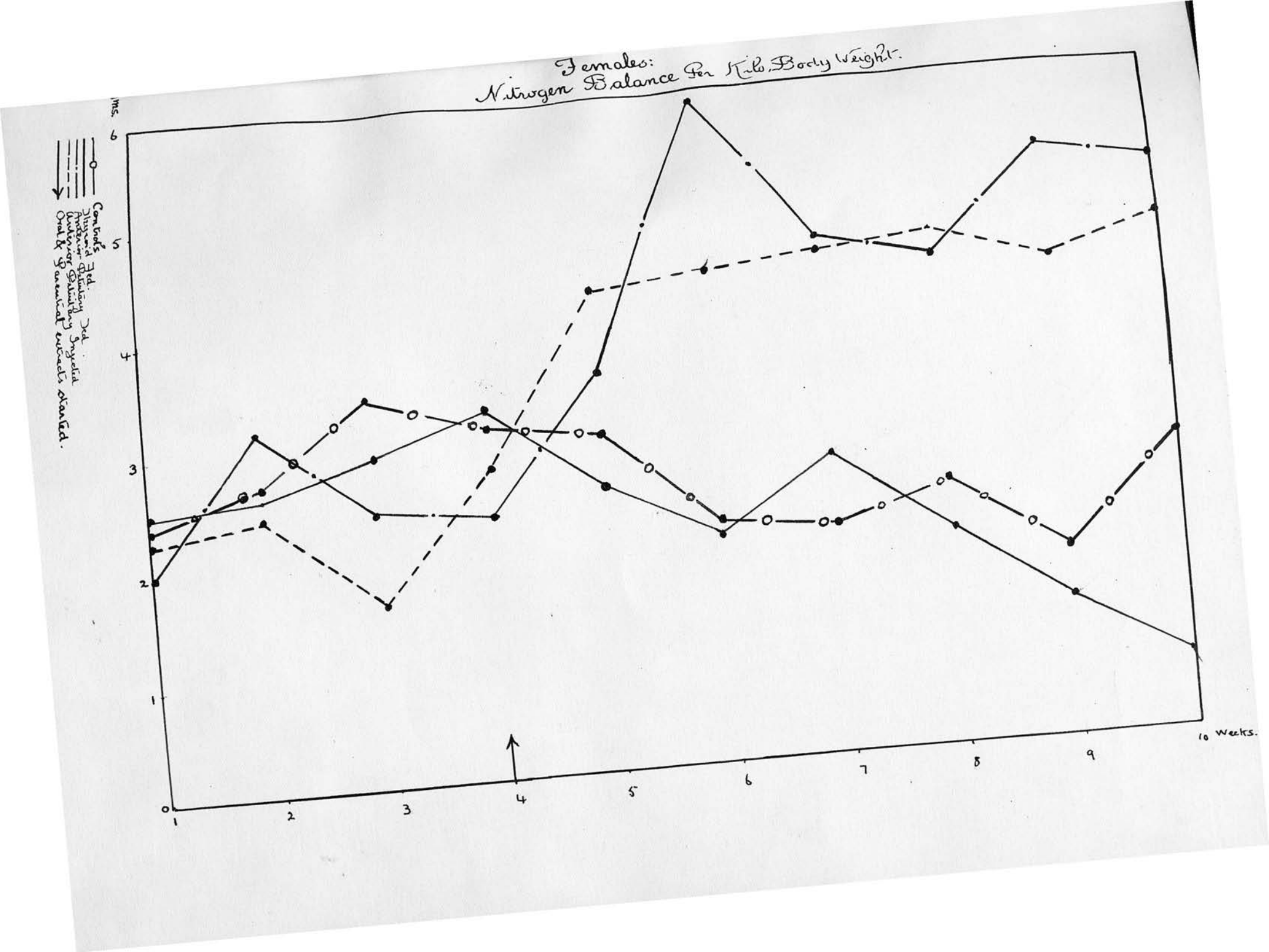
The nitrogen metabolism showing a decreased positive balance of nitrogen, indicated that such a decreased effect was also at its maximum about the sixth or seventh week of thyroid feeding when in some instances the balance became negative. It however showed a general parallelism with that of nitrogen excretion.

(See Tables I & V.)


Controls
Thyroid Fed
Anterior Pituitary Fed
Anterior Pituitary Injected
Oral & Parenteral Administration
Of Extracts Started.
Males:
Total Nitrogen of Urine and Faeces.
gm.
5
4
3
2
1
1
2
3
4
5
6
7
8
9
10 weeks


Males.
Nitrogen Balance Per Kilo. Body Weight
5
4
3
2
+1
0
-1
1
2
3
4
5
6
7
8
9
10
Weeks.
Controls.
Thyroid Fed
Anterior Pituitary Fed.
Anterior Pituitary Injected.
Oral & Parenteral Administration of Extracts Started.


Females:
Total Nitrogen of Urine & Feces.
6 Gms.
5
4
3
2
1
1
2
3
4
5
6
7
8
9
10 Weeks.
Controls
Thyroid fed.
Autolysed Pituitary fed.
Autolysed Pituitary injected.
Oral & Parenteral Administration
of Extracts Administered.


Females:
Nitrogen Balance per Kilo. Body Weight.
Controls
Thyroid fed.
Anterior Pituitary fed.
Anterior Pituitary Injected
Oral & Parenteral extracts standard.
gms.
6
5
4
3
2
1
0
1
2
3
4
5
6
7
8
9
10 Weeks.

THE EFFECT ON CALCIUM METABOLISM OF THYROID ADMINISTRATION.

Within recent years the metabolism of inorganic salts has occupied and stimulated a vast amount of research. Many views were advanced to interpret the different factors that have a controlling influence over calcium metabolism.

It is now an established fact of physiology that the autacoid of the parathyroids controls calcium metabolism by keeping the blood calcium at a steady level, but there are other autacoids that play an important part in calcium metabolism; namely those of thyroid gland and the anterior lobe of the hypophysis.

Parhon (28) showed that there was a marked loss of calcium in rabbits fed with lethal doses of thyroid.

Aub, Bauer, Heath and Ropes (29) found that hyperthyroidism in the human subject was associated with a marked increase in calcium excretion. In 50% of the cases of exophthalmic goitre investigated, they found that the elimination of calcium was 231% above the average excretion per kilo of body weight in the control cases.

Another two cases of thyroid adenoma with manifestations of hyperfunction were also investigated by these authors who found that calcium excretion was raised/

raised to almost twice the normal and that this rise in calcium output fell definitely after subtotal thyroidectomy.

In six cases of myxoedema, the above mentioned authors found that calcium excretion was 40% below the normal level. Thyroid administration to those six patients resulted in an increase in calcium excretion.

Hunter (30) attempted to elucidate by animal experiment whether thyroxin would influence calcium excretion by increasing its output after parathyroidectomy. He performed thyro-parathyroidectomy on dogs and cats. He then determined, by experiment, the minimum dose of parathormone that just kept the animals without tetany. He subsequently injected large doses of thyroxin every second day. The cats became very ill and died and the dogs suffered from tetany and proved unsatisfactory.

Percival and Stewart (31) believed that the influence by any endocrine organ other than the parathyroids was an indirect one. They also found that intravenous injection of parathormone caused an increase in serum calcium but without any increase or decrease in calcium excretion.

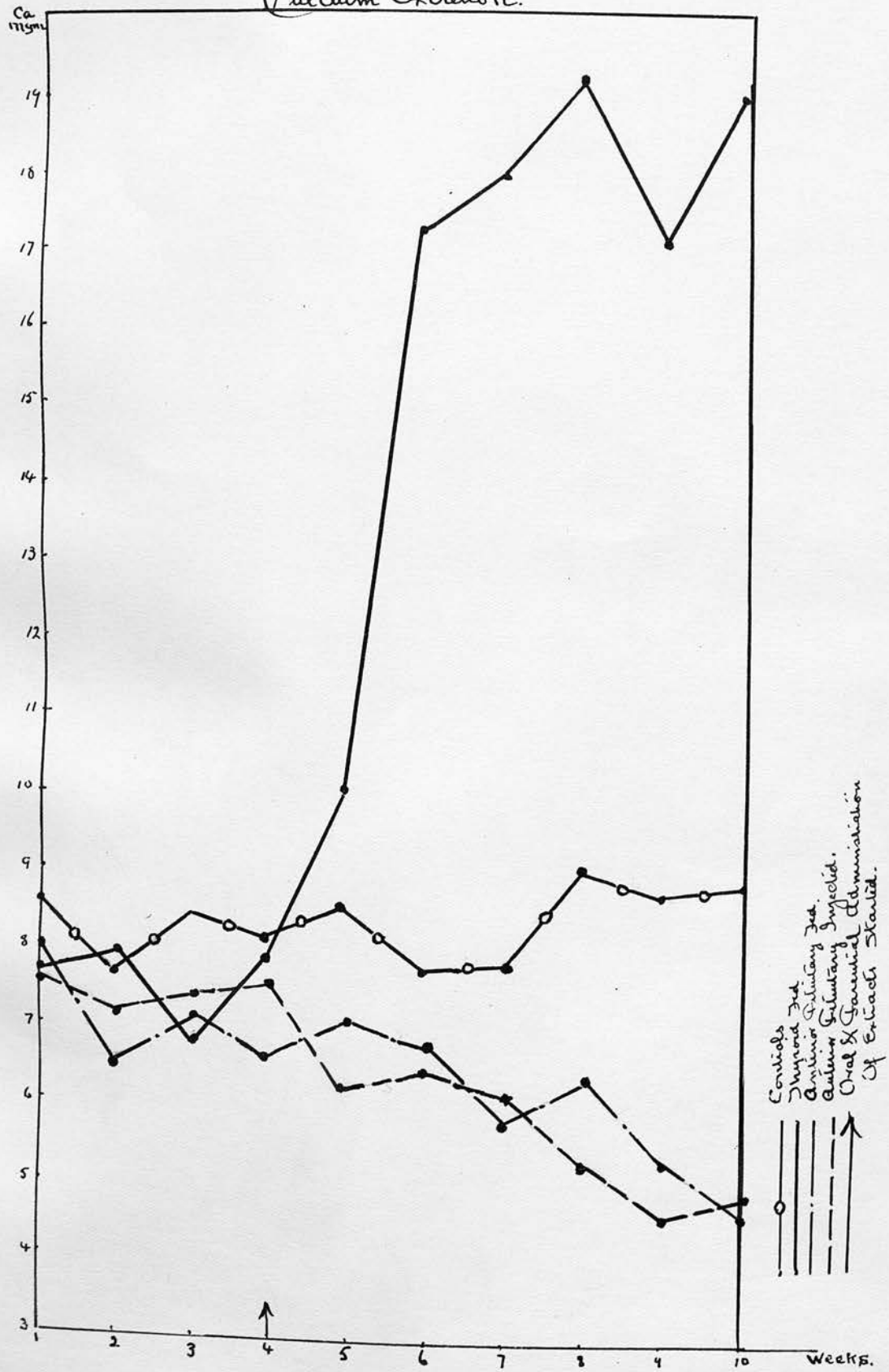
In estimating the calcium excretion I have taken the urinary output as an index to its metabolism, as it is very difficult to differentiate relatively, in the foecal calcium, between the unabsorbed surplus and/

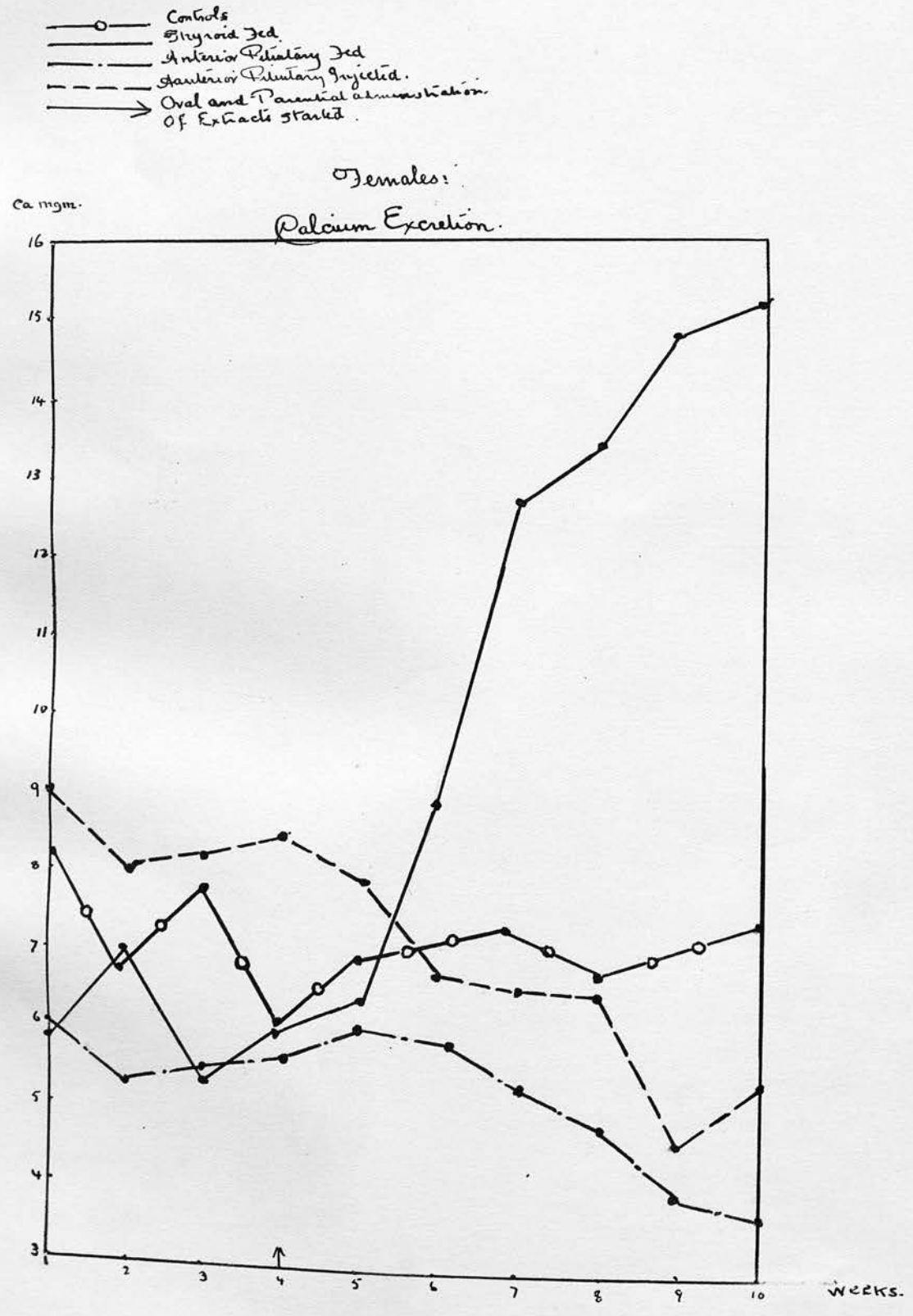
and the re-excreted calcium.

Shohl and Pedley's (32) method of calcium estimation in the urine was at first used, but due to the fact that in digesting the urine of the rat with ammonium persulphate and sulphuric acid, more than three hours were needed for such digestion with continual addition of persulphate of ammonia, and yet at the end of that duration, the solution remained, in some cases, a little opalescent. Such opalescence however, did not interfere with the estimation of the calcium but made the results obtained open to criticism. It was therefore discarded and Hoffman's (33) method was then adopted. It was, by far, a more reliable method than that of Shohl and Pedley and proved, in the case of the urine of the rat, to be much less time consuming.

From the data obtained in this investigation, one feels little hesitation in stating that thyroid administration exerted a definite and pronounced effect on calcium metabolism in the animal body. Such results as were obtained indicated that thyroid administration increased the output of urinary calcium to a great extent. This increase in output was apparent from the first week of thyroid feeding, the total calcium excretion was about 32% higher than the highest level of excretion obtained in any of the four weeks that preceded thyroid administration in the/

Males:
Calcium Excretion.




Controls
Thyroid Fed.
Anterior Pituitary Fed
Anterior Pituitary Injected.
Oral and Parental administration
Of Extracts started.
Females:
Calcium Excretion.
Ca mgm.
16
15
14
13
12
11
10
9
8
7
6
5
4
3
1
2
3
4
5
6
7
8
9
10
WEEKS.

the male but not until the second week in the case of the female when the rise was only about 28% above normal, being insignificant in the first week. The increase in excretion continued to rise and by the end of the sixth week it was about 130% when calculated per 100 cc. of urine and about 144% in the total calcium excretion above normal in the male.

In the case of the female, however, the rise in urinary calcium excretion attained a height of about 65% per 100 cc. of urine and about 118% in total output above normal.

THE EFFECT OF THYROID ON PHOSPHORUS METABOLISM.

The main route of excretion of phosphorus is by way of the kidneys.

Briggs' (34) colourimetric method was used in estimating the urinary phosphorus.

As in the case of calcium, thyroid administration influenced the metabolism of inorganic phosphorus, as its output was definitely altered by such administration.

Very little to be found in literature on this subject. Schafer (3) using young white rats showed that there was an increase in phosphorus excretion by administration per os of fresh sheep thyroid. This increased output did not appear until the dose started/

started with(0.75 gm.)was doubled.

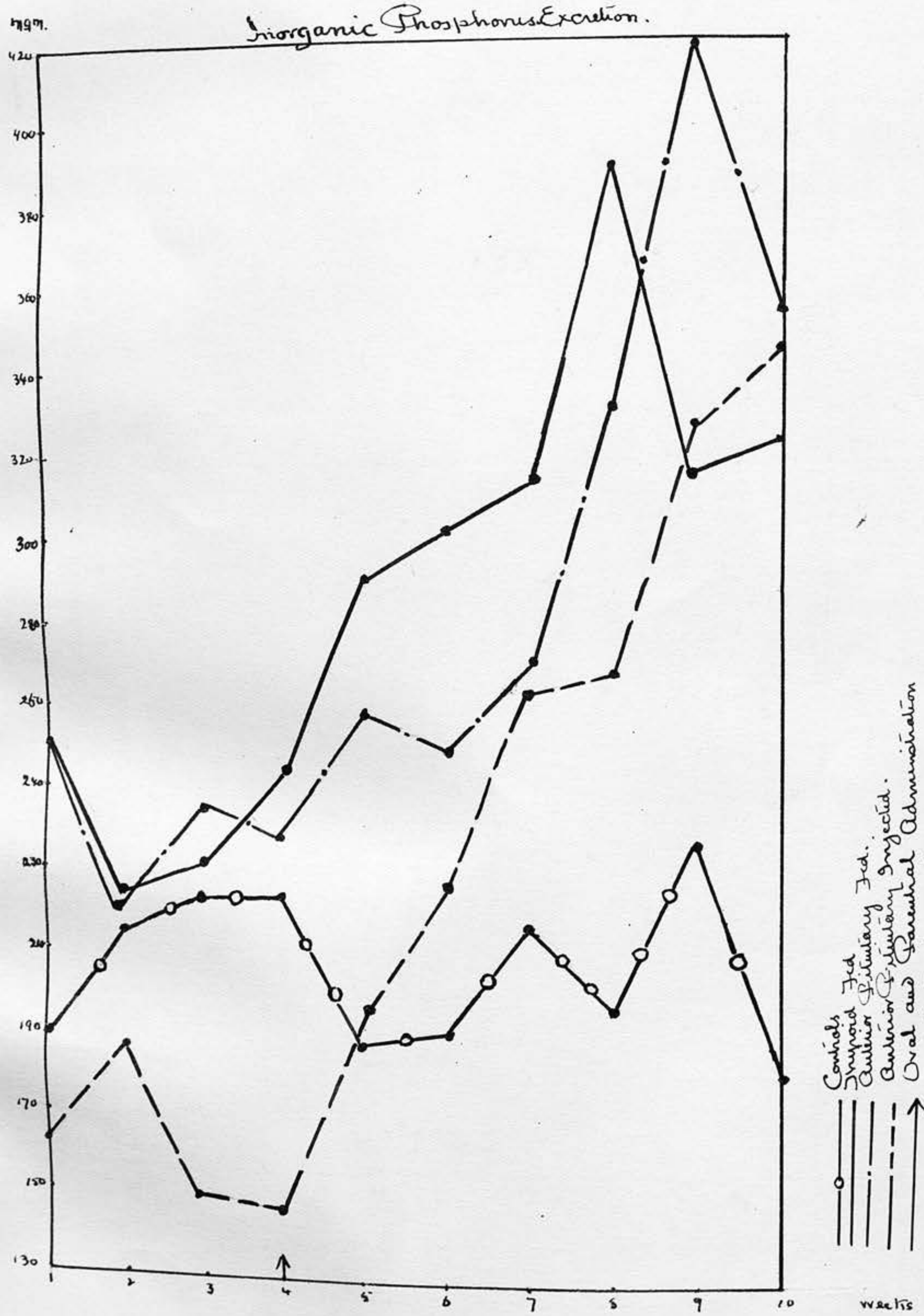
In the present investigation, it was found that oral administration of thyroid extract in a daily dose of 0.05 grm. per rat per diem produced an increase in phosphorus output. This increased excretion, as in the case of calcium, started to show soon after thyroid feeding was begun in the male but not until the second week in the case of the female, the rise in excretion in the first week although much higher than the week preceding it yet when the total output was considered, this increase in excretion appeared within the normal range. The increase in total phosphorus output attained about 17% over normal excretion in the male after one week's administration. The maximum level of excretion, however, was reached by the end of the fourth week of administration when it was 38% above normal, after which, the level of excretion showed some fluctuations. Such a rise calculated per 100 cc. of urine was found to be about 25% higher than the normal level of excretion.

