STUDIES ON THE SIGNIFICANCE

OF VARIATIONS

IN THE COLLOID CONTENT OF

THE THYROID GLAND.

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THESIS SUBMITTED FOR THE DEGREE OF M.D.

BY

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PREFACE.

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This work was commenced during the tenure of a Carnegie Research Scholarship to which I was appointed in the years 1922-1923 and 1923-1924, and was carried out in the Institute of Physiology at the University of Glasgow.

I wish to express my thanks to Professor Noël Paton for permitting me to examine the thyroid glands of the dogs used by him in his experiments on rickets, and also for his very kindly encouragement throughout the work. To Dr Norman Cruickshanks I am indebted for permission to refer to the records of the Pathological Department of the Glasgow Maternity Hospital. I wish to acknowledge the kindness of Messrs Parke, Davis & Co., who gave me a supply of their product "Metagen".

The photographs have been prepared by Mr John Bell of the Institute of Physiology. I am greatly indebted to him for the care which he took in their production.

I. THE THYROID GLAND IN THE FULL-TIME HUMAN FOETUS AND IN THE NEWLY BORN INFANT.

Introduction.

Various descriptions have been given of the appearance of the thyroid gland of the human subject about the time of birth. The majority of workers have recorded a condition very different from that seen in the normal adult gland, and several have advanced hypotheses to explain it.

It would seem that typical colloid appears at some period of intra-uterine life. Muller (16) described it in foetus of 5 months, Elkes (6) in foetus from 4½ to 6½ months, Livini (12) in embryos of 62 - 66 Min., while Peremeschko (17) and Boechat (1) found it present in older embryos. Wölfer (19) however, was of the opinion that colloid formation did not commence until the end of foetal life or even till after birth. Treating this investigation from the chemical standpoint Marine (13) states that the iodine content of the thyroid increases with the increasing age of the foetus. Fenger (7,8) examined the iodine content of a large series of foetal cattle, pigs, sheep and man, and came to the same conclusion.

Immediately after birth, according to some observers, a very striking change in the histological appearance of the thyroid/

thyroid occurs. Zielinska (20) found solid collections of cells filling the vesicles without any sign of colloid. Elkes gave a similar description, and emphasised the difference between the foetal gland, and that of the newly-born.

Meroz-Tydman (15) examined a series of thyroids from still-born and newly born children and found that in both some glands were made up of elongated or rounded heaps of epithelial cells closely pressed together, while others showed vesicles with desquamating cells and no colloid, others again were composed of almost normal looking vesicles containing a little colloid, in which, however, were so many vacuoles that it had the appearance of a thin network. Hesselberg (10) considered that the normal appearance of the thyroid at least during the first week of life is one of empty vesicles and desquamating cells.

Maurer (14), on the other hand, found that after birth colloid is sometimes present. Elkes drew his conclusion that the thyroid at this age is colloid-free, from the examination of a series of thyroids from newly-born children. There was one exception, however, which showed well differentiated vesicles filled with colloid. Garnier (9) found no great difference between the thyroid of the newly-born and that of the full-time foetus. Hesselberg also was doubtful if there was any marked difference as she found, in three out of nine cases of foetus from/

from 7 to 9 months, the desquamated condition of the gland which she considered the normal appearance in the newly-born. Cooper (2) stated that during the first two weeks after birth the thyroid shows little difference from that of the full-time foetus but that practically all the vesides are empty.

Four theories as to the cause of this change in the thyroid after birth have been suggested. Wölfer after examining a series of glands from feeble and still-born children, and finding that they had few or no vesicles and no colloid, considered that, as this condition resembled the state of the thyroid before colloid formation was begun, the cause of the appearance after birth was arrested development of the gland.

De Quervain (5) suggested that the desquamation of the epithelial cells might be due to disease in the child. Ferrando (18) and Elkes found a similar condition in thyroids taken from children who had suffered from congenital syphilis. Garnier investigating the effect of infectious diseases on the thyroid gland, found, however, that desquamation was present in the thyroid of an otherwise normal child after a prolonged labour.

Elkes was of the opinion that some metabolic change, taking place immediately after birth might account for the alteration in the thyroid. Hesselberg, however, found no evidence of desquamation in the glands of various animals killed/

killed and examined shortly after birth, and disagreed with Elkes' theory, thinking that as some similar metabolic change must probably occur in the lower animals this should, if the hypothesis were correct, cause some alteration in their thyroids also.

Hesselberg held the view that the disappearance of colloid and the desquamation of the epithelial cells was due to damage to the thyroid during birth by reason of the relative narrowness of the human birth-canal. She held that this theory would explain Garnier's results mentioned above.

The present investigation was undertaken with a view to determining whether these changes in the thyroid might be related in any way to the state of development of the child, or whether they might be due to autolysis or post mortem degeneration. Neither of these possibilities has apparently been enquired into, as no reference to either has been discovered in the literature. No description of the effects of autolysis on the thyroid seems to have been published before, and so to obtain a knowledge of this before examining the human thyroids, observations were made on the post-mortem changes in the thyroid of the dog. These shall be described first.

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A. Study of the Degenerative Process in the Thyroid.

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Method of Investigation.

Seven experiments were carried out with a view to discovering the effect of autolysis in the thyroid. Cruickshank(4) carried out similar experiments with liver and kidney and his method was followed in this case. After trying various media he found that he obtained the best results by keeping the tissue in a moist chamber, and in several of his experiments he was successful in obtaining autolysis similar to that seen in the body and in foetal tissues.

The method in detail was as follows. Previous to the removal of the thyroid a sterile Petri dish was prepared in the foot of which was a layer of blotting paper soaked in salt solution, both paper and solution having been sterilized. The neck of the dog was then shaved and washed carefully - no antiseptic could be used as autolysis is retarded by its presence. The two thyroids were removed and transferred as quickly as possible with sterile instruments to the Petri dish. Each thyroid was then cut with a sharp knife into four parts. One part was removed and fixed at once in formalin as a control, and the dish was closed. In some cases the Petri dish was kept at room temperature - to compare with the rate of change in the thyroid of a foetus kept in the mortuary - and in others it was placed in an incubator kept at a constant heat of 37°. At intervals of 24 hours a piece was removed and fixed. The tissues/

tissues were prepared by the usual paraffin method, cut, and stained with haemalum and eosin. The greatest care was taken to maintain sterility but it is doubtful if this was successful, and it certainly failed in some cases which showed batterial growth round the margins. Cruickshank also experienced very great difficulty in keeping the organs sterile and bacteriological examination proved that in about half his experiments all his precautions had been of no avail.

Results.

In the case of the thyroids kept at room temperature the degenerative process took place gradually throughout the week, the duration of the experiment. The first change was the appearance of vacuoles round the margin of the colloid masses in the vesicles, which had shrunk somewhat, possibly due to a lessening of the intra-vesicular pressure caused by diminution or liquefaction of the colloid. At this stage the vesicular cells are still cubical and ranged regularly round the vesicle. This condition is illustrated in Plate 2 which shows such a thyroid 48 hours after removal. Plate 1 shows the state of the gland immediately after removal from the body.

The lining cells of the vesicles next become detached from the basal membrane and all the colloid disappears. As the process of degeneration progresses the cells become separated from each other and come to lie free in the lumen. As this proceeds the section takes on a much more cellular appearance. The vesicles become small, and less and less

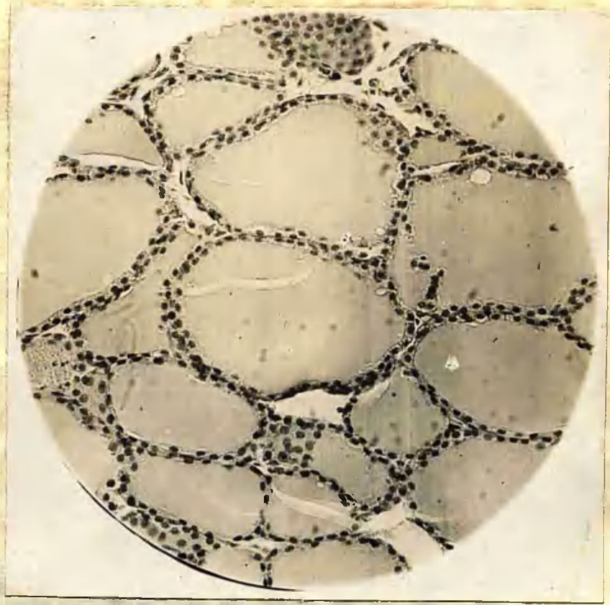

PLATE I.

Thyroid of Dog x 300.
(fixed immediately after removal).

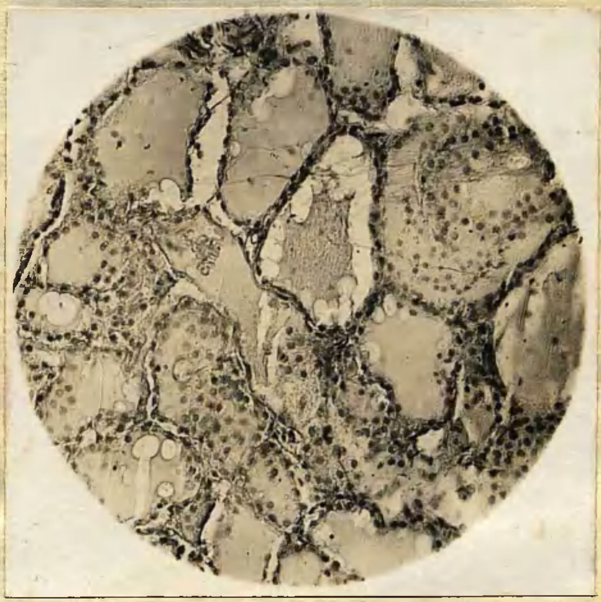

PLATE 2.

Thyroid of Dog x 300.
(fixed 48 hours after removal).

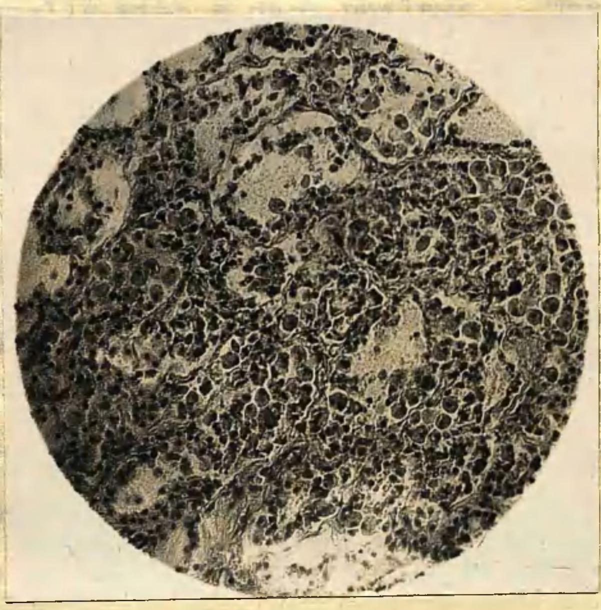

PLATE 3.

Thyroid of Dog x 300.
(fixed 7 days after removal.)

less distinct until their outline, marked by a thin strand of connective tissue, can only with difficulty be made out. The interior of the vesicles is ultimately packed with epithelial cells, many of which appear as large rounded pink-staining cells with a dark nucleus. The Thyroid shown in Plate 3 illustrates this state. It was fixed 7 days after removal during which time it had been kept at room temperature.

The rate of degenerative change in the thyroids kept in the incubator at 37° was about three times that of those kept at room temperature. The same process was observed but the change at the end of 24 hours was greater than in those kept in the cooler temperature for 48 hours. In the specimens kept in the incubator further changes than those described above were observed.

Gradually the cells and nuclei degenerated until the section presented an almost homogeneous pink appearance. For the present purposes, however, only the changes described and illustrated above are of interest.

There is one other point to which attention might be directed. Plate 1 shows a perfectly normal thyroid of a dog with large well filled vesicles, while in Plate 2 the same thyroid is shown after 48 hours' degeneration. In the latter vacuoles are very noticeable although there are none present/

present in the freshly fixed gland. It would appear that these vacuoles resulted from a process of degeneration and this is confirmed by the work described below on the thyroid of the foetus.

Many views have been expressed as to the cause of the appearance of the vacuoles which were frequently observed in the colloid. Cowdry (3) and others have ascribed them to shrinkage of the colloid in preparation. Jackson (11) believed they were connected in some way with the process of colloid formation, while Cooper (2) has advanced the opinion that they may be due to absorption of colloid into the circulation. Neither of the last two theories, however, could account for the occurrence of vacuoles post-mortem, and if Cowdry's hypothesis applies here, it would seem, as there was no shrinkage in the freshly fixed gland, that there must be some chemical change in the colloid substance before this can occur.

B. Examination of a Series of Young Human Thyroids.

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At the Glasgow Maternity Hospital routine post-mortem examinations are performed on all infant corpses whether the child has been still-born or has lived. Full details as to size and development as also to the post-mortem findings in the various organs are recorded. Through the kindness of Dr Norman Cruickshank I was permitted to examine the thyroid from a number of these cases - in all 75.

The gland was fixed in formalin after removal and weighing, and was imbedded by the paraffin method, cut, and stained with haemalum eosin.

As full details as possible were obtained in each case, special note being taken of the state of development, as it was intended to correlate this, if possible, with the histological findings in the thyroid. The age of the foetus was calculated from the date of the last menstruation to the date of birth. The former however was often difficult to ascertain and many of the ages given are only approximate. For this reason no great reliance has been placed on this, and in the later post-mortem records the last menstrual date has been omitted, so that in some of the cases the age thus calculated does not appear in the following tables. A much more accurate idea of the state of/

of development can be obtained by examination of the condition of the various centres of ossification. For this purpose the following have been noted: (i) the number of centres present in the mesosternum; the size of the centres in (ii) the astragulus, (iii) the os calcis, (iv) the lower end of the femur, and (v) the upper end of the tibia. The weight and length of the child were also noted.

As the possibility has to be taken into account of the appearances of the thyroid being due in some cases to autolytic change, the time from death or still-birth until the time of the post-mortem examination is recorded in the accompanying tables. This could be obtained accurately in the case of children who had lived, but in the others it was very difficult and usually impossible to find out how long the foetus might have been dead in utero. As the majority of craniotomies were performed on account of contracted pelvis, and as in many such cases the child is alive until the operation is undertaken, it was thought that a more accurate estimate of the time that the child had been dead before examination might be obtained in the instances where this was done, than in the other still-births, and these cases were consequently segregated.
There/

There cannot of course be the same exactitude as when the child had lived, but probably more than with the remaining still-births.

I am indebted to a house surgeon for three thyroids which he removed and fixed immediately after delivery. In these cases craniotomy was performed on living foetus, the mothers' pelves being greatly contracted. The glands obtained thus were therefore free from any suspicion of post-mortem change.

Histological Examination.

The appearances found in the thyroids were very varied. Some were made up of colloid containing vesicles, some of empty vesicles, while others presented a solid cellular appearance quite unlike that normally associated with this gland. In this description they may conveniently be grouped into two classes - those containing colloid, and those free of colloid.

1. Glands containing Colloid.

Into this class fall 23 of the thyroids examined. The gland was composed of vesicles lined with cubical epithelium and filled with colloid which stained well. Some showed a certain amount of intervesicular tissue, but where the vesicles are small this is only to be expected as the result of the section passing tangentially through the walls of some vesicles. The vesicles varied in size, but were always/

PLATE 4.

Normal human thyroid at the age of birth
x 60.

PLATE 5.

Normal human thyroid at the age of birth
showing congestion.
x 60.

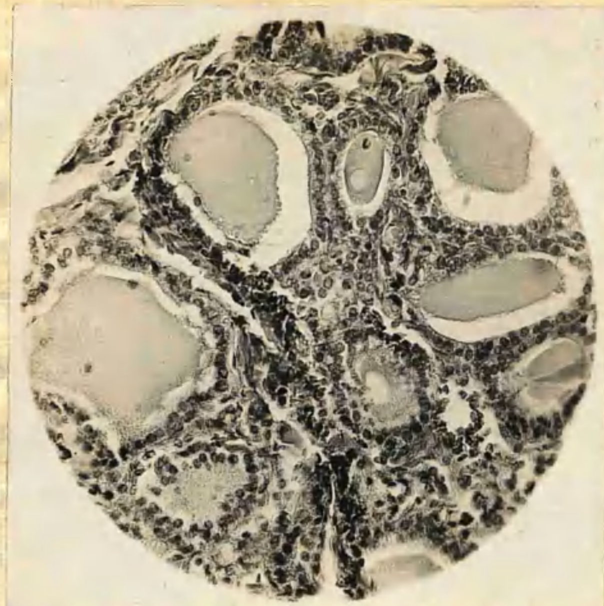


Plate 6.

Same thyroid as in Plate 4 under
high power
x 300.

PLATE 7.

Human thyroid.
(see "Histological Examination" Group 2.A.)

always much smaller than in the adult gland. Plates 4 and 5 show the low power view and Plate 6 the appearance seen under high power of the sections of two of the thyroids which were fixed immediately after death. These are more or less typical of all the glands included in this group although some showed more colloid. They were selected for illustration as there can be no doubt as to their freshness. There was little difficulty in separating this group from the next as the presence of the colloid was in every case noticeable at a glance. As regards vascularity, it will be seen that the capillaries in the thyroid shown in Plate 5 are congested while those shown in Plate 4 are more or less empty. Similarly with the others, some showed congestion, others did not. It seems possible that the congestion described by some observers as being invariably present may be due to the character of the labour.

2. Glands containing no colloid.

As has been indicated these thyroids varied from a condition in which there was merely an absence of colloid to a state in which vesicles as such can scarcely be recognised. For descriptive purposes, however, they may be divided into three types.

A. In a few thyroids there are still traces of colloid in the vesicles. This, however, stained faintly and contained large vacuoles, which were often so numerous that all that remained/

PLATE 8.

Human thyroid x 300.
(see "Histological Examination" Group 2.B.

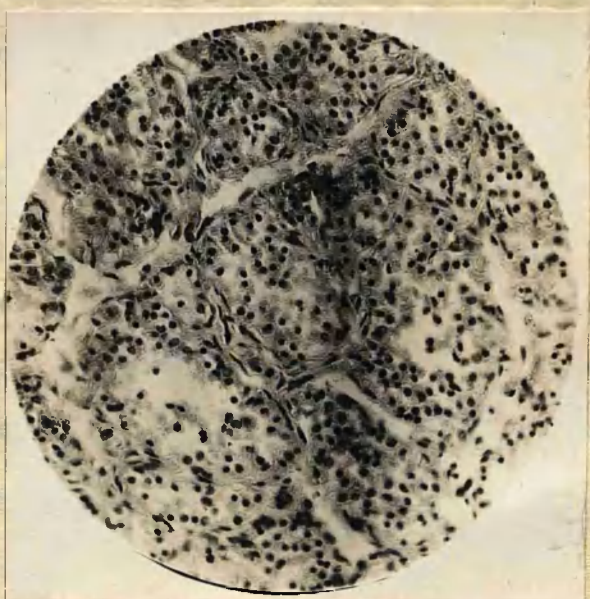


PLATE 9.