In the case of the female, the increased phosphorus output attained its maximum height by the sixth week and exhibited a big difference from that of the male; the rise in total excretion attaining a level of about 80%, and per 100 cc. of urine about 37% higher than normal.

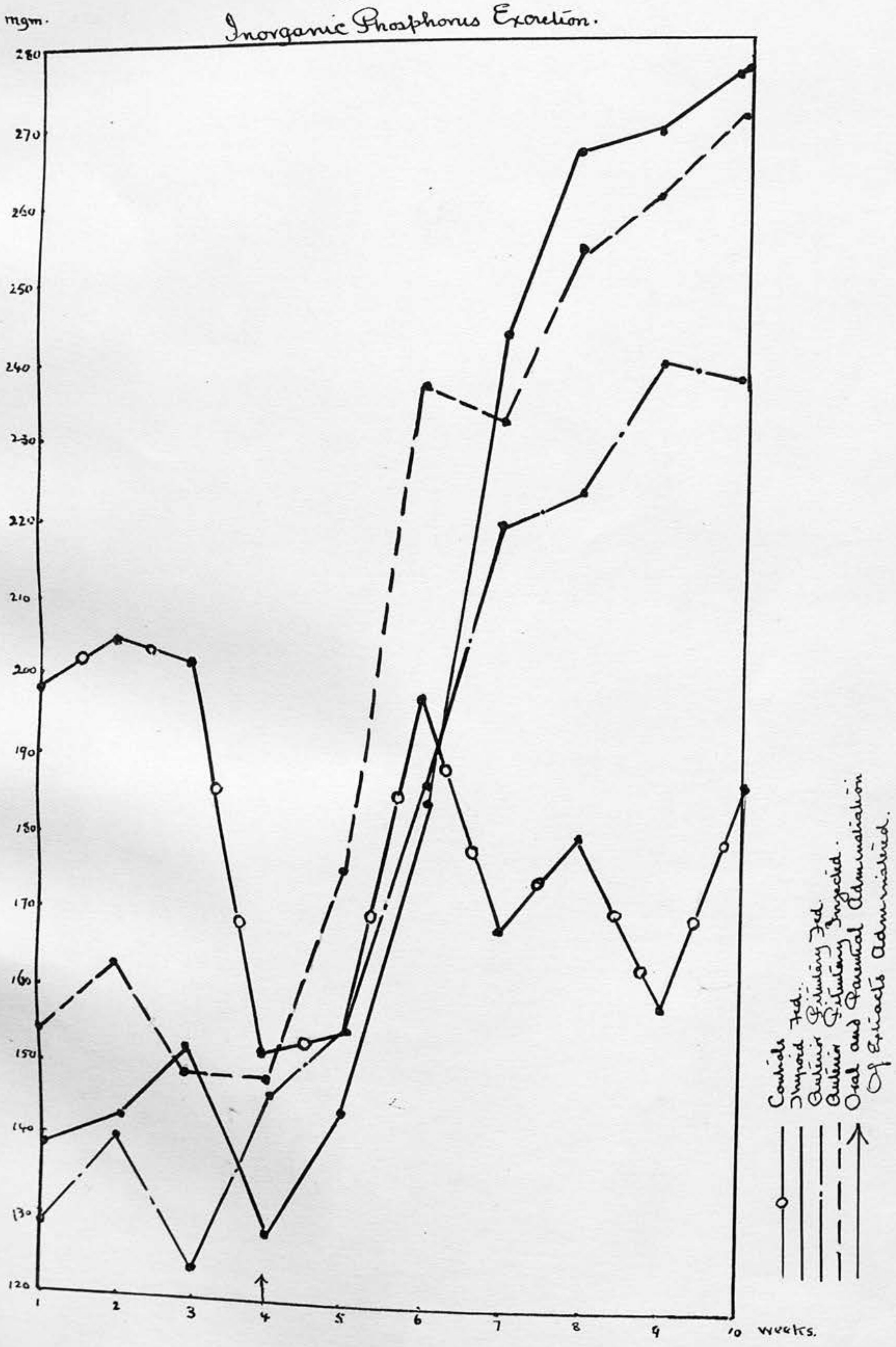
(See tables I & V.)

Males:

Inorganic Phosphorus Excretion.



Females:
Inorganic Phosphorus Excretion.



TECHNIQUE OF REMOVAL OF THE ENDOCRINE ORGANS.

At the close of the duration of each experiment, the animals were killed and the endocrine organs as well as the liver and spleen were removed from the animals and the following measures were adopted:-

In the first place each animal was weighed immediately after being killed, a general autopsy was done on each animal and any abnormalities were looked for, as well as any increase, decrease or absence of extra-peritoneal, perinephric and general body fat.

The organs were next removed and after being completely cleaned of the surrounding fat and connective tissue by means of very fine forceps and a sharp fine curved scissors, they were weighed.

The first organs removed were the suprarenal bodies due to the fact that they deteriorate rather rapidly after the death of the animal as far as their adrenaline-content is concerned. The microbalance was used for this purpose as well as for weighing the thyroid glands, the ovaries and those few pituitary bodies which were separated at this stage.

The pancreas, testes and all the other organs were weighed on the sartorius balance.

For removal of the thyroids and parathyroids great care was taken to isolate the glands without any of the muscle tissue that practically surround them/

them from the anterior, lateral and posterior aspects. To secure such a measure, the sternothyroid and sterno-hyoid muscles were cleanly removed. The oesophagus was then separated from the posterior aspect of the trachea and the glands were then exposed. (In the rat they lie opposite the uppermost rings of the trachea in relation to its lateral and postero-lateral aspects). The trachea was then cut across below the level of the thyroids and the glands cleanly dissected. By such a measure, the weight obtained, was the net weight of the thyroids including the parathyroids only.

The hypophysis of every rat was removed attached to and with the entire brain. The skull was opened into through the foramen magnum, by means of a sharp small pair of scissors insinuated under the parietal bones which were then removed thus exposing the lateral lobes of the brain. The entire brain was then mobilized along its anterior part, then turned slightly upwards and backwards and all the nerves at the base of the brain were severed commencing with the olfactory nerve. A similar procedure was then adopted to remove the basiocciput. The pituitary body was now seen and by means of a sharp cataract knife, the capsule of the gland was incised along its margin. The gland was then mobilized and removed attached to the entire brain.

No attempt was made to remove the pituitary bodies from the brain and weigh them as they were kept in that condition for the histological investigation. However, in spite of all the measures taken, some did split and these were weighed. Bone marrow was also taken from the femur of each animal for histological purposes. No other organ was removed except the liver and spleen.

METHODS OF TISSUE FIXATION USED.

Several fixatives were used according to the organ investigated:-

Fixative:	Organ:
Zenker formal; Formol saline and Regaud (35)	Hypophyseal body.
Suza & Lane's solution (36)	Pancreas.
Formol saline Chloral and Formol saline	Thyroid glands.
Suza and Regaud	Ovaries.
Suza and Regaud	Testes.
Cramer (37) Osmic acid Vapour and Bichromate.	Suprarenal.
Suza and Regaud	Liver and Spleen.

The duration for fixation varied for different tissues but never exceeded 24 hours except in the case of those fixed in Regaud's solution in which case/

case, 120 hours were needed, the solution being changed once every 24 hours.

After carrying the tissues through the grades of alcohol they were treated according to Peterfi's (38) method of methyl benzoate followed by benzole. This method of clearifying gives a much better result than either zylol or carbon tetrachloride followed by carbon disulphide. The tissues are made also less brittle and correspondingly easier to cut.

The tissues were cut generally at a thickness of 4 to 5 μ depending on the tissue. The suprarenals in particular were cut at a thickness of 6 - 7 μ. Serial sections were cut in the case of the adrenals, thyroids and ovaries.

Different methods of staining were adopted. The thyroid glands were stained with anilin blue and orange G of the modified azan method (Mallory) (39). A few sections were stained by Delafield's haematoxylin followed by eosin, chromotrop, Congo red or saffranin. The Mallory stain, however, was the one of choice as it was pointed out by Hewer (40) that such staining was a successful differential one for illustrating the different phases of activity of the thyroid gland. Part of Hewer's table of differential staining for the thyroid gland is quoted:-

Reagent./

Reagent.	Alkaline.	Acid.
Weigert's Resorcin-fuchsin	Pink	Orange
Anilin Blue & Orange G: Modified Azan.	Blue	Orange
Haematoxylin & Congo red	Pink	Blue

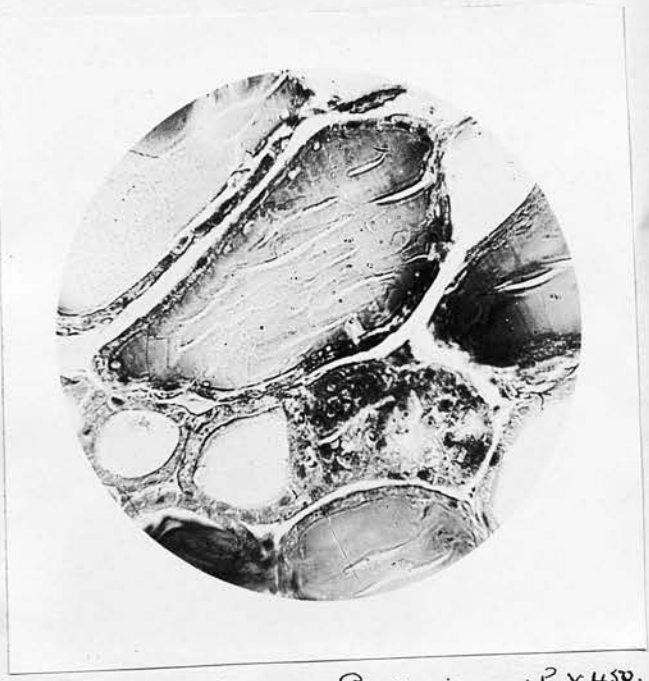
According to Hewer, the freshly secreted colloid indicative of increased activity responds to the alkaline reaction whereas the old colloid indicative of inactivity gives the acid reaction.

The pituitary bodies attached to the brain were stained by Leishman; methylene blue and alcoholic eosin, but the best results were obtained by Delafield's haematoxylin followed by water-soluble eosin or chromotrop.

The adrenals were treated by osmic vapour according to the histo-chemical method of Cramer (37). The sections after removal of the paraffin by zylol were treated with turpentine which dissolves the fat but leaves the adrenaline granules and the globoid bodies of Cramer unaffected.

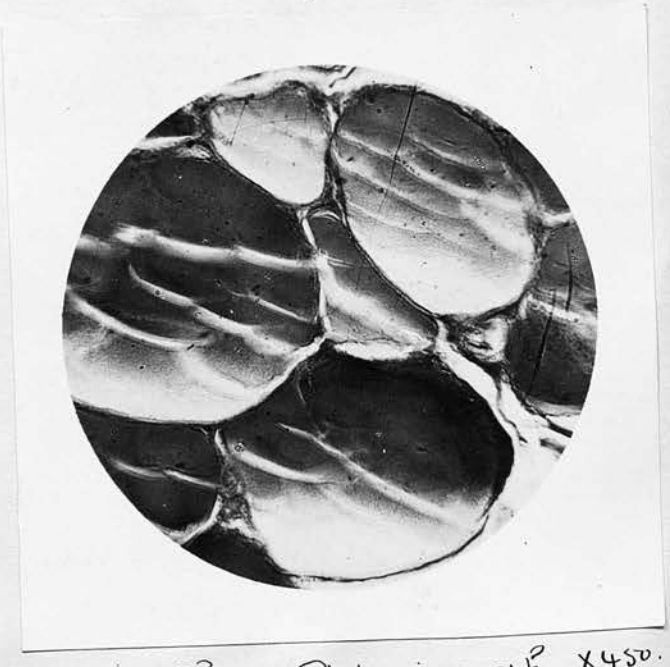
The testes were stained by the long method of Heidenhain (41). The sections were treated with ammonium iron alum for 36 hours followed by a good rinse in tap water after which they were placed in Heidenhain haematoxylin for 36-48 hours followed by a wash in tap water and then differentiated in ammonium/

Fig. (1).



Normal Thyroid. Photomicrograph X 450.
Note flat and low cuboidal epith. of vesicles.
Aniline blue & Orange G. preparation.

Fig. II.



Thyroid Gland. Photomicrograph X 450.
Animal fed with thyroid extract for six weeks
The epithelium is almost entirely absent.
Aniline blue & Orange preparation.

ammonium iron alum and the differentiation controlled by tap water. The stain was made much sharper by placing the slides in running water for 30 minutes.

Some of the sections were also counterstained by Benzolicht Bordeaux.

The ovaries were stained by Delafield's haematoxylin and counterstained by water soluble eosin. The sections were kept in haematoxylin twice as long as was necessary for ordinary staining or until they were much overstained and then they were differentiated with 1% solution of picric acid.

The pancreas was stained with Heidenhain haematoxylin and counterstained by saffranin. Delafield's haematoxylin followed by eosin or chromotrop had also been used.

The liver and spleen were stained by Delafield's haematoxylin and eosin.

The bone marrow was stained by May Greenwald stain.

THE THYROID OF THE NORMAL CONTROL RAT.

The thyroid of the albino rat lies in relation to the antero-lateral, the lateral and postero-lateral aspects of the uppermost rings of the trachea: and is covered over by the sternohyoid and sterno-thyroid muscles on these aspects and by the oesophagus on/

on the posterior aspect. It is very small and triangular in outline and when carefully removed from the animal, it always contains the parathyroid gland which is embedded in its substance at the upper postero-medial angle. The parathyroid is easily seen by the naked eye as a speck of glistening whitish yellow material. It is almost always single but occasionally one meets in the rat with two parathyroids, one in each angle of the upper part of the thyroid, the second one lies in the posterolateral angle in a straight line with the other.

THE HISTOLOGICAL PICTURE.

The gland presented a number of vesicles with a certain amount of intervesicular connective tissue. The vesicles were lined by flattened epithelium which occasionally assumed the short cubical type. The majority of these vesicles were filled with colloid which, in some of them, completely filled the entire vesicular space. In very few vesicles, however, the colloid was slightly shrunken from the lining epithelium. Fig. 1.

The vesicles exhibited great variation in size, some being very much larger than others, but on the whole they were of moderate size. They were generally rounded or oblong in shape.

THE/

THE STAINING CHARACTER OF THE NORMAL GLAND.

The epithelium of the vesicles stained readily with Delafield's haematoxylin counterstained by eosin, chromotrope or Congo red or by anilin blue and orange G.

By the first method the nuclei of the epithelial cells were stained blue of a moderate density, the cytoplasm was stained pink.

By Mallory anilin blue and orange G. stain however, the normal thyroids presented a characteristic appearance. The nuclei of the vesicular epithelium were stained orange, and the cytoplasm was stained pale blue. The colloid however exhibited a staining peculiar in character. In some of the vesicles the colloid stained deep blue, in some others deep orange, but the majority of vesicular colloid was stained with both dyes, being partly deep blue and partly deep orange. The orange stain, however, predominated and as it was shown by Hewar, such predominance of orange indicated inactivity on the part of the thyroid gland.

THE EFFECT ON THE THYROID GLAND OF THYROID ADMINISTRATION.

It was reported by some investigators that the thyroid gland showed only indications of rest or inactivity with thyroid administration. In the present work it was found that thyroid extract orally administered/

administered produced a striking result. The gland as a whole exhibited signs of extreme inactivation. In some parts of the thyroid, the epithelial cells of the vesicles showed marked degenerative changes while in the remaining parts of the gland the epithelium was completely absent. The cells seen were extremely flattened. J-g.(2)

The vesicles were very markedly enlarged and distended with colloid, which stained almost entirely by the orange dye; very little blue was visible. In some vesicles the colloid was much thicker than normal.

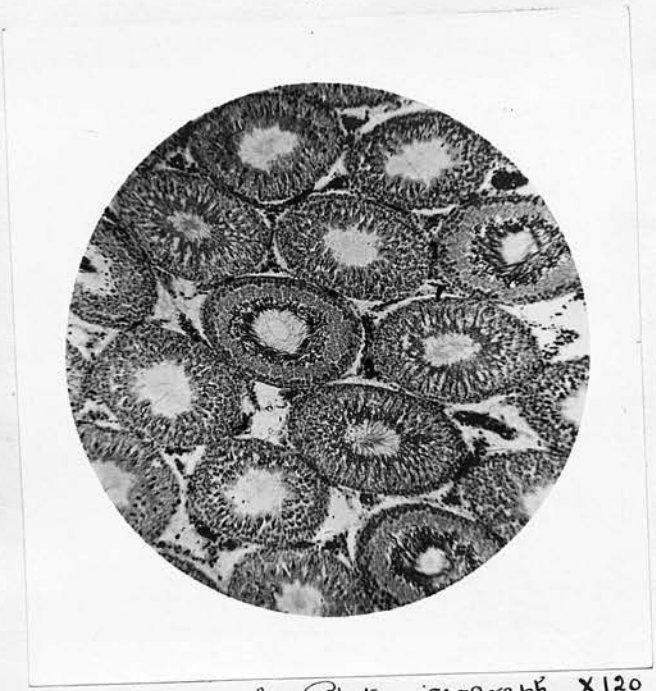
By the haematoxylin eosin staining, some of the nuclei seen were very deeply stained by the basic dye.

The connective tissue supporting the vesicles was very markedly decreased in amount and some parts reduced to a minimum or altogether absent.

No hyperplasia of epithelium was ever noticed in the thyroid glands with experimental hyperthyroidism.

It is of interest to mention, that the above described results of thyroid administration were obtained by feeding of the extract for a minimum of six to seven weeks. After four or five weeks administration, the degeneration was not so apparent and after/

Fig. III.



Testicle. Normal. Photomicrograph X120.
Heidenhain iron haematoxylin preparation.

Fig. IV.

Testicle: Photomicrograph X 120.
Animal fed with thyroid Extract for six weeks.
Heidenhain iron haematoxylin preparation.
Degenerated tubules. Hypertrophy of interstitial tissue.

after three weeks administration, the histological picture was only that of marked inactivation with very flattened epithelium.

THE EFFECT ON THE SEX ORGANS OF THYROID ADMINISTRATION.

THE TESTIS: THE CONTROL ANIMAL.

The testicle of the normal adult white rat resembled any other mammalian adult testicle. The seminiferous tubules were of normal size. They however contained few spermatozoa. The interstitial tissue and the strands of intertubular connective tissue and blood vessels exhibited no abnormality of hyperplasia, hypoplasia, hypertrophy or atrophy. J-g(3)

THE EXPERIMENTAL TESTICLE:

A very striking result was obtained by oral administration of desiccated thyroid extract. The weight of the organ was invariably found to be decreased than those of the litter male controls.

This result is contrary to the results obtained by Hoskins and by Herring and by Cameron and Carmichael who reported that such administration produced hypertrophy of the organ with increase in weight.

The seminiferous tubules showed advanced degenerative/

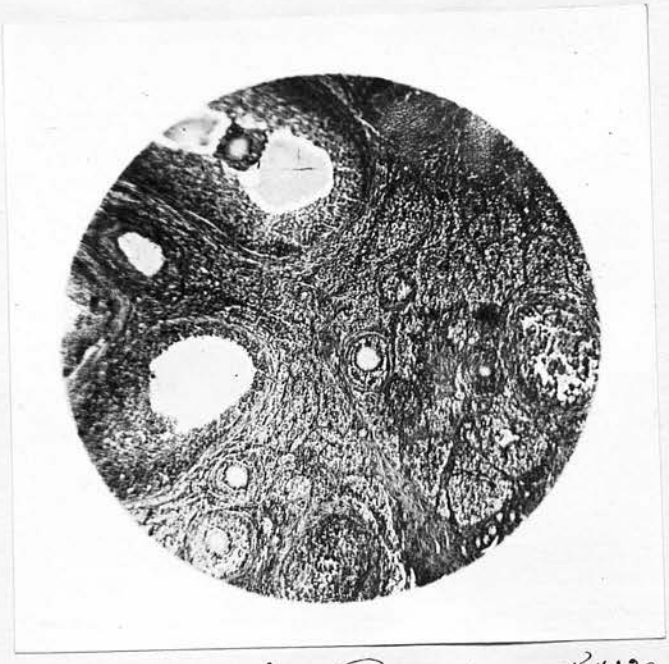
degenerative changes. A very great number of these tubules showed complete degeneration leaving only a remnant of the layer of spermatogonia cells with no nuclei to be seen and the outline of the cells was more or less obliterated. Complete degeneration of some tubules with absolute absence of any kind of cell was obtained in some parts in which case the connective tissue membrane was the only structure to be seen. Some tubules, however, still contained spermatogonia cells and occasionally some spermatocytes were also seen but in these the supporting cells of Sertoli were absent. The tubules that remained unaffected by degeneration contained practically no spermatozoa and the tubule as a whole presented a vacuolated appearance with the nuclei of the cells exhibiting piknosis in some and karyolysis in others, both phenomena are indications of starting retrogression towards degeneration. Fig(4)

In spite of the marked degeneration of the externally secreting part of the testicle, the internally secreting part or interstitial tissue of the organ showed a definite hypertrophy. The blood vessels were markedly dilated and engorged with blood corpuscles. With high magnification no mitotic figures were seen in the interstitial tissue.