Human Thyroid x 300.
(see"Histological Examination Group 2.C.

remained of colloid was a thin network between these vacuoles. The walls were lined with cubical cells more or less regularly arranged though here and there they had become detached and were escaping into the lumen. (Plate 7).

B. Many of the sections presented a characteristic, clear appearance. The glands contained no colloid and the protoplasm of the cells stained rather faintly. Vesicles were clearly discernable but many of the cells composing their walls had separated from the basal membrane. When they still retained their attachment they were of more columnar shape. (Plate 8).

C. A considerable number of the thyroids had a very cellular appearance. In many cases it was only with difficulty that the outline of vesicles could be distinguished, marked out by a thin line of connective tissue cells. The vesicles were filled with desquamated cells of more or less rounded shape (Plate 9). As in the thyroids of type B, the protoplasm of the cells stained more faintly than in those glands which contained colloid. and the nuclei were much darker.

In this group as in the first some glands were very congested, while in others there were few blood cells visible.

Results.

It will be seen from Table I that 8 of the 16 thyroids had vesicles filled with colloid while the remainder were colloid-free. Study of that table reveals similar variation in the state of development, age, weight and length, and duration of life in the infants of both groups, colloid-containing and colloid-free. One fact, however, may be observed, namely that in every case in which colloid was found to be present the post-mortem examination had been carried out within 24 hours of death, whereas in the others with only one exception this was delayed for two or three days.

Similarly from Table II, treating of still-born children on whom craniotomy was performed, it will be seen that with one exception the post-mortem examination on the six infants with thyroids containing colloid were also carried out within 24 hours of delivery. In three cases where the thyroid showed desquamation of the epithelial cells and no colloid, the autopsy was performed from one to two days after delivery and in five, in which this was delayed for three to four days, all the glands showed vesicles solid with desquamated cells.

Table III gives the particulars of the remaining still-born children not dealt with in Table II. In 9 cases out of 40 the thyroid contained colloid. In 6 of these the post-mortem/

TABLE I. Children who lived.

No.	No. of pregnancy.	General Description. Sex.	Age (appr.) Days.	Weight lbs.	oz.	Length Ins.	Duration of life. days	Time dead before P.M.	Ossification. Meso-sternum.	Astragalus.	Os calcis.	Femur.	Tibia.	Weight Grams.	Thyroid. Histological Examination.
2	2	M	280	6	11½	21	5	1	1-5	4	5	2	1	1.8	Vesicles numerous and distinct, Well filled wihh colloid.
22	9	M	221	3	7	17¼	7	1	1-4	4	3	-	-	1.05	
29	2	F	229	3	7¼	17½	1	½	1-3	4	7	-	-	1.1	
30	2	F	230	3	14¾	17	1	½	1-3	5	5	3	-	1.1	
31	1	F	287	6	8½	21	4	1	1-4	5	8	7	3	1.15	
36	1	M	245	4	0¼	18¾	5	1	1-2	4	7	2	-	.92	
42	5	M	-	7	10¾	22	3	½	1-3	5	7	4	-	1.55	
54	1	M	-	6	5½	21	5	½	1-4	5	5	6	2	1.	
23	1	M	274	4	7	18½	4	2	1-3	5	8	4	-	.8	Cells becoming detached from basal membrane. No colloid.
43	3	M	225	4	13½	18¼	2	2	1-5	4	6	-	-	11.5	
44	2	F	218	2	11¾	16¼	¼	2	1-4	2	5	-	-	.7	
56	3	F	-	3	15½	18½	10	1	1-3	4	7	3	-	2.	
60	10	M	-	5	10	20½	1	3	1-5	7	9	5	-	-	
47	1	M	280	4	5¾	20½	8	2	1-6	5	8	5	4	1.	
50	5	M		3	3½	17	2	3	1-3	6	5	-	-	1.1	Vesicles small and pakked with cells. No colloid
55	1	F	-	2	3½	15	1	2	1-4	2	5	-	-	1.	

TABLE II. Still births - Craniotomies.

No.	No. of preg-nancy.	General Description. Sex.	Age (appr.) Days.	Weight lbs.	Weight oz.	Length Ins.	Time from birth to P.M. Days.	Ossification. Meso-sternum.	Astra-galus.	Os calcis.	Femur.	Tibia.	Thyroid. Weight. Grams.	Histological Examination.
21	8	M	181	6	5¾	10½	2	1-4	7	8	4	-	1.7	Numerous vesicles well filled with colloid.
58	2	F	-	-	-	-	1	1-5	4	10	6	3	-	
68	2	F	-	6	7½	20½	1	1-5	4	5	5	2	-	
69	-	-	-	-	-	-	0	-	-	-	-	-	-	
70	1	M	-	6	14¾	21	0	1-6	5	7	4	5	-	
71	14	M	-	6	13	20¾	0	1-3	9	9	7	4	-	
46	1	M	295	7	14	22¼	1	1-3	7	8	6	3	-	
51	1	M	290	6	14½	22¾	2	1-4	5	10	5	2	3.95	Cells becoming detached from basal membrane.
66	4	F	-	6	0	19½	2	1-4	4	8	3	-	2.57	
4	3	F	275	5	9½	20¾	4	1-4	3	4	2	1	-	
7	2	F	300	6	13	21	3	1-3	6	7	3	-	1.0	Vesicles small and solid: packed with cells, and ill defined. No colloid.
14	3	M	255	5	13	21½	4	1-4	7	9	3	1	1.5	
26	1	F	277	5	0½	20½	4	1-3	6	9	5	4	.8	
28	1	F	244	6	9	20¾	4	1-5	5	8	6	5	1.3	
													2.55	

TABLE III. Still births.

No.	No. of pregnancy	Sex	Age (appr) Days	Weight lbs.	Weight oz.	Length Ins.	Time from birth to P.M. Days	Meso-sternum	Astragalus	Os calcis	Femur	Tibia	Thyroid Weight Grams	Thyroid Histological Examination
18	2	F	280	7	10	20¾	1	1-4	6	7	3	-	1.8	Distinct vesicles well filled with colloid.
37	7	F	-	3	15¼	16	1	1-2	2	4	-	-	1.4	
41	1	M	250	4	13½	18¼	1	1-4	4	7	1	1	1.53	
49	2	F	-	3	4½	17	½	1-4	3	7	2	1	.53	
62	1	F	-	6	1½	19¾	1	1-4	6	8	5	-	1.7	
64	5	M	-	8	10	21	1	1-3	7	7	1	5	-	
19	13	M	270	6	3	19¼	2	1-5	4	5	3	-	1.1	
24	8	F	-	7	1½	20¾	2	1-4	5	7	5	4	1.5	
67	3	M	-	8	1½	21	2	1-5	7	10	4	4	-	
3	2	M	268	8	3	21¼	1	1-4	4	6	2	-	3.5	All these show what is here described as degeneration in greater or less degree. Some show the start of the processs of the separation of epithelial cells from basal membrane while others present the solid appearance described in the text. None has colloid.
15	3	M	255	5	12½	29¼	1	1-5	4	5	5	-	1.4	
34	4	M	-	2	7¼	18	1	1-5	5	5	-	-	1.45	
40	1	F	212	3	7	7½	1	1-4	4	5	-	-	1.13	
45	12	F	266	7	11	20¾	1	1-3	8	7	3	-	2.7	
52	1	F	274	6	2½	19¾	1	1-4	5	8	2	-	1.35	
65	2	M	-	4	12¾	20	1	1-4	6	6	5	1	-	
5	1	M	290	6	5	21	2	1-5	4	7	4	-	1.9	
8	1	M	285	7	15½	21¼	2	1-5	5	7	6	4	2	
10	1	M	276	7	8½	21¾	2	1-5	5	7	6	5	1.95	
11	1	M	271	8	11¾	22½	2	1-4	6	9	6	5	4.2	
12	1	M	273	8	1¾	21½	2	1-5	6	8	5	1	1.3	
16	1	M	277	6	0¼	-	2	1-4	5	4	3	-	1.3	
20	1	M	294	7	10¼	21½	2	1-5	9	9	6	-	2.35.	
27	6	M	238	3	2½	19	2	1-3	3	7	2	-	.65	
32	1	F	293	6	14¾	21½	2	1-4	8	8	6	4	1.75	
33	4	M	-	6	7½	21	2	1-5	6	8	5	4	2.	
35	2	M	-	7	7½	20	2	1-4	5	7	5	4	2.42	
53	3	M	236	4	11	19¼	2	1-4	2	6	2	-	1.17	
57	1	F	-	3	5	17	2	1-2	3	5	-	-	2.	
63	8	M		6	8½	19½	2	1-4	5	7	1	-	-	
1	2	M	290	8	5	22½	3	1-4	3	6	3	1	1.8	
6	7	F	286	6	1	20	3	1-4	5	7	4	5	1.9	
9	1	M	290	7	3½	20¼	3	1-5	5	7	6	4	2.	
17	1	M	256	6	3	20	3	1-4	6	7	3	-	1.8	
38	1	F	-	2	9½	15½	3	1-4	2	4	-	-	.57	
59	1	M		8	8	21	3	1-4	6	10	6	3	-	
61	2	M		6	3½	21	3	1-3	3	6	4	-	-	
25	3	F	255	6	12½	21	4	1-4	6	6	3	-	2.15	
72		-	-	-	-	-	4	-	-	-	-	-	-	
73		-	-	-	-	-	4	-	-	-	-	-	-	

mortem examination was performed within 24 hours and in 3 within 2 days. Of the other 31 thyroids which were free of colloid 7 were examined one day after birth while the remaining 24 were not fixed for two, three or four days.

A careful study of these tables convinces one that these striking variations in the histology of the thyroid cannot be due to variations in development. whether reckoned by age, weight and length, or extent of ossification. Neither do they seem to be related to the sex of the child or the number of the pregnancy. Thus one has to consider whether these variations may be accounted for by the duration of time between the death of the child and the fixation of the thyroid - that is to say, whether they can be due simply to degenerative changes.

The thyroids from the three cases of craniotomy in which the glands were fixed within a very short time of death, all present a similar appearance, and all show a considerable amount of colloid. No degeneration surely can have taken place and consequently these may be looked on as normal, although as already stated they do not conform exactly to the appearance found in the adult gland. Taking this standard as the normal, Table I shows in a remarkable way that in all cases in which the gland was fixed within 24 hours of death the thyroid may be regarded as normal, while in practically every case in which it remained in the body for/

for a longer period, it may be said to be degenerated. Table II bears out this conclusion. In Table III it will be noted that in 7 cases in which the post-mortem examination took place within one day the thyroids showed the condition described above as abnormal. This may be accounted for by the fact that the clinical notes did not give any idea as to how long the child may have been dead in utero. As has been seen from the experimental work already described autolytic change takes place at a greater rate at blood temperature than at room temperature. Thus if the child were dead in utero for a comparatively short time it is highly probable that by the end of 24 hours after delivery, any degenerative change might be very much more advanced than if the child had lived till the time of birth. This then may be the reason for the colloid-free condition appearing so early in these cases.

In practically all cases in which the sectio was performed two days after birth, as also in all cases where this was done at a later date, the thyroid presents this abnormal condition. Thus it would appear that Table III also confirms the findings of the other two tables.

To sum up therefore it will be seen that in all recent thyroids a considerable amount of colloid was observed, while in those which remained in the body for a longer time, colloid was absent and the gland epithelium was desquamating.

Table II/

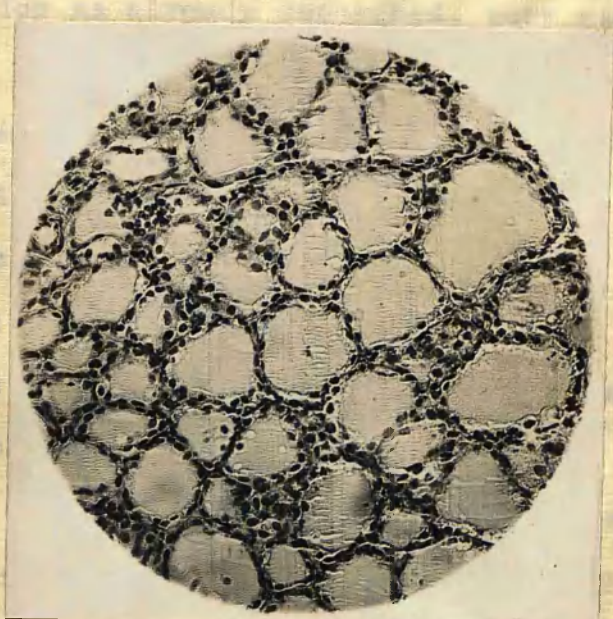


PLATE 10.

Thyroid of full time foetal guinea-pig.
x 300.

Table II shows very clearly, as also does Table I to some extent, that the compact cellular appearance of the gland due to complete desquamation was found particularly in those thyroids which had had a longer time in which to degenerate than in those showing the commencing separation of the cells.

In the investigation of the degenerative process in the thyroid of the dog as already described, very similar appearances were observed. After the colloid disappeared the epithelial cells began to separate from the basal membrane and as this process advanced, desquamation became complete. The gland then closely resembled those human thyroids described in the preceding paragraph. One may conclude therefore that the appearances in all those glands grouped as abnormal can be explained simply as the result of post-mortem degeneration.

Hesselberg examined the thyroids of dogs, cats, and rabbits immediately after birth, and always found colloid-filled vesicles, without any sign of epithelial desquamation, although she considered this desquamation to be normally present in the child during the first three weeks of life.

I have on several occasions examined the thyroid of full-time guinea-pigs removed by Caesarean section and have invariably found well-filled vesicles, and no desquamation. (Plate X) This would seem to confirm the view that the colloid state is normal and that desquamation is abnormal, although Hesselberg held a different opinion. That worker sought to explain

the/

the difference between man and animals, in that the former showed desquamation and the latter did not, by suggesting that owing to the narrow birth canal in the human subject the thyroid was injured during delivery. If this were so one would expect to find desquamation very frequently in a first-born child but not so often in the children of a multipara when the birth is usually easier. This is not borne out by this work as will be seen by reference to the accompanying Tables, I, II and III. Moreover a large proportion of still-births are due to difficult labour, yet a considerable number of still-born children in this series had normal thyroids. Garnier found that after a prolonged labour the child's thyroid showed desquamation. In such a case, however, it would seem that there was every possibility of the child being dead in utero for some considerable time in which case autolysis would account for the change he described.

Conclusions.

1. The changes which occur in the thyroid gland post-mortem may be summarized thus. First vacuoles appear in the colloid which ultimately disappears completely. The epithelial cells then become separated from the basal membrane. Eventually the vesicles become filled with desquamated cells and the gland presents a solid cellular appearance.

2. As the formation of vacuoles in the colloid may take place after death, these cannot always be regarded as evidence of activity on the part of the thyroid.

3. The thyroid gland of the full-time human foetus and also of the newly-born infant appears to be composed normally of small vesicles lined with cubical epithelium and filled with colloid which stains well. The glands may or may not be congested.

4. When variations from this standard occur, there is evidence that they may in many cases be due to post-mortem degeneration.

5. No relationship was found to exist between the condition of the thyroid in the full-time foetus and the development of the child as reckoned by the extent of ossification.

--------oOo--------

II. THE INFLUENCE OF DIET, ETC., ON THE CONDITION OF THE THYROID AND ON GROWTH.

Introduction.

Various types of thyroid have been described in animals of the same species. Watson (64) examined the thyroids from a series of white rats and grouped them into four types. Each presented a distinctive appearance varying from a condition in which the vesicles were filled with colloid to one in which the vesicles were entirely empty. Similar types were described by Douglas (37) in the thyroids of fowls. Forsyth (39) reported great variation in the thyroids of birds of the same species. Recently Crawford and Hartley (36) have found that great variations are to be observed normally in the thyroid of the rabbit. From the work of several observers it would appear that these differences in the histological appearances of the thyroid may be accounted for, in part at least, by the kind of diet on which the animal is fed. The results of these workers are mentioned later.

The following experiments were carried out to investigate further the influence of diet on the colloid content of the thyroid. Some observations were also made on the influence of thyroid extract and of adrenalin. These are described first. Thereafter an account is given of the investigations on the effect of milk and butter, of cod liver oil, and of a protein diet with particular reference to its tryptophane content.

General Notes on Experiments.

In the following experiments young white rats were used. They were kept in wire cages with a tray of sawdust at the foot. Each cage contained two to four animals. The cages were kept thoroughly clean, the tray being scrubbed with hot water every day and covered with fresh sawdust. McCarrison (52) has described changes in the thyroid of rats kept in dirty cages, so that it would appear that care in regard to cleanliness is of importance in such experiments as those described below. The animals were kept at a fairly constant temperature as a precaution against erroneous results, Hart (42) having recorded variations in the amount of colloid in the thyroid of rats kept at different temperatures.

In every case the controls were of the same litter as the experimental animals.

All animals were weighed twice a week, this being done in the morning before feeding.

Two basal diets were used to which were added the substances whose effect on the thyroid was to be tested. The first used was that given to adult stock rats in the laboratory, and consisted of -

Diet A:- porridge, cabbage, turnip.

The adult rats appeared healthy on this diet and maintained their weight. It was found, however, that it was not so suitable for young rats. This has already been discovered by Watson and/

and Hunter (67). It was desired to retain a carbohydrate diet, and the second diet was made up of

Diet B:- dried bread, potato (boiled).

All animals had an ample supply of water.

At the end of the experiments the animals were killed by chloroforming. The organs were removed at once, weighed, and fixed in formalin. Although the thyroids were removed with the utmost care, it was found that in some cases small pieces of connective tissue or even of muscle were adhering to the glands when examined microscopically. In small rats, the thyroids being of minute size, their weights could not be regarded as accurate and are consequently disregarded in the following account of the experiments. McCarrison compared the size of thyroids from rats by placing all those of one experiment side by side and photographing them. This method, however, is open to the objection that it causes delay in fixation.

The thyroids were prepared by the paraffin method. Serial sections were cut and stained with haemalum and eosin.