Such results as above described were obtained by

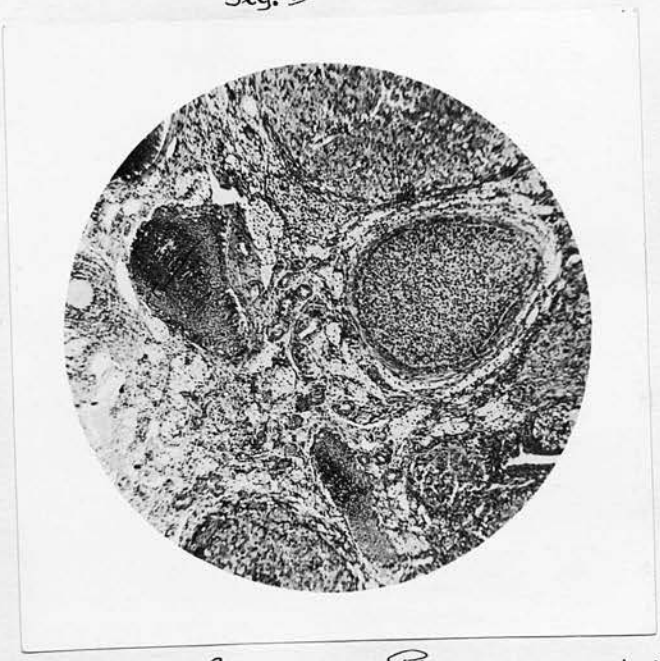
continued/

Fig. V.



Ovary. Normal. Photomicrograph X120.
Delafield's haematoxylin and
chromotrop.

Fig. VI



Ovary. Thyroid fed. Photomicrograph X120.
Animal fed on thyroid extract for
seven weeks.
Showing increased lutein and very pronounced
increase by hyperplasia of the interstitial
tissue. Also marked dilatation of blood
vessels.
Delafield's haematoxylin & chromotrop
preparation.

continued administration of thyroid extract for a minimum six to seven weeks.

THE OVARY:

The Normal Ovary of the Control Rat.

The ovary of the white adult rat is a very small solid organ which is generally rounded in shape but may be elongated. It lies in close association to the fallopian tube which, in the rat, is a minute thin coiled tube. The coils of the fallopian tube are difficult to identify from the tissue of the ovary but by careful manipulation the tube can be cleanly severed from the substance of the ovary. Great care must be exerted in the removal of the tube as failure to do so introduces an appreciable error in the weight of the gland. Fig-(5)

The Histological Picture:

The organ is composed of connective tissue constituting the ground substance or stroma of the gland in which there are an appreciable amount of spindle shaped cells that stain clearly by the basic dyes. This connective tissue contains also blood vessels along which the interstitial tissue is seen. The germinal layer constitutes a thin stratum of epithelial cells which are more cuboidal than columnar.

The Graffian follicles are scattered throughout the/

the gland. They vary in size depending on the degree of maturation. The greater number of these follicles were small containing a small ovum. The small Graffian follicles under the layer of germinal epithelium that normally exist in the ovaries of other mammals were not observed in the normal rat. Fig (5).

The Experimental Ovary.

The ovary showed a decrease in weight with prolonged thyroid feeding. Fig(6)

The organ showed an increase in the number of corpora lutea. Indeed, the major part of the gland was made up, besides the connective tissue stroma and the interstitial tissue, of moderately large sized corpora lutea the cells of which were seen filling the large cavity of the follicle. These cells were arranged in columns which radiate towards the centre of the follicle.

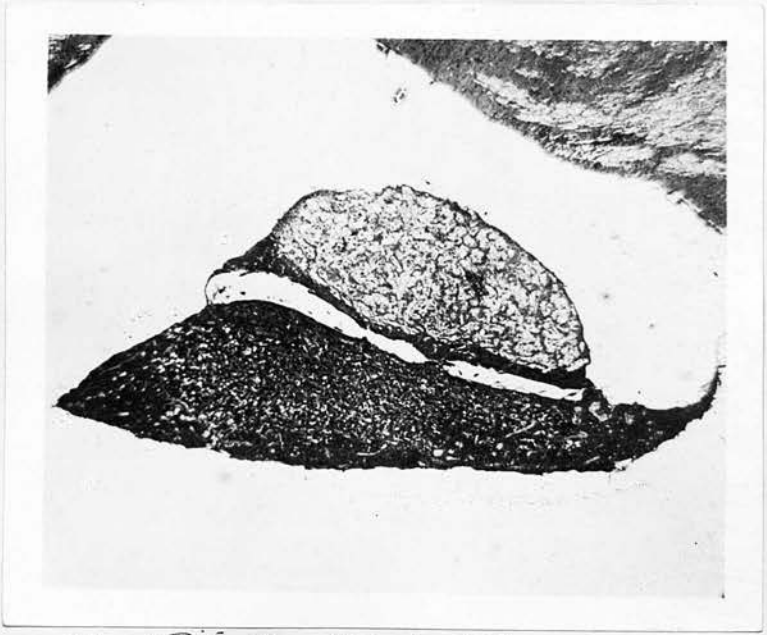
The corpora lutea were also very vascular, capillary blood vessels permeated the entire luteal tissue.

The blood vessels in the medulla of the ovary showed very marked dilatation.

The interstitial tissue of the ovary showed a remarkable hypertrophy and hyperplasia the cells being very much more numerous and slightly larger than those of the control ovaries.

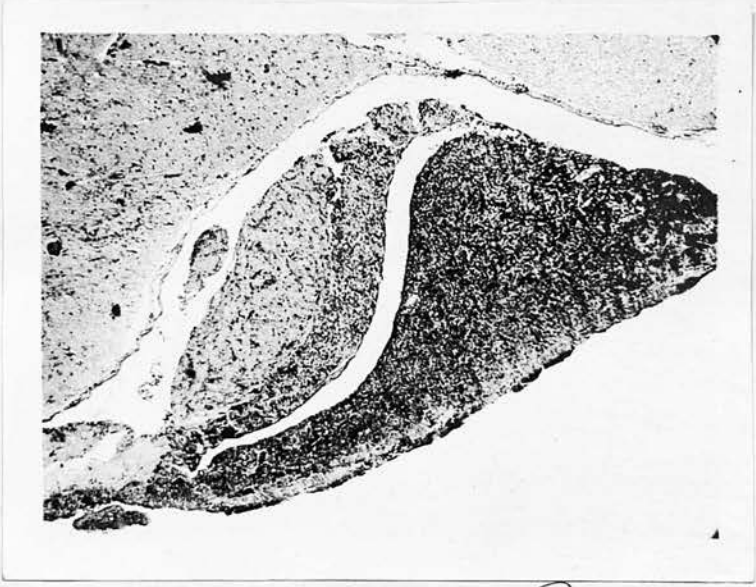
The/

Fig. IX.



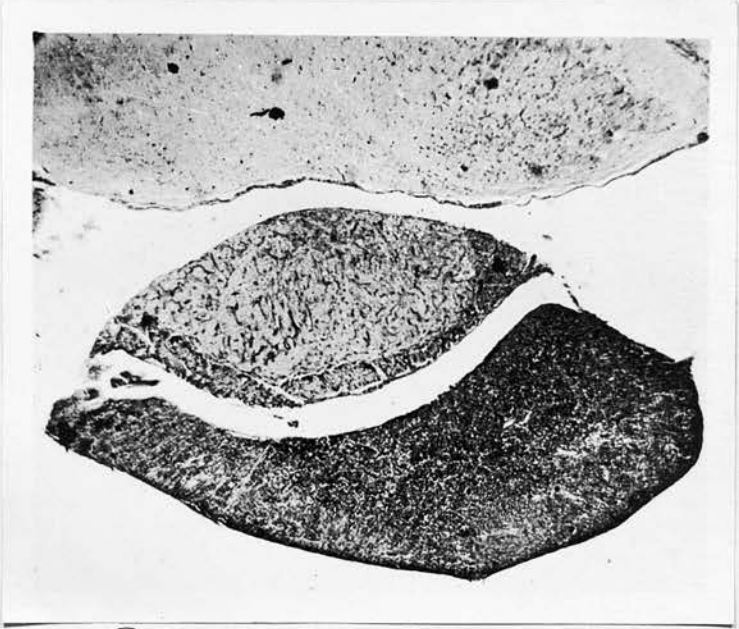
The Pituitary Body. Photomicrograph X 60.
Animal fed on thyroid extract for seven weeks. Showing marked atrophy in all the component parts of the gland. Degenerative changes quite marked at such low magnification. Delafield's haematoxylin and Chromotrop preparation.

Fig. VII.



The Pituitary Body. Normal. Photomicrograph X 60.
Delafield's haematoxylin and chromotrop preparation.

Fig. VIII.



The Pituitary Body. Photomicrograph X 60
Animal fed on thyroid extract for four weeks. Showing slight enlargement particularly noted in the pars nervosa.
Delafield's haematoxylin and chromotrop preparation.

The connective tissue stroma of the experimental ovary showed a very distinct atrophy throughout the entire organ.

THE INFLUENCE ON THE HYPOPHYSIS OF THYROID ADMINISTRATION.

The Normal Pituitary Body of the Control Rat:

The pituitary body of the adult white rat is a very tiny organ that lies in a shallow sella turcica characteristic of the rat. It is surrounded by a connective tissue capsule firmly adherent to the edges of the sella turcica and which makes it difficult to remove the gland attached to the brain, especially when one considers that the infundibulum in the rat is exceedingly tiny and thread like.

When removed attached to the base of the brain, the pituitary body contrasts in colour with the brain substance being more pinkish.

In outline it is more or less pyramidal. In section it presents the appearance of an equilateral triangle with the apex of the triangle placed anteriorly and the base placed posteriorly. Fig (7)

The Microscopical Appearance. Fig. (10)

The pars anterior occupies nearly one half to three fifths of the entire organ. It is separated

from the pars posterior by a distinct fairly wide cleft that runs along the entire length of the gland. The pars intermedia exhibits a fairly constant and uniform thickness and is juxtaposed to the pars nervosa without many irregularities at the line of union.

The cells of the pars anterior are composed of chromaphobe and chromaphil variety. The oxyphil cells are more in abundance than the basiphil cells. The chromaphobe and chromaphil cells are in the form of a compact mass permeated by a large number of moderately dilated capillaries whose walls are so thin that they simulate the sinusoids of other endocrine organs. These capillaries are almost always engorged with red blood corpuscles.

The oxyphil cells are generally uniform in size and shape, although slight variation in shape or even size was sometimes also seen.

The cytoplasm of the oxyphil cells presented a fine granular appearance. The granules stained by the acid dyes, but less readily than oxyphil granules of other mammals, for example the cat. The nuclei of the oxyphil cells were centrally placed and were rich in chromatin, staining therefore, deeply with basic dyes.

The basiphil cells varied very widely, some being smaller and others larger than the oxyphil cells. The cytoplasm is granular, such granules however/

however, are coarser than those of the oxyphil cells and they stain readily by the basic dyes. The nuclei are also centrally placed and are rich in chromatin.

The greater bulk of the cells of the anterior lobe, however, consisted of chromaphobe cells which constituted about 60% of the total number of cells in the anterior lobe leaving only 40% for both types of the chromophil variety. The chromaphobe cells were nearly of the same size and shape.

The cytoplasm is clear and non-granular and reacts slightly to the acid dyes. The nuclei of these cells were also centrally placed.

The Pars Intermedia:

The cells of the pars intermedia are large octahedral cells, larger in size than the cells of the pars anterior. They contain a relatively large rounded centrally placed nucleus with chromatin material scattered all over its substance. The cytoplasm is clear with moderately coarse neutrally stained granules. Many fusiform cells of the reticulo endothelial system are also showing. Fig(13)

The cells of pars intermedia exhibit a sharply defined margin between them and the pars nervosa but only occasionally some of the cells project a little into the substance of the pars nervosa.

Very few capillary blood vessels were seen in this/

this part of the gland.

The pars nervosa exhibited microscopically the appearance of a fine network of fibres with some neuroglia elements. One or two small hyaline masses of colloid "Herring's bodies" were seen amongst the fibres. A number of large capillary blood vessels were also observed.

The cells of the pars tuberalis resembled those of the pars intermedia and they spread in a very thin strip along the base of the brain and the third ventricle.

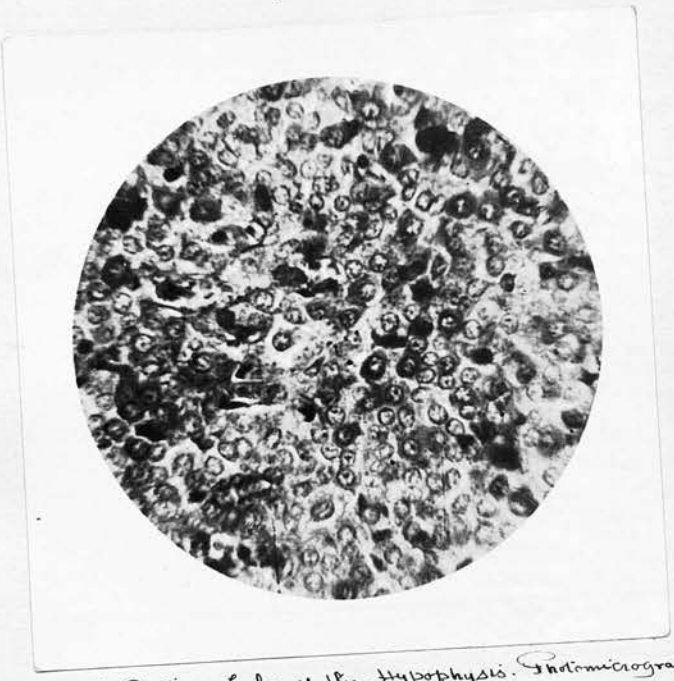
The Experimental Pituitary Body:

Oral administration of desiccated thyroid extract produced two kinds of results as far as the pituitary body was concerned. One of the results was obtained by feeding thyroid extract for three to four weeks, and the other by six to eight weeks administration.

In the first result, the short administration caused some hypertrophy measurable by the naked eye Fig.(8) as well as by weighing the gland, the increase in weight was three milligrams on one occasion and four milligrams in the other occasion. Such weighing of the glands was, however, not in view but only done when accidental splitting of the pituitary bodies during removal did happen and although no definite conclusion could be arrived at from weighing one or

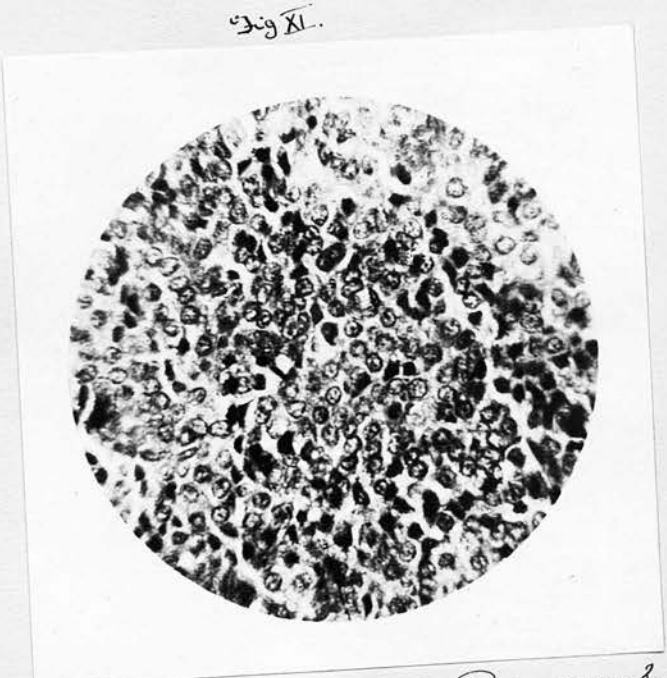
two/

Fig. X.



Normal Anterior Lobe of the Hypophysis. Photomicrograph X 450.
Delafield's haematoxylin & Chromotrop preparation.
Oxyphil cells appear dark in the photograph
while the cells of the Chromaphobe variety appear light.

Fig XI.



Anterior Lobe of the Hypophysis: Photomicrograph X 450.
Delafield's haematoxylin and Chromotrop preparation.
Very few oxyphil cells showing. Some nuclei
of the oxyphil cells showing pyknosis. Slight
increase in the reticulo endothelial system cells.
Animals fed with thyroid extract for four weeks.

two glands yet taken with the obvious naked eye size of the gland, hypertrophy might be considered as a result.

In the second result, the long feeding of thyroid extract caused the reverse of what was mentioned above. The gland on removal was more flattened, distinctly much smaller on its ventral aspect and paler than the control ones. Only one pituitary of such kind was accidentally split and thus was weighed: the decrease was six milligrams. Fig.(9)

THE HISTOLOGICAL PICTURE:

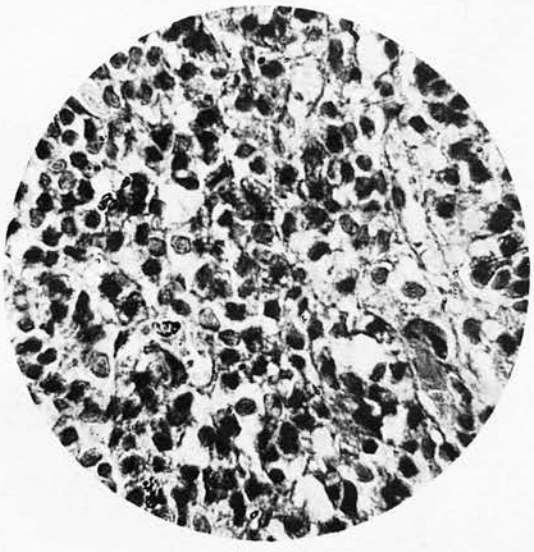
The Pars Anterior: First series of results.

The cells of the anterior lobe were the ones to exhibit the most marked features of the effect of thyroid extract. Fig.(11).

The cells are still differentiated into chromophobe and chromaphil cells, but instead of the oxyphil chromaphil cells being in abundance, they were decreased to a minimum, and a preponderance of the basiphil cells was evident.

The oxyphil cells present were much smaller in size and the chromatin of the nuclei was very much denser than normal. No change was showing in the chromaphobe cells. The sinusoid-like capillaries were numerous and slightly dilated. Slight spacing between the cells was noticed in parts of the anterior/

Fig. XII.



Anterior Lobe of the Hypophysis. Photo-micrograph X450
Animals fed with thyroid extract for seven weeks. Delafield's haematoxylin and Chromotrop preparation.
The oxyphil cells have almost entirely disappeared.
The anterior lobe presents a picture of degeneration.

anterior lobe.

The Pars Intermedia:

The pars intermedia showed no definite change to note.

The Pars Nervosa:

The pars nervosa showed a distinct hypertrophy, the microscopical appearance at or nearly at the same level of the gland was about 20% larger than normal.

The number of Herring's bodies in the pars nervosa were appreciably increased.

In the cleft of the pituitary there was also a little amount of colloid showing, which was entirely absent in any of the normal pituitaries.

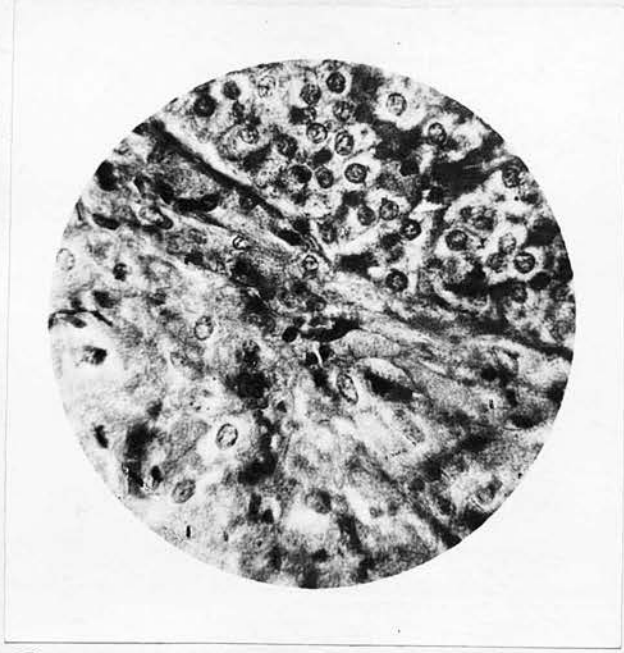
Second Series of Results:

The Pars Anterior:

The compact nature of the control anterior lobe was no longer seen. The texture was much looser and the whole anterior lobe presented a reticular framework which was more obvious than normal due to the degeneration of the glandular elements. Fig(12).

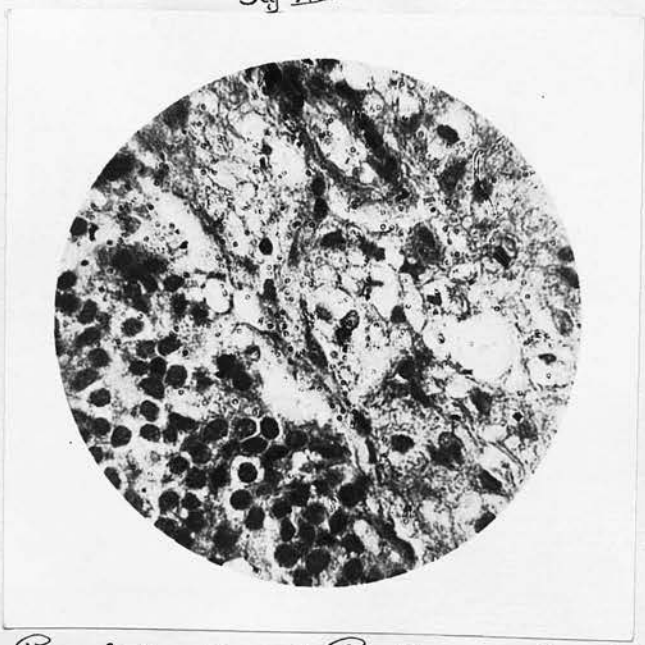
The oxyphil variety of cells appeared to have almost entirely disappeared by degeneration. An occasional cell was seen here and there but on its way to degeneration, the chromatin of the nucleus condensed, i.e. in piknosis, and the outline of the cell shrunken and the cytoplasm stained but very poorly/

Fig. XIII.



Pars Intermedia and Pars Nervosa. Normal
Photomicrograph X 450.
Delafield's haematoxylin and chromotrop preparation.

Fig XIV.



Pars Intermedia and Pars Nervosa: Thyroid fed.
Photomicrograph X 450
Animals fed on thyroid extract for seven weeks
Delafield's haematoxylin and chromotrop preparation
The nuclei of cells of pars intermedia showing pyknosis.
Pars nervosa showing degeneration.

poorly.

The basiphil cells showed slight decrease in number.

The Pars Intermedia:

The pars intermedia showed a distinct decrease in size. The cells were very much smaller than normal. The chromatin material showed more aggregation together to form a densely stained mass. The open chromatin meshwork of the normal nucleus was no longer showing. The cytoplasm appeared denser than normal. The cells of the reticulo-endothelial system were more prominent but probably this was only due to the degeneration of other elements. Fig. (14).

The Pars Nervosa:

The pars nervosa showed definite degeneration giving the appearance of open meshwork between degenerated looking ependyma elements and fibres. No Herring's bodies were observed. Fig. (14)

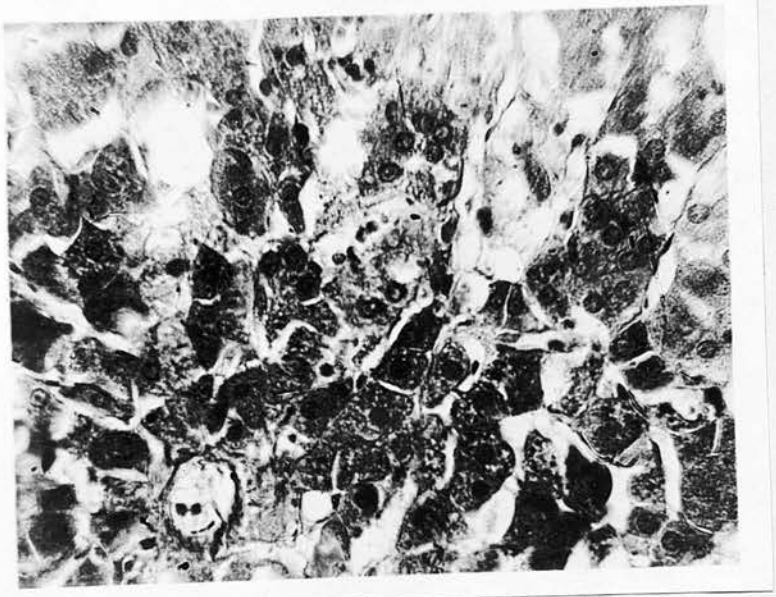
THE EFFECT ON THE SUPRARENAL OF THYROID ADMINISTRATION.

The Normal Control Suprarenal:

The suprarenal of the adult white rat consists, as in the case of other mammals, of two parts, the cortex and medulla, the two being enveloped by a thin capsule of connective tissue.

The/

Fig XV



The Suprarenal Body. Zona Reticularis & part of Medulla showing. Photomicrograph X 450. Normal. Osmic acid vapour (Cramer) preparation.

Fig XVI

The Suprarenal Body. Zona Reticularis and medulla showing. Photomicrograph X 450.
Osmic acid vapour preparation.
Animals fed with thyroid extract for six weeks.
The amount of Colloid is very marked in the zona reticularis. Medulla showing a marked increase in the adrenalin granules.

The cortical subdivision into zona glomerulosa, fasciculata and reticularis was not distinct in the rat except in the case of the first mentioned zone which was clearly seen in thin sections, being composed of the characteristically arched rows of cells.

The cells of the zona glomerulosa and the zona fasiculata particularly in its outer half were as a rule loaded with lipoid material which stained black by Cramer's osmic vapour method. The cells of the inner half of the fasiculata and those of the reticularis were practically free from lipoid and only here and there a little amount of lipoid was seen in them.

In the cells of the zona reticularis there were also present few black globules, the "glodoid bodies" of Cramer which are supposed to be adrenalin or precorsers of adrenalin. The cells, as a whole, were large and granular, the granules staining brown by the osmic vapour. Their nuclei stain dark brown by osmic.

Blood vessels are abundant and fairly large in the zona reticularis. They permeate the entire zone and reach the medulla where they become changed into wide sinuses.

The medullary cells are of two kinds, the dark and the white variety according to whether they stain darkly or lightly by osmic acid vapour. The white variety/

variety preponderates.

The medullary cells contained very little adrenaline granules. Fig.(15).

THE EXPERIMENTAL SUPRARENALS:

HISTOLOGICAL PICTURE.

The Cortex:

In this part of the gland, the picture was appreciably altered by oral administration of desiccated thyroid extract. The lipoid material as a whole, was very much more abundant throughout the entire cortex. The inner half of the zona fasiculata and all the zona reticularis were swept over by an abundant lipoid material.

After treatment with turpentine, there were seen in the cells of the zona reticularis a great increase in the globoid bodies of Cramer which were much larger and coarser than normal. According to Cramer such a microscopical picture indicates a hyperactive cortex.

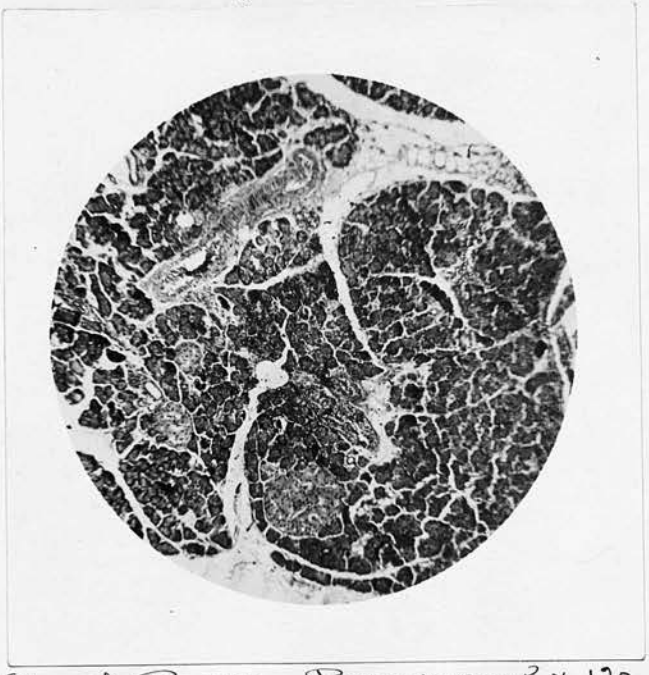
The Medulla:

The histological picture of the medulla also exhibited an appreciable difference from normal.

The predominance of the white variety of medullary cell was much more marked than in the control.

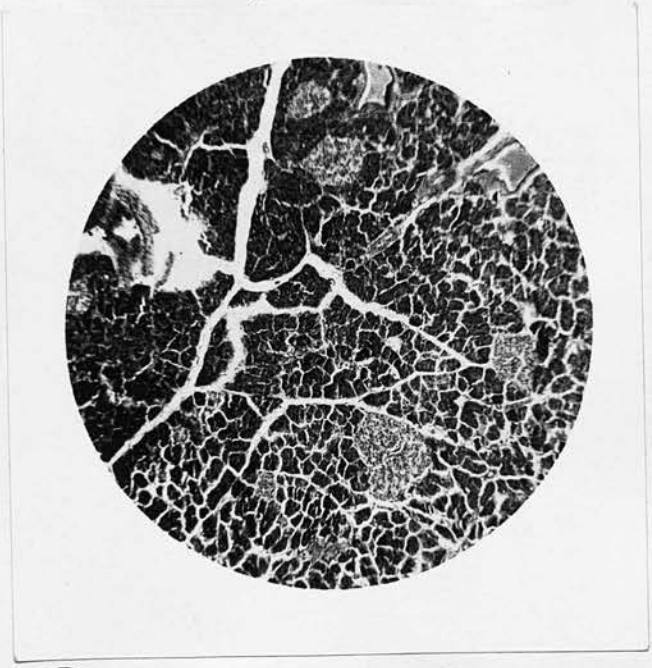
Black adrenalin granules were seen in abundance in/

Fig. XVII.



Normal Pancreas. Photomicrograph X 120
Heidenhain iron haematoxylin and Saffranin preparation.

Fig. XVIII.



Pancreas. Thyroid fed. Photomicrograph X 120
Heidenhain iron haematoxylin and Saffranin preparation.
Alveoli smaller: Zymogen granules increased.
Texture of organ much denser than normal.

in different parts of the medulla indicating a very great increase in adrenaline formation. Fig(16)

The sinusoids were greatly dilated and contained numerous greyish granules and some red blood corpuscles staining brown by the osmic vapour.

THE INFLUENCE OVER THE PANCREAS OF THYROID ADMINISTRATION.

The Normal Pancreas of the Control Rat:

The pancreas of the rat is a diffuse and very thin organ. Microscopically it presented a looseness in texture, the lobules being widely separated from one another by connective tissue. The alveoli exhibited but very little variation in size. The alveolar cells were angular and were of different sizes some being larger than others. Each cell showed a subdivision into an outer darkly stained and an inner granular less deeply stained zones. The inner part of the cell occupied the major part of its size. The granules of this zone stained readily by acid dyes: and they are zymogenic in nature. Fig(17.)

The nuclei of the alveolar cells showed variation in size and in the amount of chromatin they contained. They stained bluish black by Heidenhain iron haematoxylin and occupied almost a constant site in the cell, being situated a little outer to the junction of the outer and inner zones of the cell.

The/

The islets of Langerhan were seen scattered all over the pancreas and more numerous in some parts than others. They varied greatly in shape and size, some being fairly large in size and others were relatively very much smaller. In shape some islets exhibited a circular, and others an elongated or irregular appearance. The cells of the islets showed a clear subdivision into A and B cells. Their nuclei were rounded and centrally placed in the cell. The chromatin was distributed all over the nucleus giving it an appearance of slight vacuolation.

The Experimental Pancreas:

Short durations of thyroid administration of a minimum of three weeks and long duration of a maximum of eight weeks affected the pancreas in the same way in that the organ responded to both by hypertrophy and increased weight.

THE HISTOLOGICAL PICTURE:

The microscopical picture was distinctly and obviously changed. The organ appeared much more compact and denser than the normal organ, the lobules being much more nearer the one to the other. The amount of connective tissue separating the lobules was greatly decreased in amount. Fig.(18)

The alveoli as a whole, showed a decrease in size, being appreciably smaller than normal. There was/

was not an appreciable corresponding decrease in the size of the nuclei but they stained very deeply by Heidenhain iron haematoxylin.

The zymogen granules showed a definite increase in amount over the normal.

The blood vessels were slightly more dilated than normal.

No obvious histological change was observed in the structure of the islets of Langerhan.

THE INFLUENCE ON THE PARATHYROIDS OF ADMINISTRATION OF THYROID EXTRACT.

No histological change of any kind was obtained by which any inference could be made as to the effect of thyroid administration on the parathyroid glands. It was thus concluded that at least, in the present investigation, no inter-relationship between these two endocrine organs had been demonstrated. Such a result is contrary to that reported by Kojima (21) who in 1917 stated that the cells of the parathyroid gland showed an increased size.

THE INFLUENCE ON THE LIVER AND SPLEEN OF ADMINISTRATION OF THYROID EXTRACT.

No histological change of any significance to note.

THE INTERRELATIONSHIP OF THE ANTERIOR LOBE OF THE HYPOPHYSIS WITH OTHER ENDOCRINE ORGANS.

TABLE II. MALES.

No.of weeks	No.of animals in Group	Wt.of Group in Grams	O_2 Consumption.	Amount of food consumed per week.	Total Nitrogen of food consumed	Total Nitrogen of urine	Total Nitrogen of foeces	Total Nitrogen of Urine + foeces
1	3	800	1175	330	4.620	1.575	1.805	3.380
2	3	805	1150	333.5	4.676	2.123	0.802	2.925
3	3	799.5	1010	323	4.536	2.127	0.773	2.900
4	3	802	1165	338	5.166	2.917	1.116	4.009
5	3	789	1205	355	4.970	2.378	1.064	3.442
6	3	810	1290	369.5	5.180	1.024	1.773	2.797
7	3	815	1560	374.5	5.250	2.382	0.380	2.762
8	3	827.5	1510	353.5	4.956	1.448	0.990	2.438
9	3	835	1320	389.0	5.446	1.498	0.917	2.415
10	3	849	1535	363.0	5.082	1.283	0.952	2.235

TABLE II. (continued)

Nitrogen Balance	N.balance per kilo of body weight.	Amount of Urine voided per week	Calcium output in mgms. per 100 cc. urine.	Total Calcium excreted	Phosph.output in mgms. per 100 cc. urine.	Total Phosphorus output
1.240	1.548	175	4.6	8.05	119.1	247
1.751	2.174	158	4.2	6.6	133.4	211
1.636	2.043	188	4.0	7.5	125.0	234
1.107	1.380	165	4.0	6.6	138.8	228
1.528	1.936	178	4.0	7.1	145.0	258
2.383	2.940	169	3.5	6.75	148.8	250
2.488	3.046	185	3.2	5.9	146.1	270
2.518	3.041	201	3.2	6.4	158.5	333
3.031	3.630	230	2.3	5.3	183.0	420
2.235	3.23	215	2.2	4.73	166.8	356

TABLE III. MALES.

No. of weeks	No. of animals in Group	Wt. of Group in Grams	O_2 Consumption.	Amount of food consumed per week.	Total Nitrogen of food consumed	Total Nitrogen of urine	Total Nitrogen of foeces	Total Nitrogen of Urine + foeces
1	3	780	1125	373	5.222	1.538	1.877	3.415
2	3	785	1025	322	4.508	2.031	0.840	2.871
3	3	801	1030	323.5	4.536	2.069	0.819	2.888
4	3	790	1040	334.5	4.690	1.999	0.858	2.857
5	3	841	1420	368	4.662	1.499	1.092	2.515
6	3	837.5	1380	353	4.942	1.341	0.876	2.217
7	3	845	1470	392.5	5.472	0.969	0.572	1.541
8	3	877	1370	406.5	5.638	1.178	0.545	1.723
9	3	887	1400	434.4	6.046	1.220	0.683	1.903
10	3	912	1525	411.5	5.752	1.724	0.616	2.340

TABLE III. (continued)

Nitrogen Balance	N.balance per kilo of body weight.	Amount of Urine voided per week	Calcium output in mgms. per 100 cc. urine.	Total Calcium excreted	Phosph.output in mgms. per 100 cc. urine	Total Phosphorus output
1.807	2.312	176	4.3	7.6	92.4	162
1.637	2.082	156	4.6	7.2	120.0	187
1.648	2.035	168	4.4	7.4	89.5	150
1.833	2.320	168	4.5	7.6	88.2	148
2.071	2.46	153	4.1	6.3	128.8	195
2.525	3.02	157	3.5	6.5	130	216
3.931	4.65	164	3.7	6.1	140	264
3.915	4.451	189	2.8	5.3	142	268
4.143	4.651	230	2.0	4.6	143.2	328
3.392	3.85	240	2.0	4.8	144	346

TABLE VI. FEMALES.

No.of weeks	No.of animals in Group	Wt.of Group in Grams	O_2 Consumption.	Amount of food consumed per week.	Total Nitrogen of food consumed	Total Nitrogen of urine	Total Nitrogen of foeces	Total Nitrogen of Urine + foeces
1	3	587	845	271	3.794	1.074	0.964	2.038
2	3	585.5	875	278	3.892	1.078	0.899	1.977
3	3	587	890	280	3.934	1.745	0.868	2.613
4	3	594.5	837	280	3.934	0.967	1.558	2.525
5	3	618.25	960	310	4.356	0.902	1.246	2.148
6	3	624.5	965	366	5.138	0.812	0.953	1.765
7	3	632.5	1120	326.5	4.578	0.792	0.833	1.625
8	3	642.5	1280	302	4.242	0.690	0.728	1.418
9	3	653.0	1130	331	4.634	0.494	0.723	1.217
10	3	671.0	1140	365	5.162	0.513	0.752	1.265

TABLE VI. (continued)

Nitrogen Balance	N.balance per kilo of body weight.	Amount of Urine voided per week	Calcium output in mgms. per 100 cc. urine.	Total Calcium excreted	Phosph.output in mgms. per 100 cc. urine.	Total Phosphorus output
1.756	2.075	142	4.2	5.95	91	129.2
1.915	3.255	140	3.8	5.32	100.4	140.6
1.321	2.45	140	3.9	5.45	87.7	122.8
1.409	2.37	147	4.1	5.78	100.4	147.6
2.208	3.552	140	4.4	6.16	111	155
3.373	5.952	175	3.4	5.95	106.4	186
2.953	4.592	175	3.1	5.43	126.6	220.5
2.824	4.394	175	2.8	4.9	128.2	224
3.417	5.23	200	2.0	4.00	120	240
3.897	5.05	187	2.0	3.74	138.5	239.5

TABLE VII. FEMAL

No.of weeks	No.of animals in Group	Wt.of Group in Grams	O_2 Consumption.	Amount of food consumed per week.	Total Nitrogen of food consumed	Total Nitrogen of urine	Total Nitrogen of foeces	Tota Nitro of Ur + foe
1	3	677	976	317	4.438	2.016	0.872	2.888
2	3	676.5	983	322	4.522	2.109	0.784	2.893
3	3	668	975	340	4.774	2.208	1.405	3.685
4	3	675	976	340	4.760	1.665	1.219	2.884
5	3	701.75	1045	393	5.502	1.501	1.045	2.546
6	3	714	1130	382.5	5.362	1.248	1.002	2.250
7	3	724	1210	370	5.180	1.137	0.816	1.953
8	3	734	1300	380	5.351	1.177	0.723	1.900
9	3	741	1310	370	5.180	1.265	0.795	2.060
10	3	745	1340	419	5.866	1.554	0.929	2.483

TABLE VII. (continued)

Nitrogen Balance	N.balance per kilo of body weight	Amount of Urine voided per week	Calcium output in mgms. per 100 cc. urine.	Total Calcium excreted	Phosph.output in mgms. per 100 cc. urine.	Total Phosphorus output
1.550	2.28	180	5.0	9.0	85.5	154
1.629	2.41	186	4.4	8.15	87.7	163
1.089	1.63	162	5.1	8.25	92.5	150
1.876	2.78	163	5.2	8.45	91	148.4
2.956	4.21	194	4.1	7.95	91.0	176.6
3.112	4.351	185	3.7	6.85	128.2	237
3.227	4.445	215	3.1	6.65	107.5	231
3.351	4.550	213	3.1	6.60	120.0	255.5
3.120	4.210	208	2.3	4.75	126.1	262
3.383	4.53	216	2.5	5.4	126.3	273

THE ANTERIOR LOBE OF THE HYPOPHYSIS.

It is an amazing fact that medical history can show few revolutions of thought more astounding than the development of our knowledge regarding the varied functions of the pituitary body. The profound influence that the anterior lobe of the hypophysis possesses in relation to growth and metabolism has been attributed by some investigators to the neural centres in the floor of the third ventricle of the brain.

In reviewing the literature one is struck by the fact that in spite of the large number of papers appearing and the vast amount of experimental research done on the subject, the results published have in the majority of cases, proved utterly contradictory.

THE INFLUENCE ON GROWTH OF ADMINISTRATION OF THE EXTRACT OF THE ANTERIOR LOBE OF THE PITUITARY BODY.

As early as 1912 (3) Schafer showed that oral administration of fresh whole pituitary did stimulate growth in the young growing white rat.

Marinus (42); Robertson (43) and several other investigators reported results similar to those of Schafer. In 1921 Evans & Long (5) injected an extract of fresh anterior lobes, intraperitoneally into/

into rats just as they were weaned and reported a very remarkable acceleration of growth. Their experimental animals showed an increase of 250 grams over those of the controls.

Dott (44) in 1923 showed that administration per os of relatively large doses of anterior lobe, produced acceleration of growth in kittens and puppies.

Putnam, Teel and Benedict (6) in 1928 showed that they were able to demonstrate the effect of the anterior lobe of the pituitary body on growth by using alkaline extracts.

Hewitt (45) in 1929 reported that he did confirm Evans'and Long's results.

There are, however, a body of workers who deny the influence of the anterior lobe of the hypophysis over growth.

Lewis and Millar (46) in 1913 reported their negative results.

In 1922 Canan and Drummond (47) observed a similar negative effect.

Reitchart (48) in 1928 stated that administration of the anterior lobe of the pituitary had practically no influence in stimulating growth.

Larson, Bergeim, Barker and Fisher (49) in 1929 pointed out that intraperitoneal injection of an extract of the anterior lobe of the pituitary body had no effect whatever on the growth of the rat.

Korenchevesky (8) in 1930 using glycerol extract of/

of the anterior lobe reported a negative result.

Yet another group of investigators not only denied the fact that administration of the extract of the anterior lobe did not influence growth but definitely stated that such extract retarded growth and even stopped it.

Such results were reported by Thompson & Johnston (50) in 1905; later by Cushing (51) in 1909 and still later by Pearl (52) in 1916.

From this short review of the literature it appears that the results published show a great deal of perplexing contradictions.

In the present work, however, by the simple technique of sterile extraction and administration in the case of the fresh extract prepared by myself, the demonstration of the effect of the anterior lobe of the hypophysis on growth was a definite and positive finding in both the adult white rat and white mouse.

The effect on growth of the dry extract of Park Davis and Company, however, was definite but not as marked as that by injection. There was an appreciable difference in weight between the animals fed on this desiccated extract and those injected with the freshly prepared extract. A high increase in weight by feeding the desiccated extract was very marked in the white mice the gain being about 35% higher than the controls while by injection the increase in weight

was/

was 50%.