Histological Examination.

Great variation was found in the microscopical appearances of these thyroids. Some showed large thin-walled vesicles lined with large columnar cells. A closer study, however, revealed that these differences probably depended on the amount of colloid in the gland.

The thyroids in these experiments may be grouped roughly into four types, which resemble those described by Watson (64).

1. Vesicles distended with well stained colloid. Cells flattened to a remarkable degree (Plate 11). This had apparently been effected by great intra-vesicular pressure as at points the intervesicular wall was thinned till it appeared as a thin line (Plate 11.A) or seemed even to have disappeared (Plate 11.B). The thyroids of all rats receiving thyroid extract were of this type.

2. Vesicles well filled with colloid but lined with cubical cells. "Interstitial tissue" of varying amount. As this depends apparently on the section passing tangentially through walls of vesicles (the view held by Jackson (45)) it naturally follows that the thicker the walls are the more of this tissue is likely to appear. Hence in this and the following two types these intervesicular cells were more numerous than in Type 1. Plate 12 shows the thyroid of an animal receiving cod liver oil (Exper,9); Plate 13 that of one to whose food potassium iodide was added (Exper.10); and Plate 14 depicts the thyroid of/

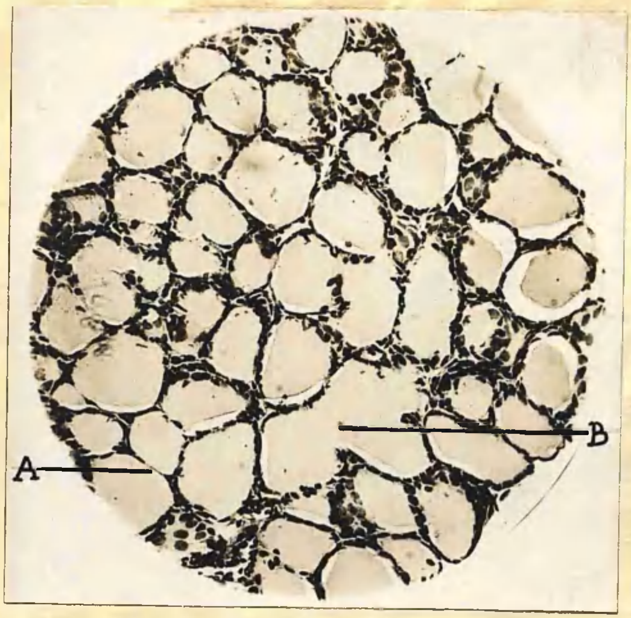


PLATE 11.

Thyroid of rat receiving thyroid extract
x 300.

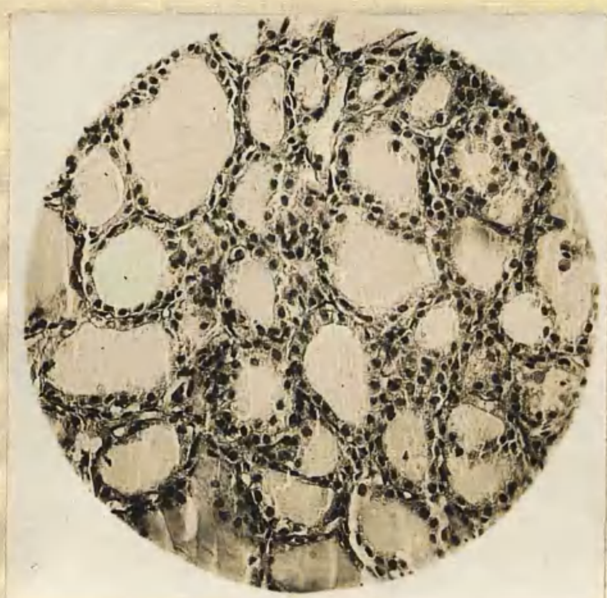


PLATE 12.
Thyroid of rat receiving cod liver oil.
x 300.

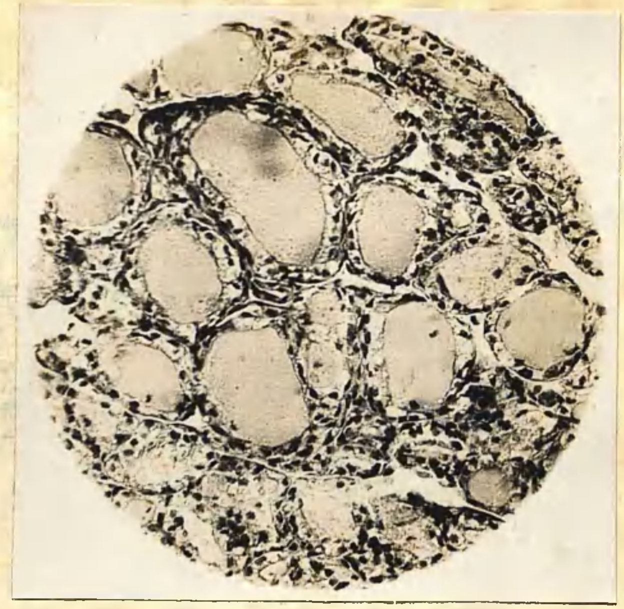


PLATE 13.

Thyroid of rat receiving potassium iodide. x 300.

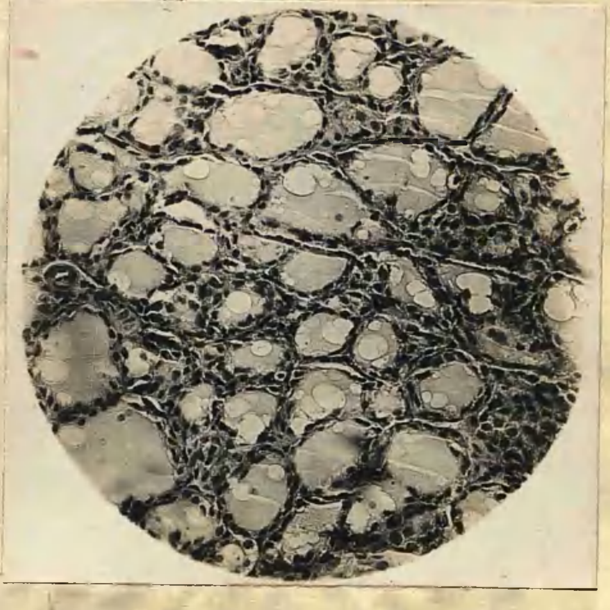


PLATE 14.

Thyroid of rat fed exclusively on meat. x 300.

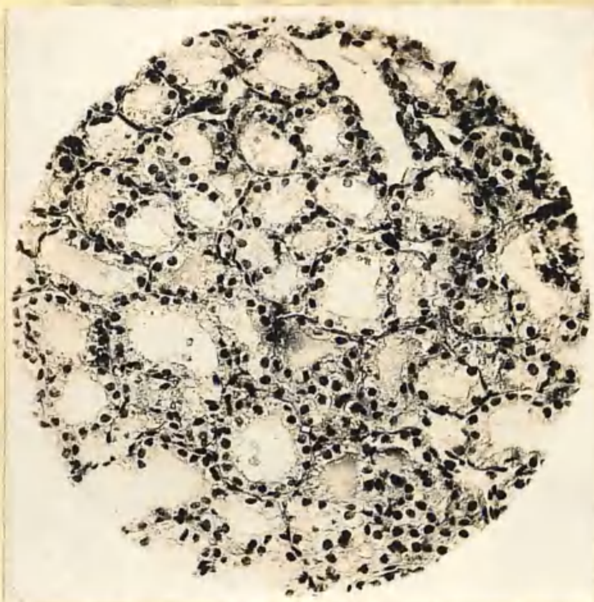


PLATE 15.

Thyroid of rat receiving milk.
x 300.

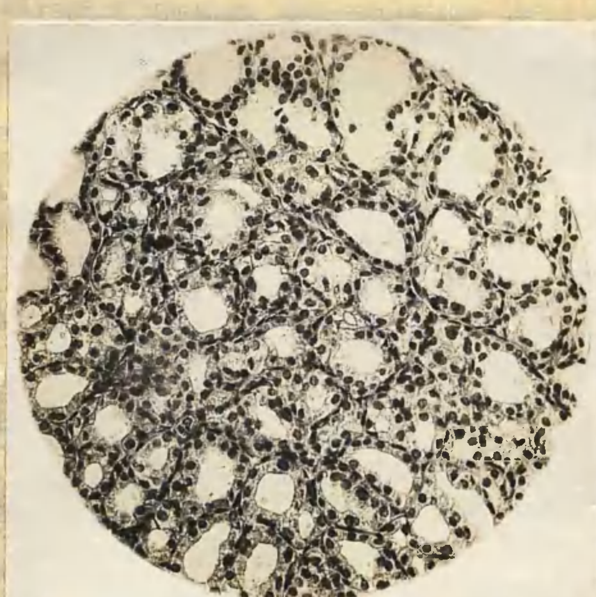


PLATE 16.

Thyroid of control rat.
(Exper.1.)
x 300.

of a rat fed exclusively on lean meat (Exper, 6).

3. Small amounts of colloid but cells filled with granules which do not take on stain well. It seems possible that these have to do with the formation of colloid and this type shows more evidence of colloid than that next to be described. This was the condition of the thyroids of animals receiving milk (Plate 15).

4. Vesicles small with very little or no colloid and lined by high cubical or columnar cells (Plate 16). As will be seen from Table 1 this type was very commonly found. It resembles the condition described by McCarrison as Goitre in rats.

In all these types the peripheral vesicles were larger than those in the centre. Where there was only a rather poor amount of colloid in the gland, as in Type 3, the peripheral vesicles were filled although the central ones might be practically empty. Schafer (58) describes this in his account of the normal structure of the thyroid.

According to Ribbert (57) after removal of part of a thyroid regeneration occurs first in the subcapsular zone and this he suggests is due to more active blood supply. It seems possible that this theory might also account for the larger peripheral vesicles in the normal gland. Courrier (33) described a bi-polar action of the vesicular cells, i.e., with a function of discharging secretion either into the vesicle or into the blood stream and this view is supported by the work on mitochondria in these cells by Bensley (25) and Cowdry (34).

Thus/

Thus when the blood carries to the thyroid more material for the formation of its secretion than would be required for body purposes at the moment the excess would, it seems possible, be stored most in the vesicles nearest the blood stream. In this way the peripheral vesicles would get the first opportunity of storing and what they could not deal with would be carried on to the inner vesicles.

TABLE IV. - Thyroid Extract, Iodine and Adrenalin.

Expt.	Average weight of rats. Gms.	Diet.		Duration of experiments.	Percentage gain in Weight.		Thyroid Appearances.	
		Experimental rats.	Control rats.	Weeks.	Exptl.	Controls.	In exptl rats.	In control rats.
1	43	Diet A + thyroid extract.	Diet A	$3\frac{1}{2}$	22	63	Vesicles completely filled with colloid.	Very little or no colloid.
2	51	Diet A + adrenalin (hypoderm.)	Diet A + Saline (hypoderm)	$6\frac{1}{2}$	66	61	No colloid.	Ditto.
3	61	Diet A + Thyroid Extract + Adrenalin (hypoderm)	ditto.	$6\frac{1}{2}$	26	61	Vesicles well filled with collid but less than in Expt.1.	Ditto.
7	91	Lean meat + Thyroid Extract.	Lean meat	3	26	39	Vesicles completely filled with collid.	Considerable amoun of colloid present
10	-	Diet B + Kl solution.	Diet B.	2	-	-	Well filled vesicles.	Very little, or no colloid.

A. Effect of Thyroid Extract, Iodine and Adrenalin.

A synopsis of the experiments is given in Table IV. The method of administration of thyroid extract was that described by Cameron and Carmichael (27). The amount of this substance to be given was calculated from the body weight of the animal in the proportion of 1:5,000. This appears to be about the maximum dose which rats can take as the animals all looked ill and had marked glycosuria. The dose of thyroid extract was, in Experiments 1 and 3, mixed into a paste with flour and water and in Experiment 7 mixed with small pieces of meat and administered to the rats individually before they received their day's food.

The adrenalin solution was given hypodermically, each rat receiving .2 c.c. of a 1/200,000 solution daily. A similar quantity of sterile saline was injected into the control animals at the same time.

Discussion of Results.

Thyroid. Experiments 1 and 7 show that the administration of thyroid extract to rats which are receiving a diet composed either of carbohydrates or of proteins, causes a great increase of colloid in the thyroid gland. This is confirmatory of the work of several observers. (Cameron and Carmichael (27), Courrier (33), Fordyce (38) and Kojima (47). The effect of iodine in increasing the colloid is seen in Experiment 10. This/

This also has been demonstrated by Cameron and Carmichael.

In these experiments (Nos. 1 and 7) the thyroid extract supplies the animal with much more hormone than is required for body purposes and the gland appears to store as much as possible of the excess. When, therefore, the thyroid is found to be in the "colloid state" one may assume that the thyroid has a reserve of secretion and does not require to secrete actively for the immediate needs of the animal. On the other hand when the gland shows empty vesicles with columnar cells it would appear that the thyroid is in a very active condition. This activity might account for the greater weight of the colloid free than of the colloid rich gland as pointed out by Cameron and Sedziak (28) and Mellanby (50).

The influence of injections of adrenalin was studied in Experiments 2 and 3. In the former the thyroids of both experimental animals and controls presented a similar appearance showing empty vesicles and columnar cells. In the latter the glands of the rats which were given thyroid extract while receiving adrenalin injections showed a considerable amount of colloid. Their vesicles were well filled but in the centre were small and all were lined with cubical epithelium - a condition decidedly different from that found in the thyroids of the rats in Experiments 1 and 7 which had large fully filled vesicles with flattened cells. Although the/

the quantity of thyroid extract was administered in exactly the same proportion (1:5,000 body weight) in all the experiments in this case (Exper.3) it did not cause to the same extent the filling up of the vesicles. It would appear, therefore, that the injections of adrenalin had interfered with this action.

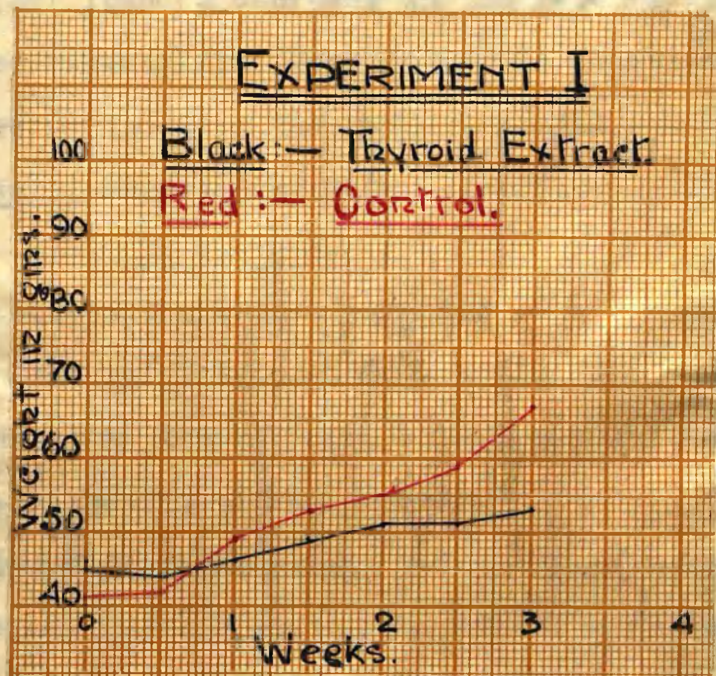
Several workers have performed experiments to determine the influence of adrenalin on the thyroid gland. Changes in that gland indicative of active secretion have been described by Osokin (56) after the injection of adrenalin. Schmid (60) however obtained negative results in similar experiments. Cannon and Cattell (29) found that the injection of adrenalin caused a difference of electrical potential in the thyroid. Crawford and Hartley (36) injected adrenalin into rabbits but could form no conclusions as to its effect on the thyroid because the varied appearances observed in that gland were, they considered, within the range seen in the normal gland, which, they state, differs markedly in different rabbits under similar conditions.

The experiments here described seem to bear out the view that injection of adrenalin causes increased activity of the thyroid gland.

Growth.

Varying opinions have been expressed as to the effect of the administration of thyroid extract on the rate of growth.

Moussu (51) stated that small doses stimulate growth, and/


EXPERIMENT I
Black:— Thyroid Extract.
Red:— Control.
Weight in grms.
100
90
80
70
60
50
40
0
1
2
3
4
Weeks.

Graph 1.

and Schafer (59) obtained a similar result in young rats. Hoskins (44) concluded that thyroid extract had no effect on growth.

Bircher (26), Herring (43), Carlson (30), and Cameron and Carmichael (27) found a decrease in the rate of growth.

In the three experiments described above in which thyroid extract was added to the diet the rate of growth was markedly decreased. Graph 1 illustrates this clearly.

Conclusions.

1. Thyroid extract administered to young rats in doses of 1:5,000 of body weight causes a great increase in the colloid content of the thyroid, and marked decrease in the rate of growth of the animal.
2. The injection of adrenalin into young rats receiving thyroid extract interferes to some extent with the storing of colloid in the thyroid vesicles. Thus it seems possible that adrenalin has a stimulating effect on the thyroid gland.

B. Effect of Milk and Butter.

Some workers have stated that milk has some effect on the thyroid. Watson (65) found that rats and mice fed on bread and milk had thyroids with vesicles filled with well stained colloid while those of animals fed on flour and water had much less distinct vesicles containing a clear poorly-staining secretion. Tsuji (62, 63) observed hypertrophy of the thyroid in rats receiving 2-3 c.c. of milk daily and considered that milk contained some substance acting hormonically on the thyroid.

A diet rich in butter fats according to Mellanby (50) causes great hypertrophy of the thyroid. That worker fed pups on diets rich in various fats and found that the dog receiving butter showed as remarkable a change in that gland as a five-fold increase. McCarrison (53) working with pigeons found that their thyroid showed active hyperplasia when the bird was fed on any other fat than cod liver oil or on a diet free of fat.

Experiments 4 and 5 were designed to reinvestigate these results. In the former each rat received 1½ gms. of butter per day. If more than this was given the food was refused by the animals. The butter was melted and mixed with porridge. In experiment 5 milk was mixed with porridge and milk given in the drinking dish instead of water.

The/

TABLE V. Milk and Butter.

Expt.	Average Weight of rats. Gms.	Diet.		Duration of Experiments. Weeks.	Percentage Gain in weight.		Thyroid Appearances.	
		Experimental rats.	Control rats.		Exptl.	Controls.	In Experimental rats.	In control rats.
4	55	Diet A + butter	Diet A	9½	151	115	Vesicles empty.	Vesicles empty.
5	54	Diet A + milk	Diet A	9½	118	115	Vesicles contain a little faintly staining colloid.	Vesicles empty.