The technique for recording the weight of the animals was the same described in detail in the section dealing with the effects of thyroid administration.

The injected rats and mice showed a pronounced increase in weight. In some experiments the animals (rats) showed an increase in weight of about 105 grams.

In the case of feeding the extract the gain was 43 grams in the male animals and 31 grams in the case of the female above the controls.

(See Tables II & VI, III & VII)

It is interesting to note that the animals had a very good appetite. Towards the later weeks of the experiment they had nearly just as good an appetite as the thyroid fed animals.

When the animals were killed at the close of the experiment it was noticed that there was a definite increase in the amount of fat in the body of the animals fed and in those injected with anterior pituitary extract, the injected animals, however, had more fat than the fed animals. This fat included subcutaneous, extraperitoneal, mesenteric and perinephric fat. No attempt was made at weighing this fat as it was very diffuse and its weight would certainly be inconsistent, and the results fallacious.

The controls although possessed xx a good deal of/

of fat, yet it was not to be compared with that of the experimental animals administered with anterior pituitary extract.

Although the conception now established is that deficiency of the autacoid or autacoids of the anterior lobe of the hypophysis, whether by disease in humans or experimentally produced in animals, causes the well known syndrome of dystrophia adiposo genitalis in which, besides other clinical features, there is marked adiposity, yet one has no hesitation in stating that administration of anterior pituitary extract caused a marked increase of body fat, contrary to Korenchevesky (8) who stated that the effect of such administration was to decrease the amount of body fat.

THE EFFECT ON GROWTH OF THE ENDOCRINE ORGANS OF ADMINISTRATION OF ANTERIOR PITUITARY EXTRACT.

The individual organs of known and suspected internal secretion were subjected to the same technique as was previously mentioned in the previous section.

In the animals injected with fresh anterior pituitary extract, it was found that the thyroid glands, the suprarenals, the testicles, the ovaries, the pancreas, the liver and spleen showed a remarkable hypertrophy.

The/

The weight of these organs was very much higher than that in the case of the controls and in some organs the weight,e.g. the pancreas and liver, was even higher than that obtained by thyroid administration particularly in the case of the injected animals in which the results were as follows:-

The suprarenals showed an increase in weight of about 71%, the thyroid glands showed an increase of 85%, the testicles of about 21%, the ovaries of about 77%, the pancreas showing an increase of 81%. The liver showed an increase of about 65% and the gain in the weight of the spleen was about 36% over those of the control animals in the male. In the case of the female, however, the increase in weight was not as high as in the male animals, the rise being 66% in thyroids, 60% in the adrenals, 39% in the pancreas, 77% in the ovaries, 34% in the liver and 20% in the spleen above those of the controls.

By feeding of the anterior pituitary extract, however, the increase in weight of the organs, although much above those of the controls, yet it was not just as high as in the case of administration by injection.

The suprarenals showed an increase in weight of about 35%, the thyroids gained about 51%, the testicles increased about 16%. (The ovaries showed an increase in weight which was nearly parallel with that of the injected/

TABLE C.

Weights of Endocrine Organs of Animals Fed with Anterior Pituitary Desiccated Extract.

Wt. of Animal	Sex	Wt. of Thyroids	Wt. of Adrenals	Wt. of Pancreas	Wt. of Ovaries	Wt. of Testicles	Wt. of Liver	Wt. of Spleen	Duration of administration of Extract.
280	M.	38	50	1.22		2.51	10.9	1.27	
286	M.	36	51	1.212		2.60	11.03	1.34	six weeks
283	M.	39	42	1.12		2.84	10.86	1.21	
230	M.	31	54	1.115		2.68	8.02	0.995	
238	M.	34	53	1.14		2.31	9.12	1.102	seven weeks
241	M.	32	50	1.10		2.40	9.86	1.182	
259	Average	35	50	1.15		2.55	9.97	1.168	
300	M.	31.0	46	0.96		2.2	8.990	0.965	three weeks
322	M.	35	45	1.15		2.66	10.20	1.111	four weeks
335	M.	44.5	66	1.686		2.84	12.85	1.480	eight weeks
226	F.	34.5	56	1.01	57		8.00	0.910	
221	F.	36	58	1.04	58		8.91	1.00	six weeks
224	F.	35	53	0.985	60		8.89	0.98	
200	F.	37	60	0.920	71		6.380	0.73	
205	F.	32	60	0.930	65		8.100	0.85	seven weeks
213	F.	30	59	1.08	70		8.551	0.96	
214	Average	34.5	56.5	0.989	63.5		8.13	0.895	

TABLE D.

Weights of Endocrine Organs of Animals Injected with Anterior Pituitary Extract.

Wt. of Animals	Sex	Wt. of Thyroids	Wt. of Adrenals	Wt. of Pancreas	Wt. of Ovaries	Wt. of Testicles	Wt. of Liver	Wt. of Spleen	Duration of administration of Extract.
301	M.	40	65	1.42		2.71	11.72	1.20	
303	M.	39	70	1.36		2.79	12.81	1.36	six weeks
308	M.	50	67	1.32		2.995	13.10	1.32	
249	M.	40	72	1.195		2.89	12.32	1.001	
251	M.	41	64	1.28		2.56	13.14	1.015	seven weeks
255	M.	50	60	1.36		2.54	14.82	1.232	
278		43.3	66.3	1.318		2.66	12.99	1.188	
Average									
315	M.	32	50	1.02		2.31	9.51	0.978	three weeks
330	M.	36	50	1.22		2.69	12.61	1.065	four weeks
365	M.	50	95	1.84		2.98	15.96	1.578	eight weeks
245	F.	38	89	1.16	59		9.61	0.936	
249	F.	37	100.2	1.23	60		9.73	1.12	six weeks
251	F.	34	88	0.998	62		9.921	0.996	
218	F.	39	66	0.981	68		10.23	0.79	
220	F.	40	64	1.00	66		9.65	0.92	seven weeks
239	F.	32	72	1.20	69		9.730	0.916	
237		36.5	79.7	1.062	64		9.81	9.46	
Average									

injected animals, the gain was also about 75%). The pancreas showed an increase in weight of about 58%, the liver showed an increase in weight of about 27% and the spleen a gain of about 34% in the male over the weight of those of the control animals.

By feeding the desiccated extract as in the case of administration by injection, the female endocrine organs showed a decreased rise in weight than those obtained in the males, the gain being 14% in the adrenals, 13% in the pancreas, 13% in the liver and 14% in the spleen over those of the controls.

THE INFLUENCE ON BASAL METABOLISM OF ADMINISTRATION OF ANTERIOR PITUITARY EXTRACT.

In measuring the effects of administration of anterior pituitary extract on basal metabolism the apparatus and technique were as previously described in the section dealing with the thyroid gland.

Bernestein and Falta (53) in 1912 injected anterior pituitary extract and found that there was a slight and transient reduction in the basal metabolic rate.

Arnold and Leschke (54) in 1921 obtained inconsistent results in respect of the gaseous metabolism of two patients in whom they injected commercial/

commercial preparations of anterior lobe.

Kestner (55) in 1922 found that by injecting anterior pituitary extract he was able to raise the basal metabolic rate of patients with hypophyseal hypofunction.

Bowman and Grabfield (56) in 1926 found that the basal metabolic rate was slightly raised by oral administration of anterior pituitary extract.

Foster and Smith (57) in the same year performed hypophysectomy on rats and found that the basal metabolic rate was about 35%, lower than that of normal animals. Moreover they stated that they were able to restore it to normal by daily anterior lobe homotransplants but not by daily injections of extracts of posterior lobe.

Cushing and Davidoff (58) in 1927 investigating cases showing clinical evidence of pituitary insufficiency found that in 66% of these cases, the basal metabolism was, on an average, 14% below the normal standard. On the other hand, they found that in 46% of cases with clinical manifestations of acromegaly the basal metabolism was increased to an average of 18% above the normal standard. The same authors stated that hypophysectomy lowered the basal metabolism from 12% above normal to 7% below normal.

Lee and Gagnon (59) in 1930 reported that daily administration/

administration of anterior pituitary extract, although it stimulated growth, yet it decreased the metabolic rate (measured in terms of oxygen consumption).

Houssay and Biasotti (60) in 1931 stated that extirpation of the pituitary body decreased the basal metabolism by 40%.

It appears, therefore, from this short survey of the literature on the subject, that many discrepancies and contradictions exist. Such discrepancies may be attributed to a number of factors including activity or non-activity of the extracts used, and the time of feeding in relation to that of recording the basal metabolism, and whether there was any control on the temperature under which the animals were kept, the last mentioned being important, especially in experiments with rodents (as previously described).

In the present investigation in which all of the above mentioned factors were under complete comtrol, the results obtained indicated that administration of anterior pituitary extract definitely altered the basal metabolism by raising the rate of oxygen comsumption to a level varying between 30% in the male and 34% in the female above normal.
By injection of the fresh extract, however, the basal metabolism was a little higher than that obtained by feeding, the rise being 35% in the male and 37% in the female.

The distinction between the male and female animals was a constant finding, but to such a small difference little significance attaches.

The rise in the basal metabolism described above was obtained by considering the rise in oxygen consumption which attained its maximum, as a rule, by the end of the sixth or seventh week of administration of the extract - although in some instances this maximum was reached by the fourth or fifth week after which a slight fall in oxygen consumption occurred - compared to the maximum rise obtained in any of the weeks prior to the administration of the extract.

(See Tables II, III, VI & VII)

THE INFLUENCE ON NITROGEN METABOLISM OF ADMINISTRATION OF ANTERIOR PITUITARY EXTRACT.

As in the case of the estimations in the section dealing with the thyroid, Kjeldahl's method of estimating the total nitrogen in urine and faeces and food had been adopted. The chemicals used in all the biochemical investigations were those of the analytical reagent standard.

Malcolm (61) in 1903 found that oral administration to dogs of anterior or posterior lobe produced slight retention of nitrogen.

Thompson and Johnston (50) in 1905 chopped entire pituitary/

pituitary bodies and dried the pulp in a hot-air oven at 45° - 50°C.

They showed that feeding this dried extract to dogs produced an increased nitrogen metabolism. They catheterised their dogs morning and evening and they built their results entirely on the urinary output of nitrogen not taking into consideration the nitrogen balance.

Hewitt (62) in 1914 fed rats with small doses of bovine anterior pituitary extract simultaneously administered with thyroid extract and found that the former did not appreciably modify the action of the latter on nitrogen metabolism.

Grabfield and Prentiss (63) in 1925, by oral administration of dried extract of anterior lobe of whole pituitary body to patients on constant diet, observed that there was no effect on the quantity of nitrogen excreted per day.

Korenchovesky and Dennison (64) in 1929 reported that oral administration of the anterior lobe of the hypophysis dried at room temperature in vacuo had no definite influence on nitrogen metabolism of rats or rabbits. By subcutaneous injections of aquous solution of glycerol extracts of anterior lobe of fresh pituitary bodies, they found that such administration produced on the day of injection a decreased urinary flow and a 27% decrease in urinary nitrogen excretion/

excretion. On the days following the injection, they found that nitrogen excretion was still depressed in the rabbit but increased in the rat by 28% above normal.

Teel and Walkins (65) in 1929 working on the blood chemistry in relation to anterior pituitary administration showed that injection of alkaline extracts of the anterior lobe caused a marked drop in the non-protein nitrogen concentration in the blood.

In the light of the results obtained in the present investigation whether by oral or parentral administration, the anterior lobe of the hypophysis had a definite influence over nitrogen metabolism.

The results obtained indicated, that, by whatever route the extract was administered, it produced a definite decrease in nitrogen output. This decrease in excretion was showing by the first week of administration in the case of injection but did not clearly show until the second week when the extract was administered by mouth.

The decrease in nitrogen output in both cases of feeding and injection, continued to increase from week to week and by the fourth or fifth week in the case of injected animals and the fifth or sixth week in the fed ones, the decrease in output reached its maximum, thereafter it was sustained until the animals were killed.

The/

The nitrogen balance showed a general parallelism with nitrogen excretion. It was very markedly positive by the fourth or fifth week and was maintained at that level or thereabout until the animals were killed.

THE INFLUENCE OF ADMINISTRATION OF ANTERIOR PITUITARY EXTRACT ON THE METABOLISM OF CALCIUM AND PHOSPHORUS.

Very little is to be found in the literature on the relation of the anterior pituitary lobe to calcium and phosphorus metabolism.

Oswald (66) in 1902 fed dried preparations of the entire pituitary body and observed no change in urinary excretion of nitrogen or phosphorus.

Schafer (3) in 1912 found no influence upon the excretion of nitrogen or phosphorus by feeding the fresh entire hypophysis to young growing white rats.

Malcolm (61) in 1903 stated that, by feeding anterior lobe and the posterior lobe separately, he found such administration to produce a slightly increased retention of phosphorus and an increased output of calcium.

Thompson and Johnston's (50) results showed an increase in phosphorus excretion.

Hoffman's and Brigg's methods of estimation of calcium/

calcium and phosphorus respectively were adopted here also.

In the present work, the extracts of the anterior lobe of the hypophysis whether orally or parentrally administered had a remarkable definite effect on the metabolism of both calcium and inorganic phosphorus. Such results, however, were more marked by parentral introduction of the fresh extract than by oral administration.

THE INFLUENCE OF THE HYPOPHYSEAL ANTERIOR LOBE ON CALCIUM METABOLISM.

Calcium metabolism by parentral introduction decreased the urinary calcium output by about 55% by the end of the six or seventh week of administration. This decrease in calcium excretion was significant in the second week but it was not really well marked until the third week of administration. Thereafter the excretion progressively decreased and by the end of the sixth week it was decreased to a little over half its value prior to anterior pituitary administration.

In the case of feeding the extract, a similar result was obtained but not just as marked as in the case of administration by injection of the fresh extract. The weekly urinary calcium excretion in this case/

case was about 40% lower than the value obtained before feeding was begun.

The demonstrable effect of feeding on calcium excretion was again not significant until the third week and by the end of the experiment the weekly urinary excretion dropped from a minimum of 6.6 mgms. to 4.7 mgms. and from 5.32 mgms. to 3.7 mgms. in the male and female respectively.

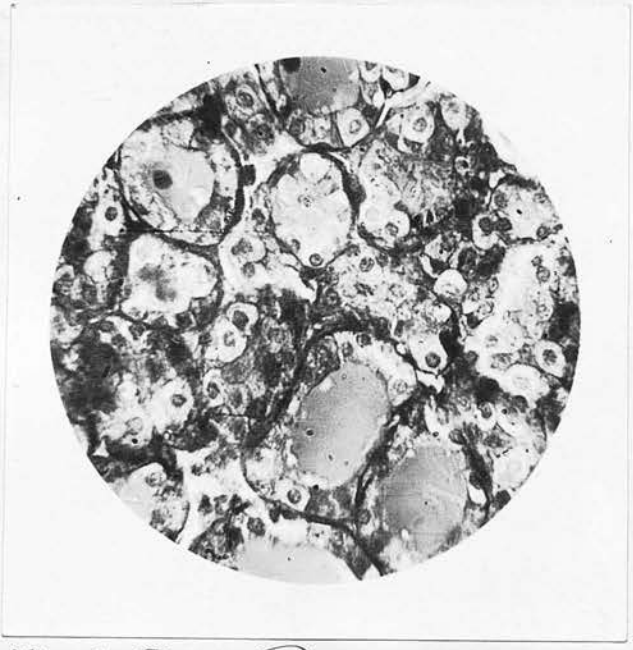
INFLUENCE OF THE ANTERIOR LOBE EXTRACT ON PHOSPHORUS METABOLISM.

Administration by injection of the fresh extract produced a very marked increase in inorganic phosphorus excretion. The maximum rise in excretion was attained in some instances by the fifth week, after which a little drop did occur particularly in the animals fed with the extract. In other instances the increase in output continued to go higher every week and the maximum was obtained at the last week of the experiment, i.e. sixth or seventh week. Such a result was particularly noted in the injected animals.

Administration by injection produced a rise of about 95% higher than normal in the male and only about 67% increase in the female.

By feeding the desiccated extract, the increase in phosphorus excretion was again not as high as that obtained/

Fig. XIX.



Thyroid Gland. Photomicrograph X450
Aniline blue and Orange G. preparation.
Animals fed with desiccated extract of the
Anterior lobe of the pituitary for six weeks.
The epithelium is high cubical and columnar.
Marked hyperplasia of epithelium of some vesicles.

Fig XX.

Thyroid Gland. Photomicrograph X450.
Aniline blue and Orange G. preparation.
Animals injected with fresh extract of the anterior
lobe of the pituitary for six weeks.
The epithelium of vesicles is high cubical
and columnar. Marked hyperplasia in some
vesicles. Colloid globules are seen in the
injected animals more than the fed ones.

obtained by injection, the rise being about 70% in the male and 62% in the female, above normal.

THE INFLUENCE OF ANTERIOR PITUITARY ADMINISTRATION ON THE THYROID GLAND.

Good and Ellis (67) in 1918 stated that in destructive tumours of the pituitary body which are frequently associated with deficient development of the sex organs, may also be accompanied by atrophic changes in the thyroid gland.

Smith (68) in 1927 showed that hypophysectomy in rats produced atrophy of the thyroids and parathyroids. Partial or complete return to normal was restored to both organs by daily homotransplants of fresh pituitary bodies. He further showed that intraperitoneal injections of an extract of bovine anterior lobe prepared according to the method of Evans & Long had no influence in restoring these organs to normal.

Loeb and Bassett (69) in 1929 stated that injection into guinea pigs of bovine anterior pituitary extract produced hypertrophy in the thyroid gland accompanied by loss in weight.

Schockaert (70) in 1932 reported that injection of anterior pituitary extract into the young duck produced hyperplasia of the thyroids.

Korenchovesky (8) in 1930 found that injection of/

of glycerol-aqueous extract of the anterior pituitary lobe in rats caused a decrease in weight of the thyroids by 12%. No histological report was given.

In the present investigation, administration of the anterior pituitary lobe substance whether orally given in the form of desiccated extract (P.D. & Co.) or by injection in the form of fresh extract produced a very marked alteration in the weight of the gland and the histological picture. A marked enlargement in size and an increased weight of the gland by such administration was a remarkable result, the rise in weight of the glands ranged between 51% and 85% over the weight of control thyroids.

Such a hypertrophy however, was not of such a high value under five to six weeks of administration. This response of the thyroids to the administration of anterior pituitary extracts was a progressive one, in so far that the longer the duration of administration the greater the enlargement and hypertrophy of the gland. The increase in weight by three weeks administration was about 14% by injection and 10% by oral administration, and by four weeks the gain was 28% by injection and 25% by feeding, above the controls, whereas by eight weeks administration the rise in weight was about 100% by injection to about 78% in the case of feeding the desiccated substance.

It/

It thus appears that very little difference was noticeable between feeding the desiccated extract and injecting the freshly prepared one as regards their effect in causing hypertrophy of the thyroid by short administration but by prolonged administration the rise in weight by injection attained a higher level than by feeding.

The Normal Thyroid.

The histological picture of the control normal thyroid was previously described. (Fig I.)

The Experimental Thyroid.

Microscopically the structure of the thyroid gland after administration of anterior pituitary extract, exhibited a remarkable contrast from that of the normal control. In place of the flattened or low cubical epithelium of the cells of the vesicles in the case of the control thyroid, that of the experimental thyroid showed a definite high cubical or columnar epithelial cells in which there was observed a variable number of intracellular vacuoles believed by Uhlenhuth[71] to be the colloid material actively secreted in the cells and passed into the vesicular space. The vesicles, all over the gland, showed a smaller size compared to the size of the vesicles in the control thyroids. (Fig (9))

The epithelium of the vesicles over scattered areas/

areas in the gland, in both the fed and injected animals showed an obvious hyperplasia, the proliferation of the epithelium of the vesicles entirely obliterating the vesicular space. In such vesicles no trace of colloid was noticed.

In between some vesicles, an aggregation of some lymphocytes staining deeply by haematoxylin was observed. These lymphocytes were not sharply brought out by the anilin blue and orange G.

The colloid was partially or entirely absent from some vesicles and when present it was distinctly thinner in character than that found in the control gland. It stained pink by eosin or Congo red. By anilin blue and orange G. however, the colloid was almost entirely stained blue and exceedingly little orange, if any, was observed.

According to Hewer (40) such a feature in the staining character of the colloid indicates marked hyperactivity on the part of the gland.

In a large number of the vesicles where colloid was present, the last showed also the presence of a number of vacuoles similar to those observed intracellularly. These vacuoles were rounded in appearance and to a certain extent refractile and did not respond to staining.

The histological picture described above was generally observed in the thyroids of the animals whether/

Fig. XXI.

Testicle. Photomicrograph X 120
Heidenhain iron haematoxylin preparation.
Animals fed with desiccated extract of the anterior lobe of the hypophysis for six weeks.
Spermatogenesis is increased. Slight atrophy of interstitial tissue. Control is Fig III.

Fig XXII.

Testicle. Photomicrograph X 120
Heidenhain iron haematoxylin preparation
Animals injected with fresh extract of the anterior lobe of the hypophysis for six weeks.
Spermatogenesis is increased. Slight atrophy of interstitial tissue. Control is Fig III.

whether administration of the extract of the anterior lobe of the pituitary was by injection or by means of feeding, although occasionally in some thyroids a very little amount of orange was observed in the case of oral administration. Fig (20)

THE INFLUENCE ON THE SEX GLANDS OF ADMINISTRATION OF ANTERIOR PITUITARY EXTRACT.

THE TESTICLE:

In the case of the sex glands, as elsewhere, the results published indicate a perplexing discrepancy.

Walzen (72) in 1914 showed that pituitary substance whether fed or injected produced a stimulating effect on the development and weight of the testicle.