The experiments and results are summarised in Table V.

The thyroids of the rats receiving milk contained somewhat more colloid than those of the controls and presented more evidence of colloid formation as described above in the histological description of the glands (see Plate 15). The thyroids of the animals fed on butter were similar in appearance to those of the controls.

These experiments have not been sufficiently extensive to warrant the forming of any conclusions.

C. Effect of Cod Liver Oil.

(See Table VI).

Experiment 9 was originally undertaken with a view to discovering the conditions of the thyroid in animals showing different rates of growth. It was hoped to produce this difference by the addition of abundant vitamines to the practically vitamine free diet which was given to the controls. For this purpose all animals were fed on Diet B to which was added in the case of the experimental animals one capsule per rat per day of "Metagen," a preparation of Messrs Parke, Davies & Co., stated to be very rich in vitamines A, B, and C. This was continued for 4½ weeks without producing any difference in the rate of growth from that of the controls. It was then withdrawn and replaced by cod liver oil on account of its reputed richness in Vitamine A. The amount of oil given was 1 c.c. per rat per day mixed in with the food.

The other experiment (No. 8) shown in Table VI was carried out in an investigation on the effect of a meat diet on the thyroid which will be described later. With a view to improving the nutrition of the animals, however, cod liver oil was added to the food of all the rats so that this experiment may with advantage be considered in this section.

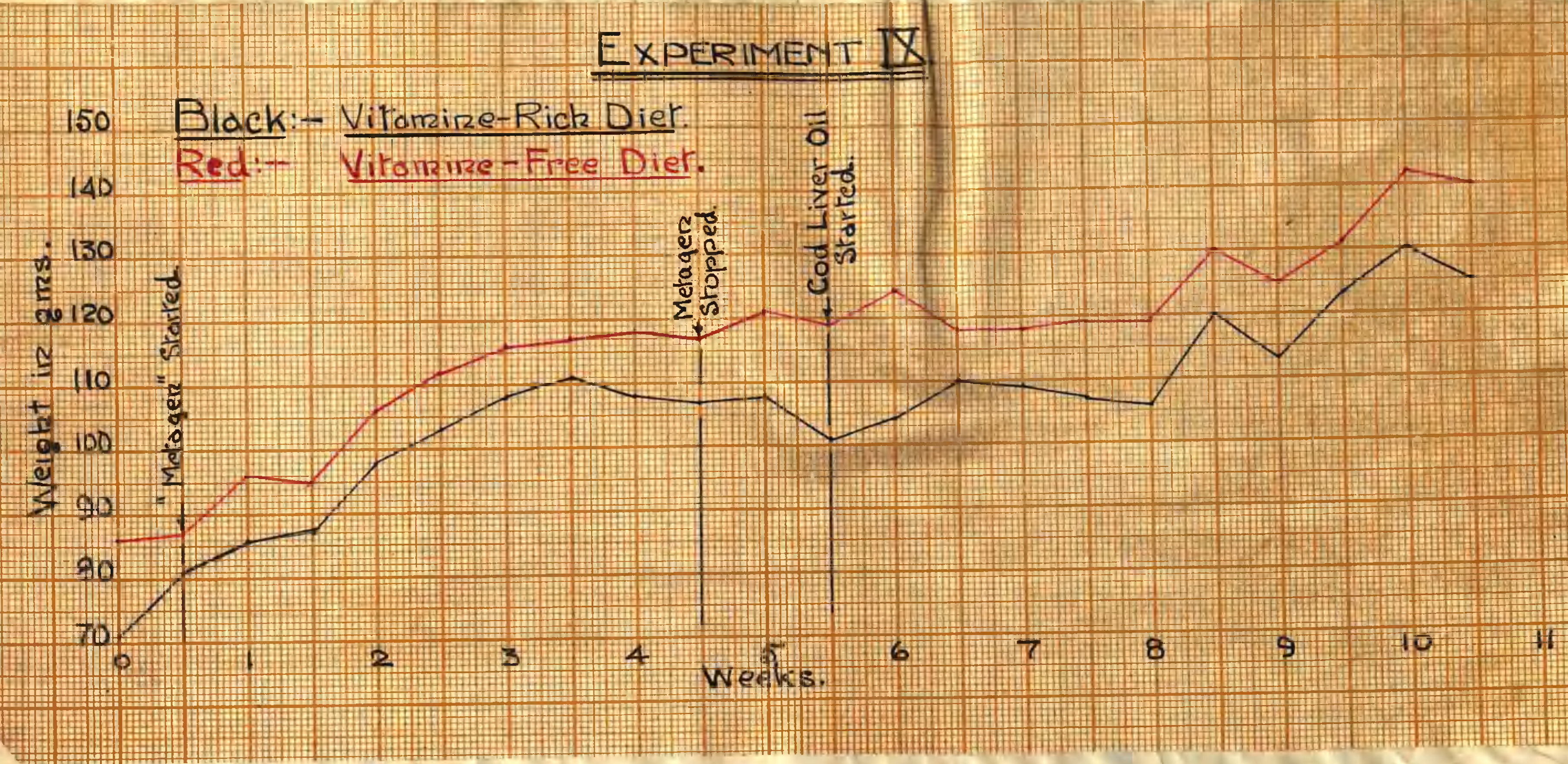
Results.

Thyroid.

In the thyroids of all the animals in experiment 8, whether they were receiving meat or gelatin the vesicles were/

TABLE VI. Cod liver oil.

Expt.	Avge. Weight of rats. Gms.	Diet.		Duration of experiment. Weeks.	Percentage gain in weight.		Thyroid appearances.	
		Experimental Rats.	Control rats.		Exptl.	Controls.	In experimental rats.	In control rats.
9	68	Diet B + Metagen (for 4½ weeks). later + cod liver oil (in place of metagen).	Diet B.	10½	54	61	Well filled vesicles.	Empty vesicles.
8	72	Diet B. + Cod liver oil + lean meat.	Diet B. + cod liver oil + gelatin.	4	94	19	Well filled vesicles.	Well filled vesicles.


EXPERIMENT IX
Black:- Vitamine-Rich Diet.
Red:- Vitamine-Free Diet.
Weight in gms.
150
140
130
120
110
100
90
80
70
"Metagen" Started.
Metagen Stopped.
Cod Liver Oil Started.
0
1
2
3
4
5
6
7
8
9
10
11
Weeks.

Graph 2.

were well filled with colloid. As will be shown later, in no other case when the food consisted of Diet B and gelatin was the presence of colloid observed. All the experimental animals in Experiment 9 had thyroids similar to the above, composed of vesicles filled with colloid (Plate 12) while those of the controls were colloid free.

It appears from these experiments that cod liver oil exerts some influence on the thyroid causing the formation of colloid. This confirms McCarrison's findings and agrees also with the work of Mellanby, who found that cod liver oil maintained the thyroid in a normal condition while all other fats caused hyperplasia.

Growth.

As has already been mentioned the addition of "Metagen" to the diet did not produce any change in the rate of growth. Reference to Table VI and Graph 2 will show that cod liver oil did not prove any more effective. Somewhat similar results have been obtained by other workers, Abel (21) and his co-workers finding that animals on a trytophane free diet were losing weight added a rich supply of vitamines with no effect. Chang (32) discovered that vitamine B tablets did not correct the malnutrition of animals on a gelatin diet.

In experiment 8, however, the animals which were fed on meat grew much better than those receiving gelatin but the thyroids of both lots presented a similar appearance. It would seem, therefore, that the difference in the rate of growth was due/

due to the meat and not to either cod liver oil or the condition of the thyroid.

Conclusions.

1. Cod liver oil exerts an influence on the thyroid causing the appearance of colloid.
2. "Metagen," a preparation stated to be very rich in vitamines added to a diet of dry bread and potatoes had no effect on the rate of growth.
3. Cod liver oil did not accelerate growth.
4. Similar appearances of the thyroid may be found in young rats growing at different rates.

D. Effect of a Protein Diet, with special reference to its Tryptophane Content.

That meat has an influence on the thyroid has been stated by several observers. Watson (66) fed fowls for a long period on raw meat and water and found that their thyroids were partly hypertrophied, while microscopically they showed a normal structure, though the vesicles were rather larger than normal. Similar results were obtained by Bauman (24) and Aeschbacher, (22), and more recently this has been confirmed by Marine and Lenhart (49).

The question arises as to what is the cause of this increase in the thyroid. As it is known that there is a marked relationship between the chemical formulae of tryptophane and thyroxin, the substance isolated from the thyroid and described as the active principle of its secretion by Kendall(46), it seemed possible that the tryptophane in the meat protein might account for this hypertrophy by supplying the thyroid with the requisite material for forming its secretion. Long before thyroxin had been discovered, however, Willcock and Hopkins (68) had suggested that tryptophane might be the mother substance of the thyroid hormone. Cramer (35) found that animals fed on a diet poor in tryptophane showed atrophied thyroids with small vesicles containing little or no colloid.

In/

TABLE VII. Tryptophane.