Goetsch (73) in 1916 observed that administration of anterior pituitary extract produced a stimulating effect on sexual development.

Klinger (74) in 1919 and Marinus in the same year reported a similar stimulating effect on the testicle.

Maxwell (75), however, in 1916 pointed out that administration of anterior pituitary extract did not influence the testicle in any way.

Kross (76) in 1922 and Smith in 1927 reported a similar negative finding.

Evans and Simpson (77) in 1926 showed that administration/

administration of anterior pituitary extract produced a delay in maturation of the testicle with a decrease in weight. They also mentioned that microscopically no evidence of degeneration was found except smaller seminiferous tubules.

Biedle (78) in 1927 observed that injection of anterior pituitary extract or emulsion caused a retardation of growth of male sex organs particularly the testicle and seminal vesicles in both rats and mice.

Larson, Bergeim, Barker and Fisher (49) in 1929 reported that injection of anterior pituitary extract caused a decrease in weight of the testicle of the rat.

Robson & Taylor (79) in 1932 using implantations of bovine anterior pituitary lobe on alternate days into rats of four weeks old, found that the testicle was less than half the size of the control. No histological details were given.

In the present work the results obtained indicated that both by feeding and by parentral introduction of the anterior pituitary extract the effect was a stimulating one with increase in weight. By injection the weight of the testicles was somewhat higher than the weight obtained by feeding the desiccated extract.

In performing the autopsy for removal of the organs,/

organs, it was quite obvious to notice the enlarged size of the organ and the marked engorgement of the superficial blood vessels of the gland in a more pronounced fashion than was seen in the normal controls.

The increase in weight by oral administration was about 16% and by parentral introduction of the extract it reached about 21% above the controls.

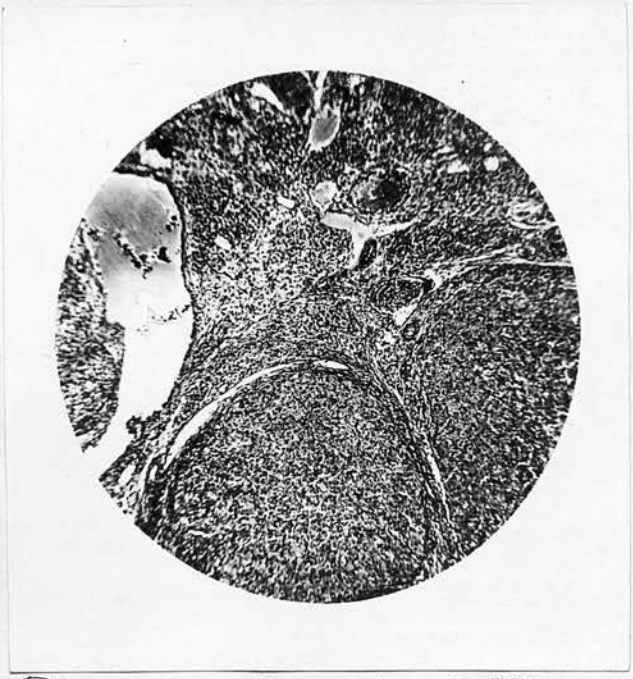
THE HISTOLOGICAL PICTURE.

In the part of the present investigation already published, I have pointed out that the histological results obtained were more pronounced by oral administration than by injection, and it was mentioned that such a result might have been due to the smaller amount of extract injected which was 100 mgms. of the fresh bovine anterior lobe substance injected every other day.

In the subsequent series of experiments performed, injections were given five times a week instead of three and as was previously described, the dose was increased from 100 mgms to 150 mgms. injected intraperitoneally for the last two or three weeks of the duration of the experiment depending on whether the administration was continued for six or seven weeks.

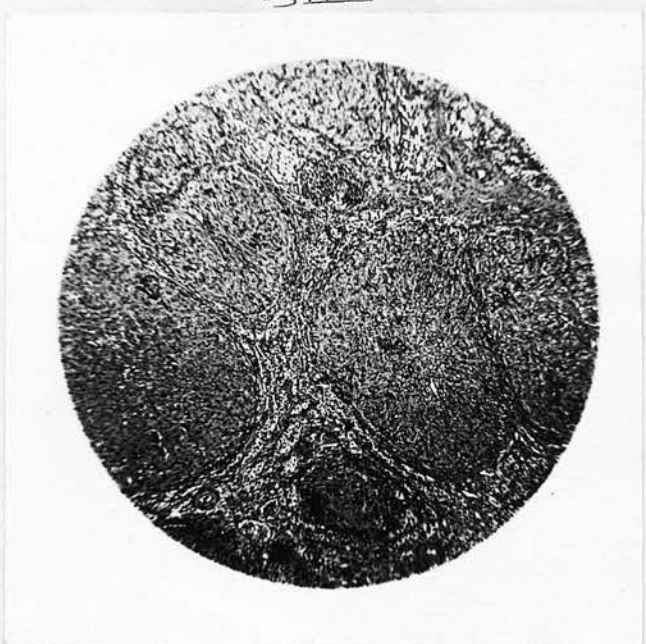
This arrangement did alter the subsequent results as far as the testicles and thyroids. However, the/

Fig XXIII.



Ovary. Microphotograph X 120
Delafield's haematoxylin and chromotrop preparation.
Animals fed on desiccated extract of the anterior lobe of the hypophysis for six weeks.
Marked increase in lutein cells. and blood vessel dilatation. Control is Fig V.

Fig XXIV



Ovary. Microphotograph X 120
Delafield's haematoxylin and Chromotrop preparation
Animals injected with fresh extract of the anterior lobe of the pituitary body for six weeks.
Marked lutein formation. Control is Fig V.

the alteration indicated that both desiccated extract and fresh extract were equally effective in stimulating spermatogenesis. The increase in weight however was found more pronounced by injection than by feeding.

Microscopically an increased spermatogenesis was observed. The lumina of the greater part of the tubules were observed to be entirely filled with spermatozoa. In many other tubules there was a very marked proliferation of the spermatocytes with a very great increase in the number of spermatids present.

Besides this increased spermatogenesis, the ~~Figs~~(21&22) interstitial tissue showed an appreciable atrophy by hypoplasia and indications of degenerative change indicated by the presence in the interstitial tissue here and there of small refractile fat-like globules.

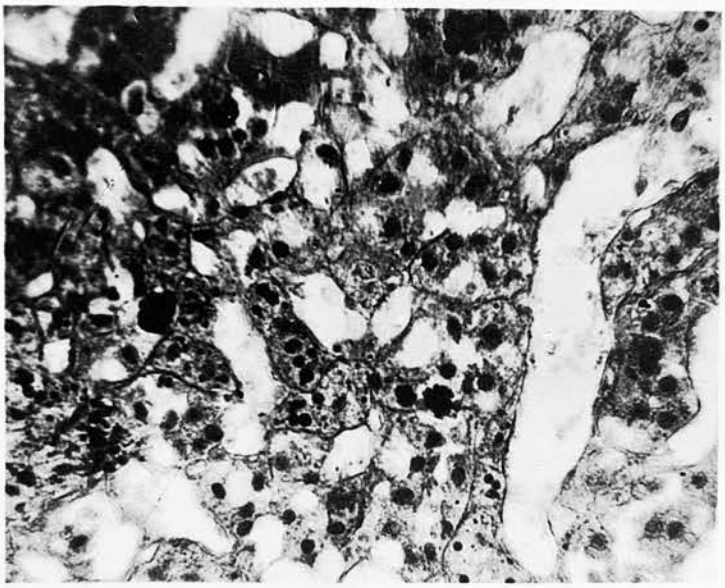
THE OVARY.

Evans and Simpson (80) in 1928 considering their previous investigations, reported by arriving at the conclusion that there is in the anterior pituitary lobe an autacoid that stimulates the ovary.

In a series of four papers by Zondek and Aschheim in 1927 and 1928 these authors described the presence in the urine of pregnant women and animals of an autacoid that stimulates the ovary. They believe that such an autacoid is secreted by the anterior lobe of the hypophysis.(81)

In the present investigation administration of anterior/

Fig. XXV.



The Suprarenal Body: Zona Reticularis and Medulla
Photomicrograph X 450.
Osmic acid vapour (Cramer) preparation.
Animals fed with desiccated extract of the anterior lobe of the hypophysis for six weeks.
The lipoid is abundant in the zona reticularis and numerous adrenalin granules in the medulla are showing. Marked dilatation of capillaries. Globoid bodies of Cramer are numerous. Control is Fig XV.

Fig. XXVI.

The Suprarenal Body. Zona Reticularis and medulla.
Photomicrograph X 450.
Osmic acid vapour preparation.
Animals injected with fresh extract of the anterior lobe of the hypophysis for six weeks.
Numerous globoid bodies of Cramer showing. The number of adrenalin granules is very numerous. Control is Fig XV.

anterior pituitary extract whether orally or parentrally introduced caused a very marked hypertrophy in the ovaries with a high increase in the weight of the gland. The increase in weight by feeding was equally marked as by injection of the extract, the gain being 75% in the former case and 77% in the latter above the weight of the controls.

THE HISTOLOGICAL PICTURE.

Practically the entire gland was made up of corpora lutea, the greater number of which were very large in size. The majority of these were almost entirely filled with lutein tissue. Hardly any Graffian follicles to be observed in many of the sections examined. Figs (23 & 24.)

The interstitial tissue showed slight atrophy being less in amount than that present in the control gland.

The blood vessels of the gland showed some dilatation.

THE INFLUENCE ON THE ADRENALS OF ADMINISTRATION OF ANTERIOR PITUITARY EXTRACT.

As far as I am aware very little work is to be found in the literature regarding the interrelationship of the adrenals with the anterior lobe of the hypophysis.

Smith/

Smith (68) in 1927 stated that hypophysectomy in the rat caused the suprarenal cortex to undergo atrophy.

Robson and Taylor (79) in 1932 reported that implantations of bovine anterior lobe substance with quinanil to prevent sepsis into rats 4 weeks old produced an increase in weight of 8 mgms. over those of the controls. No histological description given.

In the present investigation, the suprarenals of the animals both fed on desiccated extract and injected with the fresh extract, showed a very remarkable and definite increase in weight.
In the case of the injected animals the increase was about 71% in the male and 60% in the case of the females, whereas by feeding the increase was about 35% and 14% in the male and female respectively over the weight of the glands in the controls.

Thus it appears that the increased weight was much more marked in the male animals than in the female ones. It is interesting to mention in this connection that it was constantly found in the rat that normally the adrenal glands in the females were much larger in size than the adrenals of the male animals.

In considering such results as that of Korenchovesky (8) who stated that anterior pituitary extract produced a very marked decrease in body fat; it/

it is interesting to note that the adrenals were hardly visible to the eye due to the fact that the perinephric fat was so abundant to cover over and hide the suprarenals almost entirely from view, the fat elsewhere in the body was also much more plentiful than in the controls.

THE HISTOLOGICAL PICTURE.

The entire success in demonstrating the effect of the anterior lobe substance on the adrenal glands was due to the use of the histo-chemical method of Cramer which, as far as I am aware, there is no men-:tion in the literature of this sound technique being utilized by research workers investigating the changes in the adrenal response.

Before exposing the gland to the osmic vapour all care was taken to remove every visible particle of fat from the adrenal bodies which were then surrounded by a piece of gauze soaked in saline.

Microscopically the adrenal gland showed a very markedly altered picture in both its two component parts.

THE CORTEX:

The cortex was markedly loaded with lipoid material, which was spread in a denser manner over the entire/

entire cortex. The inner half of the zona fasiculata and the zona reticularis contained an abundance of lipoid which normally did not exist in the control adrenals.

Besides this abundant lipoid, the zona reticularis showed the presence of a very great increase in the Globoid bodies of Cramer considered by him as indicative of increased hyperactivity on the part of the cortex. These globoid bodies were made much sharper to see microscopically by treating some sections with turpentine for 20 minutes which dissolves the greater part of the fat and leaves these bodies unaffected. Fig(25)

THE MEDULLA.

The microscopical appearance of the medulla at once illustrated a very highly active part of the gland.

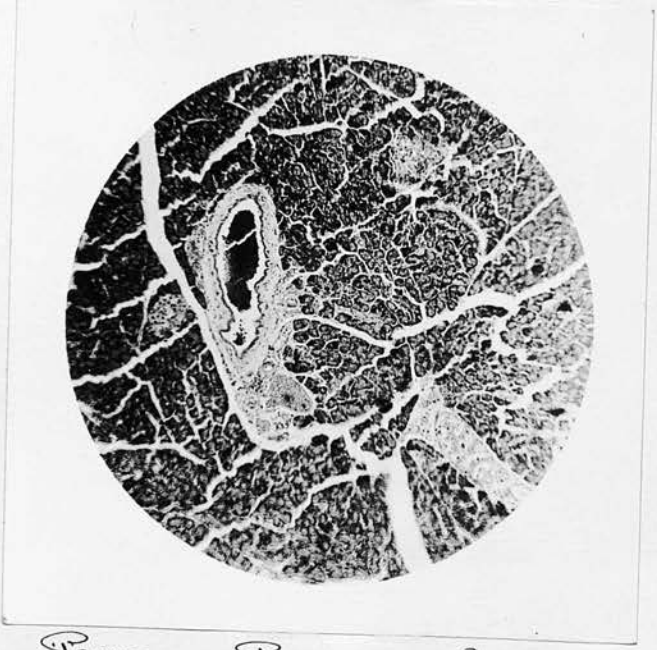
The adrenaline granules were seen in many parts of the medulla. The sinusoids were very dilated and contained numerous greyish granules. Fig.(25&26).

The histological picture although showing a great increase in the amount of adrenaline in the case of oral administration, yet it was more marked in the case of administration by injection, in which case the adrenaline granules were not only a little more numerous but also larger in size than those observed in the adrenals of fed animals. (Fig 25&26).

Fig XXVII.

Pancreas. Photomicrograph X 120
Heidenhain's haematoxylin and Saffranin preparation
Animals fed with desiccated anterior lobe extract
for six weeks.
The alveoli are smaller and the zymogen granules
increased. Normal Control is Fig XVII.

Fig XXVIII.



Pancreas: Photomicrograph X 120.
Heidenhain iron haematoxylin & saffranin preparation
Animals injected with fresh extract of the anterior
lobe of the pituitary body for six weeks.
The alveoli are smaller & zymogen granules
more numerous. Control is Fig XVII.

THE INFLUENCE ON THE PANCREAS OF ADMINISTRATION OF ANTERIOR PITUITARY EXTRACT.

Glycosuria of a character similar to that existing in pancreatic diabetes is of a common occurrence as a clinical feature in acromegaly.

As far back as 1884 Loeb (82) observed the occurrence of diabetes in some cases of acromegaly.

In 1886 Piere Marie (83) reported a similar observation to that of Loeb.

Since then a number of investigators observed the association of diabetes in disturbances of the anterior lobe of the hypophysis.

Recently in 1927 Davidoff and Cushing (58) showed that 25% of the acromegalic cases investigated by them, exhibited this clinical feature.

Such observations are in some way suggestive of some interrelation between the anterior pituitary lobe and the pancreas.

The striking observation in the present investigation regarding the influence of the anterior lobe of the hypophysis on the pancreas was, that that oral or parentral administration of this substance produced a very marked hypertrophy of the pancreas which was much more marked by injection than by feeding and in the male being more so than in the case of the female animals.

On/

On performing autopsy, the organ exhibited a marked contrast to that of the control. Instead of the diffuse thin organ, the experimental pancreas showed a more solid character and an obvious visible hypertrophy and possessing a more pinkish colour than normal.

THE HISTOLOGICAL PICTURE.

Microscopically the organ exhibited a denser and more compact appearance than that of the normal one. Fig (27028)

The alveoli and the alveolar cells showed a slight diminution in size. In some of these cells the nuclei were larger than normal. Large nucleoli staining deeply by Heidenhain's (41) iron haematoxylin were also present. The blood vessels showed a little dilatation. The connective tissue between the lobules was greatly decreased in amount.

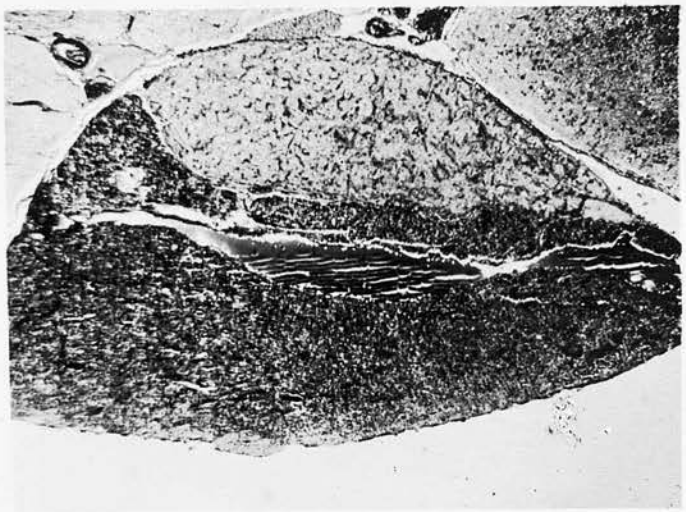
The zymogen granules appeared to be much coarser and more numerous than those of the normal pancreas.

No mytotic figures were observed.

THE ISLETS OF LANGERHAN.

These were, if anything at all, a little smaller than the average normal size in the control organ. Scattered areas in the sections appeared to contain very small islet tissue. However no significance could be attached to such an observation especially when it was considered with the negative glycosuria present/

Fig XXIX.



The Pituitary Body. Photomicrograph X 60

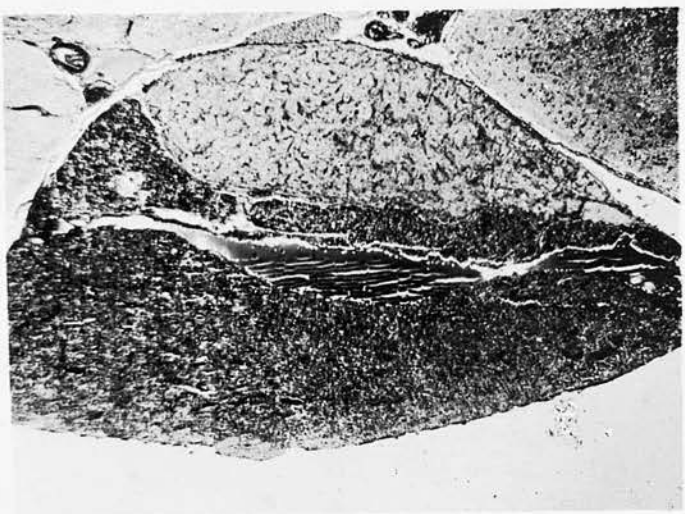
Delafield's haematoxylin and chromotrope preparation.
Animals fed with desiccated extract of the anterior lobe of the pituitary body for six weeks.
The organ is enlarged. The cleft is entirely filled with Colloid. Control is Fig VII

Fig. XXX.

The Pituitary Body. Photomicrograph X 60.

Delafield's haematoxylin and Chromotrope preparation.
Animals injected with fresh extract of the anterior lobe of the hypophyses for six weeks.
The organ shows enlargement. Control is Fig VII

Fig XXIX.



The Pituitary Body. Photomicrograph X 60

Delafield's haematoxylin and chromotrope preparation.
Animals fed with desiccated extract of the anterior lobe of the pituitary body for six weeks.
The organ is enlarged. The cleft is entirely filled with Colloid. Control is Fig VII

Fig. XXX.

The Pituitary Body. Photomicrograph X 60.

Delafield's haematoxylin and chromotrope preparation.
Animals injected with fresh extract of the anterior lobe of the hypophysis for six weeks.
The organ shows enlargement. Control is Fig VII

present in several testings of the urine of the animals fed and injected with the extract, during the experiments.

In some of the islet tissue the nuclei stained rather lightly with Heidenhain iron haematoxylin and the cytoplasm stained faintly only with saffranin or benzolicht bordeaux. In some other cells however, the chromatin of the nuclei appeared condensed and stained very deeply with Heidenhain iron haematoxylin giving the appearance of the phenomenon of pathological retrogression of the nucleus when it starts to degenerate (Piknosis). The islet tissue however still showed the presence of a distinction between the A and B cells.

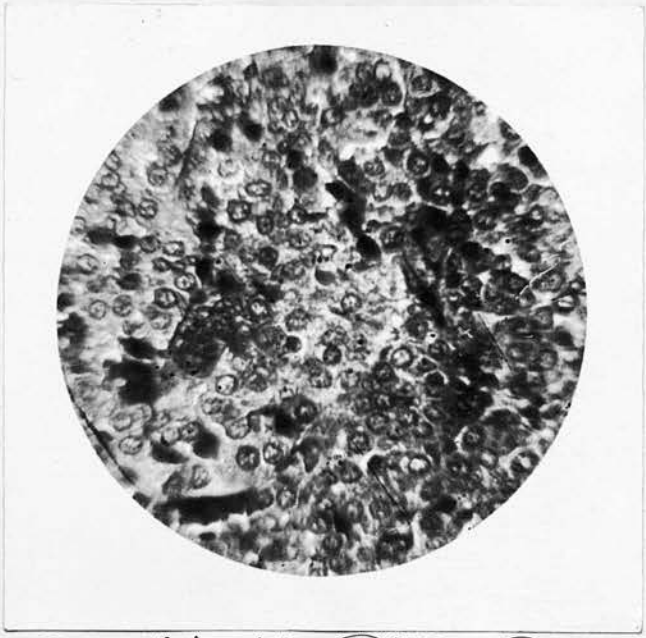
The results above described were common to both oral and parentral administration.

THE INFLUENCE ON THE HYPOPHYSIS OF ADMINISTRATION OF ANTERIOR PITUITARY EXTRACT.

Whether by oral administration or by parentral introduction the substance of the anterior lobe of the pituitary body produced a change in the histological picture of the organ.

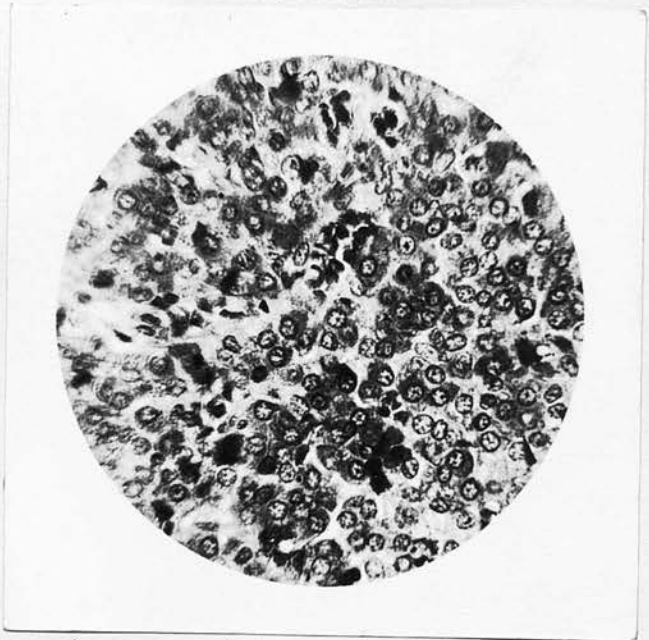
As was previously mentioned removal of the pituitary body for weighing purposes was not attempted in favour of the histological investigation as the arrangement/

Fig XXXI.



Anterior Lobe of the Pituitary Body.
Photomicrograph X 450.
Delafield's haematoxylin and Chromotrop preparation.
Animals fed with desiccated extract of the Anterior lobe of the pituitary Body for six weeks.
Very marked increase in the oxyphil cells. Control is Fig X.

Fig XXXII



Anterior Lobe of the Pituitary Body.
Photomicrograph X 450
Delafield's haematoxylin & Chromotrop preparation.
Animals injected with fresh extract of anterior pituitary lobe for six weeks.
Marked increase in the oxyphil cells. Control is Fig X.

arrangement of the histological picture of the organ changes when it is removed from its attachment to the base of the brain and a proper sagittal plane is difficult to obtain in the small pituitary body of the rat. But judging from the naked eye appearance of the size of the gland and also by its size in section seen microscopically, one feels inclined to state that the organ showed some enlargement by such administration of this substance, and was a little redder in colour than normal. Figs.(29&30)

THE HISTOLOGICAL PICTURE.

The normal gland of the control was described in the section dealing with the effects of thyroid administration.

THE EXPERIMENTAL PITUITARY BODY.

The desiccated anterior lobe substance and the fresh extract were equally remarkable in their effect on the hypophysis.

THE PARS ANTERIOR:

HISTOLOGICAL PICTURE.

Oral administration of the desiccated substance and injection of the freshly prepared extract of the anterior lobe produced similar effects on the pars anterior of the experimental pituitary bodies.

The/

The anterior lobe in both cases showed an apparent increase in size. The remarkable effect produced, however, was the tremendous increase in the number of the oxyphil cells with a slight tendency towards decrease in the number of the basiphil cells and of the chromaphobe variety. The oxyphil cells were larger than normal and showed a relatively enlarged centrally placed nuclei, and their cytoplasm stained deeper red than normal. In some parts of the pars anterior the capillary blood vessels showed a very pronounced dilatation with engorgement by red blood corpuscles. Fig 5 (31432).

THE PARS INTERMEDIA:

The cells in size and character appeared normal. There was no appreciable change to note except a slight increase in the cells of the reticulo-endothelial system.

In the cleft between the pars intermedia and the pars anterior there appeared very little colloid in the injected animals, but in the animals fed on the anterior lobe substance, the cleft was almost entirely filled with moderately thick colloid staining pink with acid dyes.

THE PARS NERVOSA:

The ependymal fibres and the neuroglia elements appeared normal. No appreciable change in the size of the pars nervosa to note.

More/

More abundant Herring's bodies than normal were observed.

In the pituitary of the animals injected with anterior pituitary extract, the pars nervosa was surrounded by a thin layer of cells between it and the base of the brain. Anteriorly these cells seemed as if they had originated from those of the pars intermedia although in character they were different from that of the cells of the pars intermedia.

THE EFFECT ON THE PARATHYROID OF ADMINISTRATION OF ANTERIOR PITUITARY EXTRACT.

No histological change of any description was produced on the parathyroids by administration of the anterior lobe, desiccated or fresh extract.

It appeared from this investigation that any interrelationship of the anterior lobe of the hypophysis and the parathyroids was not demonstrable.

THE EFFECT ON THE LIVER AND SPLEEN OF ADMINISTRATION OF THE ANTERIOR PITUITARY EXTRACT.

Beyond the fact that the liver and spleen responded by very marked hypertrophy to the administration of the anterior lobe substance there was no histological alteration in structure to note.

No/

No structural changes in the macroscopical or microscopical appearance in the bone marrow to note.

THE EFFECT OF THYROID AND THE ANTERIOR LOBE SUBSTANCE ON THE HAIR.

It is interesting to note an observation that was found constant in all the animals used. Administration of both thyroid and anterior pituitary extract, showed that they had an effect on the shedding of the hair of the animals.

In the case of the thyroid administration the effect noticed was that the hair of the animal's coat was shed in great abundance. Indeed, the funnel of the metabolism cages used to be loaded with hair every day.

On the other hand, in the case of the animals fed and injected with the anterior lobe substance, the effect was exactly the reverse seen in thyroid-fed animals. Very little hair was noticed from the beginning of the third week onwards. This effect was much more pronounced in the animals fed with the desiccated substance than those injected with the fresh extract.

THE ACETONITRITE TEST OF THE ACTIVITY OF THE THYROID GLAND.

From the observations on the thyroid glands in relation to the administration of the anterior lobe desiccated or fresh extract noted above, a conclusion was arrived at that such administration produced a very remarkable hyperactivity of the thyroid glands.

To confirm this hyperactivity, I found it advisable to make use of Reid Hunt's reaction for biologically detecting any such activity in the thyroid glands. (84)

For this purpose sixteen male white adult mice five months old, of very nearly equal weight, were used. Male mice were selected because of the observation reported by Von Zwehl (85) when trying to confirm Reid Hunt's test, that di-iodotyrosine did protect to some extent female mice against the poisonous effects of aceto-nitrite, while the same substance had no such protecting influence on male mice.

The animals were grouped into sets of four and fed on the same diet as the rats. The first group was fed with .025 gm. of desiccated thyroid extract per mouse per diem ; the second group was fed with .05 gm. of desiccated anterior lobe substance per mouse per diem. The third group was injected intraperitoneally with a daily dose of 0.5 cc. containing about 50 mgm. of fresh anterior lobe substance.

The/

The fourth group was kept as a control.

All precautions recommended by Reid Hunt (84) for the success of the test, as feeding the animals on fixed constant diet as well as feeding on such diet for some time before applying the test etc. were carried out, as the mice were kept for four weeks on the ordinary diet followed by the addition of extracts of the amounts mentioned above to their diet for six weeks. At the close of ten weeks acetonite (B.D.H.) was injected.

Dilutions were prepared of the original solution in such a way that each 0.5 cc. of sterile distilled water would contain half a minim in the first dilution, one minim in the second, one and a half minims in the third and so on.

All the animals in all the four groups received the first injection of 0.5 cc. containing half a minim. It was remarkable to notice that all the controls succumbed to the first injection. Two of the controls died two hours after the injection, the third an hour later and the fourth animal became very toxic and died five hours from the time of the injection. None of the experimental animals of any group showed any signs of ill effects.

Forty-eight hours later, every animal received 0.5 cc. containing double the dose of the previous injection with no signs of toxicity. Twenty-four hours/

hours later four times the dose was injected. One mouse of the group injected with fresh anterior pituitary extract succumbed, the rest of the animals of the same group as well as all the others appeared quite healthy and consumed their food as usual. When five times the original dose was injected one animal of the thyroid fed group died within two hours from the time of the injection and two of the animals fed on desiccated anterior lobe substance and also another mouse of the group injected with anterior pituitary extract likewise died. Six times the dose when injected twenty-four hours later proved fatal to the rest of the anterior pituitary fed group, the anterior pituitary injected group and to two of the thyroid fed group, only one remaining from the last mentioned group which died immediately after an injection given twenty four hours later containing seven times the dose that killed all the controls.

This test showed a corroborative evidence as to the response of the thyroid glands to the administration of the extracts of the anterior lobe of the hypophysis indicated by the enormous enlargement and by the microscopical picture of the thyroid glands indicative of hyperactivity as was previously noted.

QUALITATIVE/

QUALITATIVE AND QUANTITATIVE ESTIMATION OF ADRENALINE IN THE SUPRARENALS.

By the histo-chemical method of Cramer's (37) osmic vapour the present investigation revealed that administration per os of desiccated thyroid extract, desiccated anterior pituitary extract and of saline extract of the fresh anterior lobe of the hypophysis parentrally introduced, produced a stimulating effect on both the cortex and medulla of the adrenal bodies of the experimental animals.

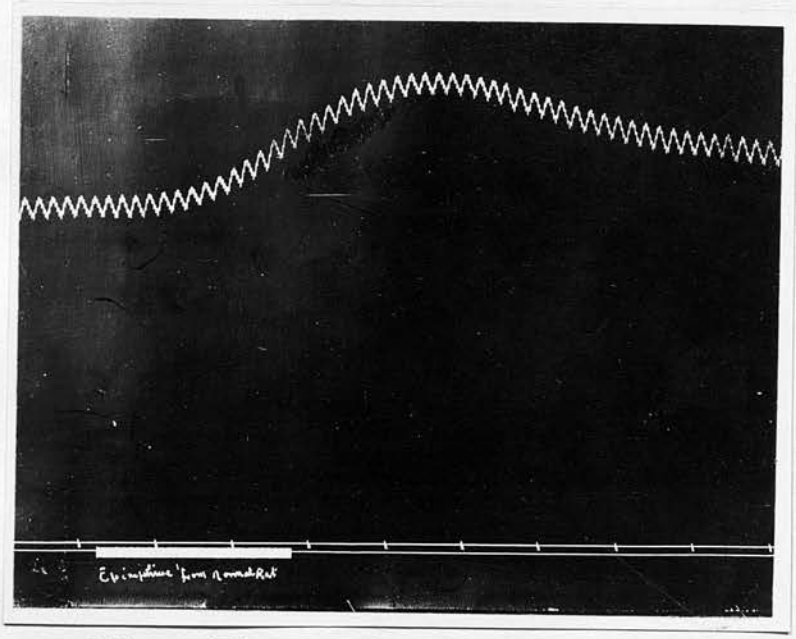
To confirm this observation of increased adrenaline secretion in the medulla of the experimental adrenals, a batch of white rats were treated in the same manner as before for ten weeks, four weeks' feeding on normal diet and six weeks of administration of the extracts.

For qualitatively estimating the amount of adrenaline in the experimental and control adrenal bodies, some of these suprarenals were extracted according to Folin, Cannon and Dennis (86) method and tested as to their effect on the blood pressure.

For the quantitative estimation of their adrenaline content some other suprarenals were extracted by means of the same technique and estimated according to Folin, Cannon and Dennis method of colourimetric estimation of adrenaline. (86)

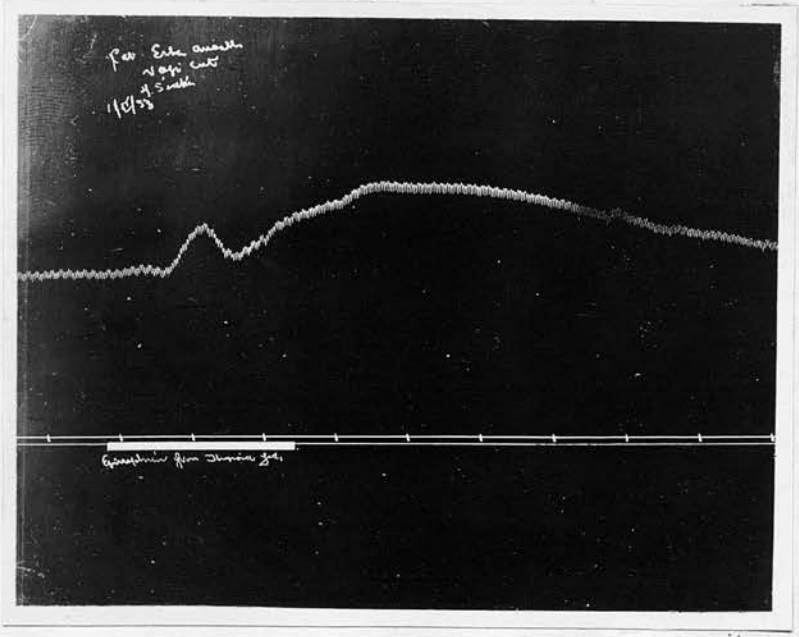
TECHNIQUE/

Fig. XXXIII



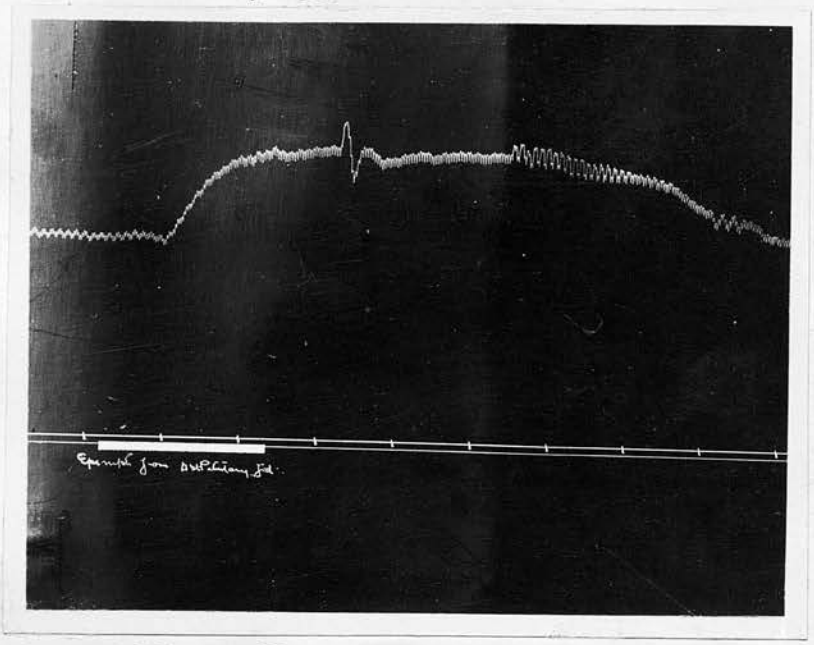
Blood Pressure tracing: Cat:
Adrenalin from normal Control rat.

Fig XXXIV



Blood Pressure tracing (Cat)
Adrenalin from adrenals of rat fed with thyroid extract for six weeks.

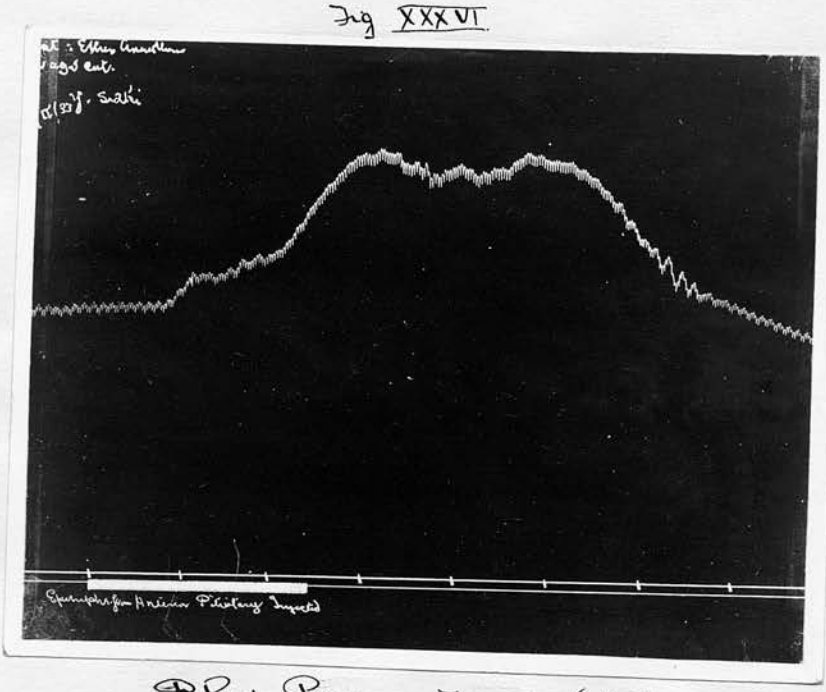
Fig. XXXV



Blood Pressure Tracing (Cat)

Adrenalin from adrenals of rat fed with desiccated extract of the anterior lobe of the pituitary Body

Fig XXXVI



Blood Pressure Tracing (Cat)

Adrenalin from adrenals of rat injected with Anterior pituitary fresh extract for six weeks.

TECHNIQUE OF QUALITATIVELY ESTIMATING OF THE ADRENALINE BY TESTING ITS EFFECT ON BLOOD PRESSURE.

A cat of three kilogram weight was anaesthetised with ether, the trachea having been exposed by a mesial incision, two rings were cut, and a tracheal tube was then inserted and tied. The common carotid artery on the left side was then exposed and freed, so also was the vagus nerve on each side of the neck.

Having cleaned the artery from its sheath a small nick in its wall was made and a blood pressure canula was introduced into the artery and tied.

Both vagi were subsequently severed.

The right femoral vein was exposed and a metal canula was introduced into it and tied. The adrenal glands having been dissected clean of any fat were weighed and extracted, the amount in ccs. of each extract being thus equal. One tenth of the amount of each extract diluted with warm saline was then injected slowly into the femoral vein and its effect on the blood pressure was recorded.

(See the accompanying tracings)

From these tracings it will be noticed that the highest rise in the blood pressure recorded was that of the extract of the suprarenals of the animals injected with anterior pituitary extract, the suprarenals of the animals fed on thyroid and those fed with anterior pituitary substance exhibit nearly the same/

same rise in blood pressure. The control suprarenals showed the lowest rise of blood pressure of the series.

This result confirmed that obtained by the histochemical technique of Cramer's (37) osmic vapour.

Quantitative Estimation of the Adrenaline Content of the Suprarenals of Normal Controls.

	Weight of animal	Weight of glands	Total adrenaline content	Percentage
	210	32	0.0277	0.0865%
	220	35	0.03195	0.1040%
	228	37	0.0278	0.0750%
Average	219	34.6	0.0316	0.0885%

Quantitative Estimation of Adrenaline Content of the Adrenals of Thyroid Fed Animals.

	Weight of animal	Weight of adrenals	total adrenaline	Percentage
	175	41	0.0643	0.1568%
	185	47	0.0653	0.1389%
	180	52	0.0725	0.1393%
Average	176	46.5	0.0678	0.143%

Quantitative Estimation of Adrenaline Content of the Adrenals of Anterior Lobe Fed Animals.

	Weight of animal	Weight of adrenals	Total adrenaline	Percentage
	230	54	0.0618	0.1144%
	238	53	0.0615	0.1162%
	241	59	0.0695	0.1178%
Average	236	55.3	0.0643	0.1162%

Quantitative Estimation of Adrenaline Content of Adrenals of the Anterior Lobe Injected Animals.

	Weight of animal	Weight of adrenals	Total adrenaline	Percentage
	249	72	0.1112	0.1544%
	251	70	0.1192	0.1720%
	255	64	0.1043	0.1630%
Average	251	68.6	0.1112	.1631%

From these tables it is apparent that the adrenaline content of the experimental animals is much higher than normal and corroborates the evidence of hyper-activity of the medulla of the adrenals, revealed by the histo-chemical technique of Cramer (37) and also by investigating the relative effects of the extracts of these glands on the blood pressure.

DISCUSSION.

The many discrepancies apparent in the literature seem at first perplexing but on closer examination they can be accounted for by the details of techniques used by different workers. Unfortunately it must be said that in some cases investigators have founded their results merely on the effect of extracts administered whether orally or parentrally introduced, without taking into consideration the control of the diet especially with regards to the amount available to the animals and to the adequacy of the vitamin content. Sometimes the importance of reducing the handling of the animals to a minimum is not mentioned to have been realised. Another factor which has received insufficient attention, as Cramer (87) has emphasised, is the temperature control. He has shown that not only is it directly important for metabolism experiments on rodents but variations therein produce significant changes in the suprarenal and thyroid glands. In some cases, also, highly complicated methods of extraction and preparation of extracts have been employed. These may well be responsible for some of the differences which have been described, for not only is there a possibility of active substances being altered during the processes but some may be lost or destroyed. Several autacoids are reported/

reported in the literature to have been separated from the anterior lobe of the hypophysis, the female gonadotropic; growth controlling and fat regulating autacoids; at least another, the sleep regulating autacoid (Zondek) (88) has been prognosticated; and it is possible that the list is not exhausted. The purpose of the present investigation was to establish the endocrine interrelationship of the thyroid gland and of the anterior lobe of the hypophysis as a whole and therefore no attempt at partition of the active substances was made.

Every precaution has been taken here to meet with all of the above mentioned factors and as is explained in the text the techniques adopted have been thoroughly and completely controlled. The extracts orally administered were those of Park Davis and Co., and that injected was fresh and prepared by a simple process that ensured the presence of all the active principles of the gland in the extract prepared, the success appears to be attributable to the strict asepsis used throughout, whether in extracting the gland or in injecting the extract.

In discussing such results as those reported in the present investigation, one is struck by the diverse functions and the multiple interrelations of the thyroid and the anterior lobe of the hypophysis with the other endocrine organs.

In/

In the light of the results obtained in the present investigation as well as that of other investigators, it seems that the thyroid gland is linked with most of the other endocrine organs. It can hardly be emphasised too strongly that it exerts its influence on the organism as a whole through these endocrine glands. The magnitude of the response depends partly on the dosage and the duration of administration and partly on the controlled technique including such details as the age and sex of the animal used and whether growth is complete or not.

My results agree with most of the other investigators who reported that thyroid administration causes increase in the gaseous metabolism by increasing the oxygen consumption to a much higher level than normal. It is not possible to reconcile such results with those that indicate that thyroid in whatever form or dose can lower the gaseous metabolism as has been reported by Kojima (21) in 1917.

In my results thyroid administration has always exerted a constant effect on the size of the body of the animals used, being chalonic in character. This retrogression in size and weight has been observed in many occasions and on animals of different age, and sex as well as the time of birth.

From Schafer's (3) work and that of others who confirmed his results, it seems that thyroid gland/

gland possesses as one of its autacoids a substance which is of a growth stimulating nature, i.e. growth hormone. Such a result, as well as those reported by some other authors, have been obtained from experiments performed on young growing rats or other kind of young growing animal. I have always used full grown adult albino rats and adult white mice and from the present results it appears that there is also, in the thyroid gland, or rather it may be, that the active principle of the organ "Thyroxin" has a diversity of functions being hormonic as far as growth is concerned in the young growing animal but it becomes chalonic when the animal attains its full growth in so far as it reduces the weight and the size of the animal. The results obtained in the present investigation regarding size and weight show inhibition. In other words the animals always became much lighter and visibly smaller than their litter mate controls. The loss started soon after thyroid administration.

Some of the endocrine organs, as has been shown by Hoskins (10) and by Herring (11) and by Cameron and Carmichael (12) responded to the administration of thyroid by hypertrophy and increase in weight. In some others, however, my results are contrary to those reported by the above mentioned authors and by others, in so far that thyroid administration produced in them an atrophy and decrease in weight instead/

instead of the hypertrophy reported. The testicles and ovaries as well as the spleen showed a distinct decrease in weight.

Particularly, perhaps, the testicles deserve mentioning, their marked decrease in weight is corroborated by the histological picture which reveals a very remarkable atrophy and degeneration of the seminiferous tubules which in parts are completely destroyed. Yet another feature is the hyperplasia and hypertrophy of the interstitial tissue of these glands which indicates a very definite link of interrelationship and association between the autacoid of the thyroid and that secreted by the interstitial tissue of the testicle.

Other organs that showed this marked atrophy as regards thyroid administration especially by prolonged administration is the pituitary body. This organ showed a very remarkable degenerative change in practically all its component parts, the pars anterior and pars nervosa showing such effects most.

The ovaries showed also a decrease in weight together with a very evident decrease in the connective tissue stroma which is likely to be the cause to account for this apparent drop in the weight of the gland. An intimate association between the thyroid and ovary is revealed by the histological picture which showed a very remarkable hyperplasia

and/

and hypertrophy of the interstitial tissue of the ovary associated with an increase in the number of the corpora lutea present.

A not infrequent obstetrical and gynecological measure in the treatment of habitual abortion and amenorrhoea of endocrine origin is the exhibition of thyroid extract. It may be that the basis of this clinical use lies in the fact that thyroid administration stimulates lutein formation on the one hand and so increases lutein production which thus ensures the embedding of the ovum in the uterine mucosa. The term lutein is used to denote the autacoid complex of the corpus luteum. Various workers have introduced a wide range of terms to denote active principles extracted from this part of the gland; among these are hythin, relaxin, progestin, hebin, luteo-lipoid, lipamin. It is not clear whether these are separate entities, or if not which are identical. In the present state of knowledge it seems desirable to include them all in the collective term lutein.

On the other hand the marked hyperplasia and hypertrophy of the interstitial tissue of the ovary and remarkable increase in the blood supply to this part of the organ justifies the impirical clinical use of thyroid extract in the treatment of amenorrhoea as by such administration the product of activity of this part of the gland is thereby increased.

As/

As far as I am aware, no histological details have been published to the effect that thyroid administration caused some degenerative changes in the thyroid gland itself which was a very definite result in the present investigation.

The suprarenal glands, on the other hand, revealed an increase in weight by thyroid administration and by quantitatively estimating their adrenaline content by the micro-chemical method of Folin, Cannon and Dennis (86) and by the histo-chemical method of Cramer (37) also by kymographic work, testing the effect of adrenalin content of the glands on the blood pressure, the results are all in concordance and harmony with the results of Herring (89) who showed that thyroid administration results in increased adrenaline in the adrenal glands of rats and cats. The histo-chemical method of Cramer revealing, in addition to the increased adrenaline in the medulla, an active cortex as well, indicated by the spread of lipoid all over the entire cortex particularly in the inner half of the zona fasiculata and the entire zona reticularis which normally show very little or no lipoid at all, also the presence in the zona reticularis of the globoid bodies of Cramer which are supposed to be adrenaline or precursors of adrenaline, being unaffected by turpentine and claimed by Cramer to indicate a hyperactive cortex.

In/

In all the autopsies performed on female animals, it was a constant feature to find that although smaller in weight yet they possessed a much larger and heavier suprarenals than the male rats. Such observation has been also reported by Donaldson (90).

Though the pancreas responds by hypertrophy to thyroid administration, yet there is no histological evidence of any change taking place in the islets of Langerhan. The more or less clinical feature of the presence of glycosuria and albuminuria in pathological hyperthyroidism, for example in Graves' disease, was not present in experimental hyperthyroidism. On one occasion only in one set of the females a weak positive Follin McIlroy reaction was found, but as this was not repeated in the many chemical tests done, it was concluded that it was a transient phenomenon of no particular significance. Such a negative result in the experimental hyperthyroidism regarding this glycosuria is suggestive of the fact that it may be a toxic feature of the disease due to altered colloid or the presence in such colloid of a toxic material and that it is not due to direct influence of the thyroid on the pancreas causing a pancreatic diabetes, although a good many of the islets in the pancreas of animals fed on thyroid showed somewhat smaller size, yet a conclusion to be drawn from such a result is certainly fallacious as the islets of

Langerhan/

Langerhan in the rat show a great difference in size.

The absence of any histological changes in the parathyroid glands indicates that this endocrine organ is not greatly influenced by thyroid administration and shows that possibly the increased urinary calcium excretion is a direct effect of the thyroid on the metabolism of this important electrolyte.

In the case of the anterior lobe of the hypophysis, the results obtained concerning the increase in weight and size of the animals justifies the belief of the presence of a growth stimulating autacoid in the anterior lobe of the pituitary. The diminished, yet positive increase in weight and size of the animals fed with the desiccated extract compared to those of the animals injected with the fresh extract indicates that either the autacoid concerned with such an effect is partially destroyed or inactivated in the process of drying or that a large proportion of this autacoid is destroyed or unabsorbed in the alimentary canal, only part of it being absorbed unaltered and thus exerting its effect, the magnitude of which, of course, is not as great as when the substance is unaffected or given directly into the blood stream. Such a drawback can, however, be overcome by increasing the dosage of the desiccated extract.

There is no doubt that administration of the anterior/

anterior lobe of the hypophysis whether per os or parentrally, causes a very significant rise in the basal metabolic rate measured in terms of oxygen consumption. Some other investigators have reported that such administration lowers the basal metabolism or has no effect on it. It is highly improbable that such results are tenable as it would seem clear that this has been due to the effect of the technique of extraction or inconsistant administration when the extract is orally given or from other disturbing factor in husbandry of the animals or of metabolic measurements.

The interrelationship of the anterior lobe of the hypophysis and the sex glands although it has been established as far as the female sex organs, yet no such conclusive evidence is given regarding the male sex organs. A body of investigators report that administration of the anterior lobe substance has a retarding effect on the male sex organs particularly the testicle. Such results as these seem to me to be untenable as I have never found even one animal to respond to such administration by atrophy or even diminished weight when compared with those of the controls. The only interpretation to such results obtained by other workers indicating inhibition on the one hand and no influence on the other hand is that in the one case the extract is most likely to

have/

have been toxic in nature, especially when considered with the decrease in weight of the animals reported by some workers to accompany administration of the extract, and in the other it is either inert or of too small a dose to produce any appreciable and demonstrable effect. The histological evidence obtained in this investigation is all in support of the stimulating influence of the anterior lobe substance over spermatogenesis.

The interrelationship between the anterior pituitary lobe and the thyroid gland is indeed a very strong and remarkable one. The anterior lobe substance whether orally administered in the form of desiccated extract or parentrally introduced in the form of fresh extract causes an amazing hypertrophy and hyperactivity of the thyroid gland associated with a degree of hyperplasia. Such results as are obtained and reported in the present investigation, although they agree with those of Schwartzbach and Uhleuhuth (91) as far as the effect on the thyroid gland of the administration of the anterior lobe extract by injection, yet they disagree with these authors' results as far as feeding the anterior pituitary lobe. The above mentioned authors in 1928 showed that the basal metabolic rate measured in the larvae of Ambyostoma trigeminum was raised to a level varying between 50% and 114% above normal, depending on the source/

source of the larva used. They obtained such results only with administration by injection but no such effect was obtained by feeding the anterior lobe substance (Armour & Co.) to these larvae. They conclude that the only effective way in causing hyperactivation of the thyroid to which they attribute the rise in the basal metabolic rate, is by parentral administration. Oral administration is equally effective in inducing hypertrophy and hyperactivation of the thyroids, to administration by injection and it seems that failure of these authors to obtain positive results by oral administration of the anterior lobe extract is due to inactivity of the extract used or non assimilation of these larvae to the anterior lobe substance.

CONCLUSIONS.

(1) Administration of desiccated thyroid extract raises the basal metabolic rate measured in terms of oxygen consumption and produces a diminution in the size and weight of both the albino rat and the white mouse.

(2) There is a marked increase in the nitrogen output and a remarkable rise in the excretion of both inorganic phosphorus and urinary calcium by such administration.

(3)/

(3) The effect of prolonged administration of desiccated thyroid causes atrophy and extreme inactivation of the thyroid glands and a distinct degeneration of the epithelium of the vesicles in many parts of the gland.

(4) Thyroid administration causes a very marked atrophy and degeneration of the seminiferous tubules with an obvious hypertrophy of the interstitial tissue, which effects indicate in the definite interrelationship between the externally and internally secreting parts of the testicle with that of the autacoid of the thyroid gland.

(5) Prolonged administration of thyroid extract causes a distinct diminution in the connective tissue stroma of the ovary accompanied by increased lutein formation and a remarkable hyperplasia and hypertrophy of the interstitial tissue of the organ with greatly dilated blood vessels to the glands. Such results as these obtained in the ovary, furnishing a definite link of interrelation and association of the two glands, perhaps makes the empirical clinical use of thyroid extract in correcting some obstetrical and gynaecological errors, a little clearer.

(6)/

(6) The suprarenal glands respond to thyroid administration by hypertrophy and hyperactivity of both cortex and medulla of the gland which indicates a firm link of interrelation between the thyroid gland and both parts of the adrenal bodies.

(7) The constituting parts of the hypophysis cerebri, particularly the pars anterior, intermedia and pars nervosa seem to be intimately interrelated with the thyroid gland. The pars anterior with thyroid administration show an early very marked decrease in the number of oxyphil cells with some degenerative changes in the nuclei of the few others that are seen. When thyroid administration is pushed a little further, a marked degeneration appears and a general almost complete disappearance of oxyphil cells in the pars anterior. The pars nervosa showing degeneration also. The pars intermedia, however, shows an early indication of degenerative retrogression in the nuclei, i.e. piknosis.

(8) No histological evidence was obtained in the pancreas or parathyroids by which any definite interrelationship/

interrelationship between these organs and the thyroid gland can be prognosticated.

(9) Administration of the anterior pituitary lobe substance whether orally given in the form of desiccated extract or parentrally introduced in the form of freshly prepared extract, raises the basal metabolism rate measured in terms of oxygen consumption and produces an increase in the size and weight of the animals. Such effect, however, is more marked by parentral administration than by feeding the anterior lobe substance.

(10) There is, by such administration, a decrease in nitrogen excretion and a very marked decrease in urinary calcium excretion. Inorganic phosphorus excretion is very much increased.

(11) There is a definite and remarkable interrelation between the anterior body of the pituitary body and the thyroid gland; the latter responding to oral and parentral administration of the anterior lobe extract by enlargement and increase in weight/

weight; and by very marked hyperactivity.

(12) Administration of the anterior pituitary extract by whatever route causes marked lutinzation in the ovary with dilatation of the blood vessels. Such dilatation of the blood vessels to the ovary was marked by oral administration.
In the testicle the effect of anterior lobe substance is to cause increased spermatogenesis with indication of slight atrophy of the interstitial tissue. Such evidences indicate a link of interrelation between both female and male sex organs with the anterior lobe of the hypophysis. There is also by such administration, a marked increase in weight of both testicles and ovaries.

(13) Administration of anterior lobe extracts orally and parentrally induces the adrenal glands to hypertrophy and increased weight. It also produces very marked hyperactivity of both the cortex and medulla. Administration by injection however produces a little more marked effect than by feeding. This indicates that there is a remarkable interrelation between the anterior lobe of the pituitary and the two component parts of the adrenal glands.

(14) By whatever route the anterior pituitary extract is administered, it causes a marked increase/

increase in the oxyphil cells of the anterior lobe. Other parts of the pituitary body show no evidence of any structural change. The gland as a whole tends to be enlarged by such administration and shows the presence of colloid in the interglandular cleft, particularly marked in the fed animals.

(15) By administration of the anterior lobe substance, the pancreas responds with very marked hypertrophy and increased weight. The alveoli are smaller and the organ as a whole is denser. There is also increase in the zymogen granules which are also coarser than normal. No histological evidence suggestive of any change in the islet tissue save that occasionally some nuclei of the cells show more condensed nucleus than normal, probably a phenomenon of retrogressive change.

(16) No histological evidence of any change in the parathyroid glands is revealed by which a conclusion can be made as to the interrelation of the anterior lobe of the pituitary with the parathyroids.

The liver and spleen show a very remarkable enlargement.

REFERENCES.

(1) Claude Bernard, (1859) Lecons sur les proprietes physiologique et le alterations pathologique de liquides de l'organisme, vol. II. 411.

(2) Quoted by Sir E. Sharpey-Schafer, John Mallet Purser lecture, University of Dublin. Irish J. Med. Science (1931)

(3) Schafer (1912) Quart. Journ. Exp. Physiol. 5, 203.

(4) Schafer (1924) Endocrine Organs: Longman and Green. Vol. I. 9.

(5) Evans and Long (1921) Anat. Record. 21, 62.

(6) Putnam, Teel and Benedict (1928) Amer. Jour. Physiol. 84, 157.

(7) Bugbee, Simond and Grimes (1931) Endocrinology, 15, 51.

(8) Korenchovesky (1930) Bioch. Journ. 24, 383.

(9) Fordyce (1912) Edin. Med. Jour. 9, 55.

(10) Hoskins (1916) Jour. Exp. Zool. 21, 295.

(11) Herring (1917) Quart. Jour. Exp. Physiol. 11, 231.

(12) Cameron and Carmichael (1921) Jour. Biol. Chem. 46, 35.

(13) Schafer, (1932) Quart. Jour. Exp. Physiol. 22, 95.

(14) Magnus-Levy (1904) Z. Klin. Med. 52, 201.

(15) Salmon (1904) Berl. klin. Wochschr. 41, 635.

(16) Steyerer (1907) Z. Exp. Path. Therap. 4, 720.

(17) Von Bergman (1909) ibid. 5, 646.

(18) Asher and Horrisberger (1921) Biochem. Zeitschr. 121.

(19) Schafer (1924) Endocrine Organs. Vol.I. 46.

(20)/

(20) Hewitt (1915) Quart. Jour. Exp. Physiol. 8, 113.

(21) Kojima (1917) Quart. Jour. Exp. Physiol. 11, 255.

(22) Janney and Henderson (1920) Arch. Internat. Med. 22, 160.

(23) Courvoisier (1917) Mitt. Grenzg. Med. Chir. 29, 270.

(24) Schondroff (1897) Arch. ges. Physiol. 47, 395.

(25) Ord and White (1893) Brit. Med. Jour. 2, 217.

Roos (1895) Z. Physiol. Chem. 27, 19.

(26) Dening (1895) Munch. Med. Wochschr. 42, 389.

(27) Dinkler (1896) ibid. 43, 513.

(28) Parhon, M. (1912) C. R. soc. Biol. 72, 620

(29) Aub, Bauer, Heath and Ropes (1929) Jour. Clin. Invest. 7, 97.

(30) Hunter, D. (1930) Lancet. 954.

(31) Percival and Stewart (1928) Physiol. Reviews. 8, 283.
(1927) Bioch. Jour. 21,301.

(32) Shohl and Pedley (1922) Jour. Biol. Chem. 50,537.

(33) Hoffman (1931) Jour. Biol. Chem. 93,787.

(34) Briggs (1922) ibid. 53, 13.

(35) Carlton, Histological Technique 1926 40.

(36) Carlton ibid. 279.

(37) Cramer (1920) Jour. Physiol. (Proc. Soc.) 104, 2

(38) Peterfi (1921) Zeitschrift Wissenschaft. Mikros. Bd. 83, 1

(39) Langly. Practical Histology 1901 311.

(40) Hewer (1927) Jour. Path. and Bact. 30.

(41) Schafer's Essentials of Histology (1929) 601

(42)/

(42) Marinus (1919) Am. Jour. Physiol. 49, 238.

(43) Robertson (1917) Endocrinology 1, 24.

(44) Dott (1923) Quart. Jour. Exp. Physiol. 13, 241.

(45) Hewitt (1929) Bioch. Jour. 23, 718.

(46) Lewis and Millar (1913) Arch. Inter. Med. 12, 137.

(47) Canan and Drummond (1922) Bioch. Jour. 16, 53.

(48) Reitchart (1928) Endocrinology 12, 451.

(49) Larson, Bergeim, Barker and Fisher (1929) Endocrinology 13, 63.

(50) Thompson and Johnson (1905) Jour. Physiol. 33, 189.

(51) Cushing (1909) Jour. Amer. Med. Assoc. 53, 257.

(52) Pearl (1916) Jour. Biol. Chem. 24, 123.

(53) Bernestein and Falta (1912) Verh. Congr. Inn. Med. 29, 364.

(54) Arnold and Leschke (1921) Z. Klin. Med. 92, 364.

(55) Kestner (1922) Munch med. Woch. 69, 796.

(56) Bowman and Grabfield (1926) Endocrinology 10, 201.

(57) Foster and Smith (1926) Jour. Am. Med. Assoc. 87, 2151.

(58) Cushing and Davidoff (1927) Arch. Int. Med. 39, 673.

(59) Lee and Gognan (1930) Endocrinology. 14, 233.

(60) Houssay and Biasotti (1931) ibid. 15, 511.

(61) Malcolm (1903) Jour. Physiol. 30, 270.

(62) Hewitt (1914) Quart. Jour. Exp. Physiol. 8, 113.

(63) Grabfield and Prentiss (1925) Endocrinology. 9, 144.

(64)/

(64) Korenchovesky and Dennison (1929) Bioch. Jour. 23, 868.

(65) Teel and Walkins (1929) Am. Jour. Physiol. 89, 662.

(66) Oswald (1902) Virchow's Arch. 169, 444.

(67) Good and Ellis (1918) Endocrinology 2, 431.

(68) Smith (1927) Jour. Am. Med. Assoc. 88, 158.

(69) Loeb and Bassett (1929) Proc. Soc. Exp. Biol.Med. 26, 860.

(70) Schockaert (1932) Am. Jour. Anat. 49, 379.

(71) Uhleuhuth Schafer's Essentials of Histology (1929) 280.

(72) Wulzen (1914) Am. Jour. Physiol. 34, 127.

(73) Goetsch. (1916) Johns Hopkins Hosp. Bull. 27, 29.

(74) Klinger (1919) Arch. ges. Physiol. 177, 232.

(75) Maxwell (1916) Pub. Univ. California Physiol. 5, 5.

(76) Kross (1922) Am. Jour. Obst. & Gyn. 4, 19.

(77) Evans and Simpson (1926) Anat. Record 32, 206.

(78) Biedle (1927) Arch. Gyn. 132, 175.

(79) Robson and Taylor (1932) Jour. Physiol. (Proc. Soc.) 75, 37.

(80) Evans and Simpson (1928) Jour. Am. Med. Assoc. 91, 1337.

(81) Zondek and Aschheim (1927) Klin. Woch. 6, 1321

(1927) Arch. Gyn. 132, 176.

(1928) Klin. Woch. 831, 1404.

(82) Loeb, M. (1884) Deutsches Arch. f. klin. Med. 34, 443.

(83) Piere Marie (1886) Revue de med. 6.

(84)/

(84) Reid Hunt (1922) Am. Jour. Physiol. 58, 257.

(85) Von Zwehl (1926) Arch. Entwicklungsmech. Organ. 109, 456.

(86) Folin, Cannon and Dennis (1913) Jour. Biol. Chem. 13, 477.

(87) Cramer (1928) Fever, Heat-Regulation, Climate and Thyroid-Adrenal Apparatus.

(88) Zondek (1932) Klin. Wchnschr. 11, 760.

(89) Herring (1915) Quart. Jour. Exp. Physiol. 9, 391.

(90) Donaldson The Rat 1924.

(91) Schwartzbach and Uhleuluth (1928) Proc. Soc. Exp. Biol. Med. 26, 389.