Expt.	Average weight of rats. Gms.	DIET.		Duration of experiments. Weeks.	Percentage gain in weight.		Thyroid Appearances.	
		Experimental rats.	Control rats.		Exptl.	Controls.	In experimental rats.	In control rats.
6	92	Lean meat	Diet B	3	52	7	Considerable amount of colloid in vesicles.	Empty vesicles.
8	72	Diet B. + cod liver oil. + lean meat.	Diet B + cod liver oil. + gelatin.	4	94	19	ditto.	Same as in experimental animals.
11.	44	Diet t + cheese.	Diet B + gelatin.	4	74	43	Very little colloid.	Very little colloid.
12.	49	Diet B + gelatin + meat extractives.	Diet B. + gelatin.	4	44	47	ditto.	ditto.
13	49	Diet B. + gelatin. + tryptophane.	Diet B. + gelatin.	8	55	70	ditto.	ditto.

In this connection, it may be of interest to note that Harries, (41) assuming that tryptophane is the basis of the thyroid secretion, has formulated a theory as to the etiology of exophthalmic goitre. He considers that the putrefactive indol-producing bacteria in the intestine are exterminated in this disease, with the result that all the tryptophane in the diet, part of which is normally broken down in the intestine, is absorbed. Consequently, he holds, there is too great a supply of this substance to the thyroid which uses it to form thyroxin in excess.

Experiments.

(See Table VII.)

The object of experiment 6 was to reinvestigate the reported increase in the thyroid in animals fed on meat. One group of rats were fed on lean meat, while the controls received a carbohydrate diet of approximately the same caloric value.

The remaining experiments were designed to discover the influence of trytophane. In Experiment 8 meat was compared with a similar quantity of gelatin, a protein lacking tryptophane. Cheese was used instead of meat in Experiment 11 on account of the Casein in it being particularly rich in tryptophane. In Experiment 13 all the rats received a diet of bread, potato, and gelatin but to one lot was added a solution of pure tryptophane.

When

When the effect of meat was compared with that of gelatin, the object was to give one diet rich in trytophane and the other tryptophane-free. These diets, however, differed also in that the meat diet contained other substances besides tryptophane. Experiment 12 was performed to discover whether the meat extractions had any influence either on the histological appearance of the thyroid, or on the rate of growth of the animal. For this purpose lean meat was pounded in a mortar and thoroughly extracted with cold water. The amount of extractives administered was obtained from a quantity of meat of the same weight as the gelatin in the diet.

Results.

The appearance of the thyroids of the rats fed exclusively on meat is shown in Plate 14. It will be seen that the vesicles contain a considerable amount of colloid. There was a marked difference between these and the glands of the controls. This experiment therefore confirms the work of Watson.

It has been pointed out in the section dealing with the effect of cod liver oil that this substance was given to all the animals in Experiment 8 with the result that all their thyroids showed similar microscopical appearances. Thus in this case the effect of the meat on that gland could not be observed.

When/

When cheese was given instead of meat the thyroids of the rats were made up of vesicles nearly empty of colloid. There was no difference between these thyroids and those of the control animals which had been given gelatin in place of cheese. It was thought that as cheese was relatively rich in tryptophane these rats would have well filled thyroid vesicles. To ascertain, however, what effect tryptophane *per se* added to Diet B has on the thyroid, experiment 13 was performed using the pure amino-acid. The results obtained were the same as in the experiment in which cheese was given. The thyroids of both experimental and control animals were similar, containing little or no colloid.

It will be observed that while meat appears to increase the colloid, cheese and tryptophane alone do not have this effect. The possibility that this was due to the meat extractions was investigated, as mentioned above, in experiment 12 which, however, did not show that these had any influence on the thyroid. *In these experiments it was found that tryptophane alone did not cause the formation of colloid in the thyroid vesicles.*

In a recent paper Chang (32) gives the result of an investigation on somewhat similar lines but he administered the tryptophane hypodermically instead of by the mouth.

He/

He found that on animals receiving bread and milk the tryptophane had no effect but when given to animals on a gelatin diet it caused an improved nutritive condition and practically normal thyroids. The gelatin diet, which he used, however, contained McCollum's Salt Mixture (55) and it may be that the addition of tryptophane was sufficient to transform that diet from an insufficient to an adequate one. It is probable that the animals in my experiments may still have been receiving a qualitatively insufficient diet even after the addition of tryptophane.

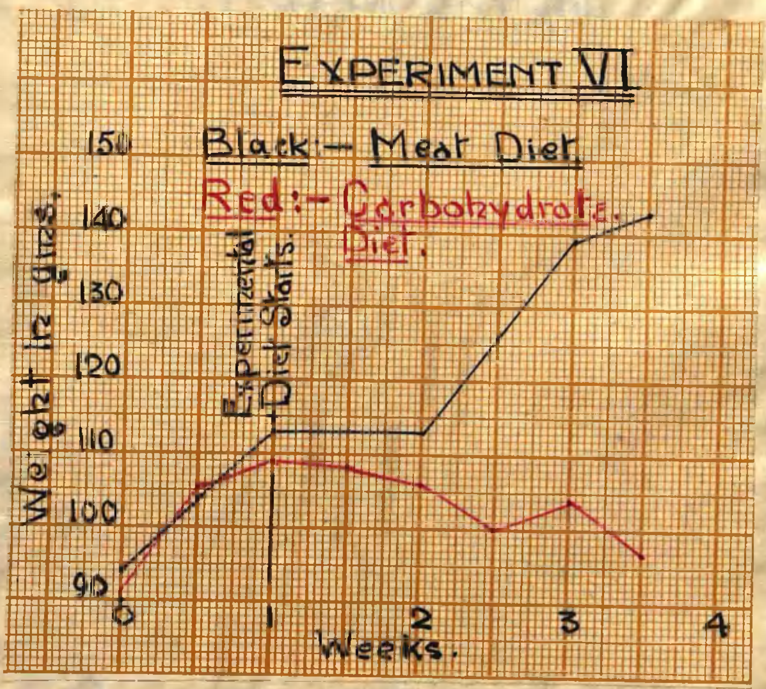
Abel, Backus, Bourquin, and Gerard (21) have recently published details of their experiments on the connection between tryptophane and thyroid function. They dewcribe normal glands in animals receiving tryptophane but here too salt mixture was part of the diet which also included cod liver oil. The latter substance probably accounted for the fact that the tryptophane-free animals nevertheless showed thyroids with a very considerable amount of colloid.

From the experiments described and from Chang's work with the injection of tryptophane into animals on a bread and milk diet it would certainly appear that an excessive supply of tryptophane does not necessarily cause the thyroid/

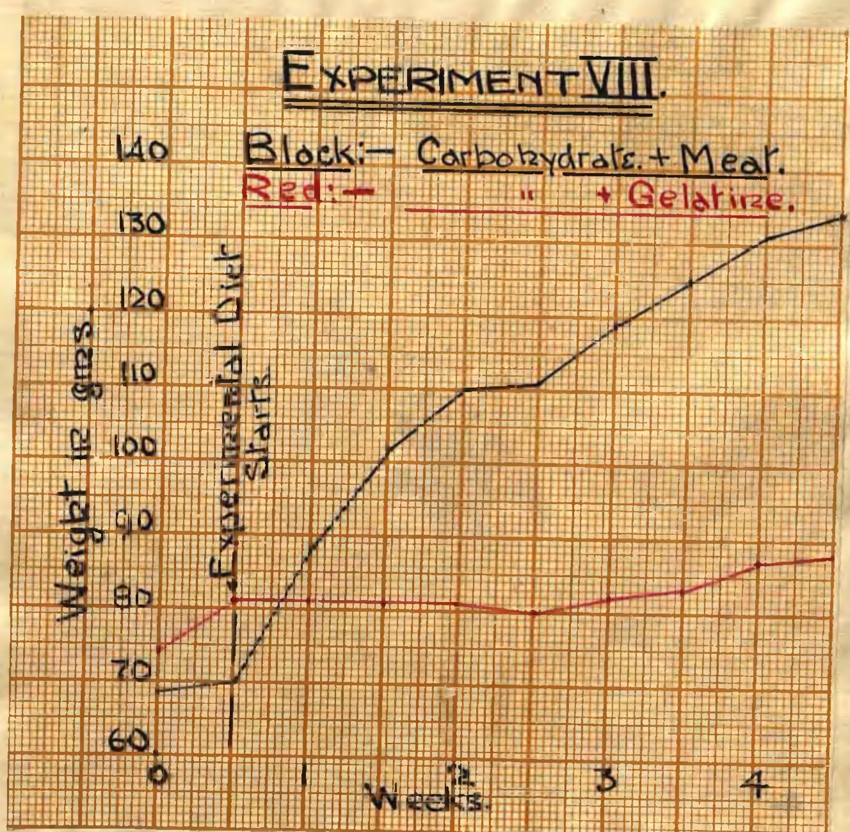
thyroid to form more secretion. It seems possible therefore that although neither tryptophane nor the meat extractives independently cause increase in the colloid content of the thyroid gland they may act together by helping to make up a properly balanced diet and so produce a better state of nutrition. It may be on account of this that the animals which Chang fed on a gelatin diet plus tryptophane showed normal glands. Meat, when added to a defective diet such as Diet B may make up an adequate diet for rats and so may account for the appearance of colloid in the thyroids of the meat-fed animals.

Attention may be directed at this point to the influence of the various diets given in these experiments on the growth of the animals. It will be noted (see Table VII) that meat caused a remarkable increase in the rate of growth compared to that of the controls (Graphs 3 and 4). Cheese had a much less pronounced effect (Graph 5), while the rats receiving tryptophane grew no better than the control.

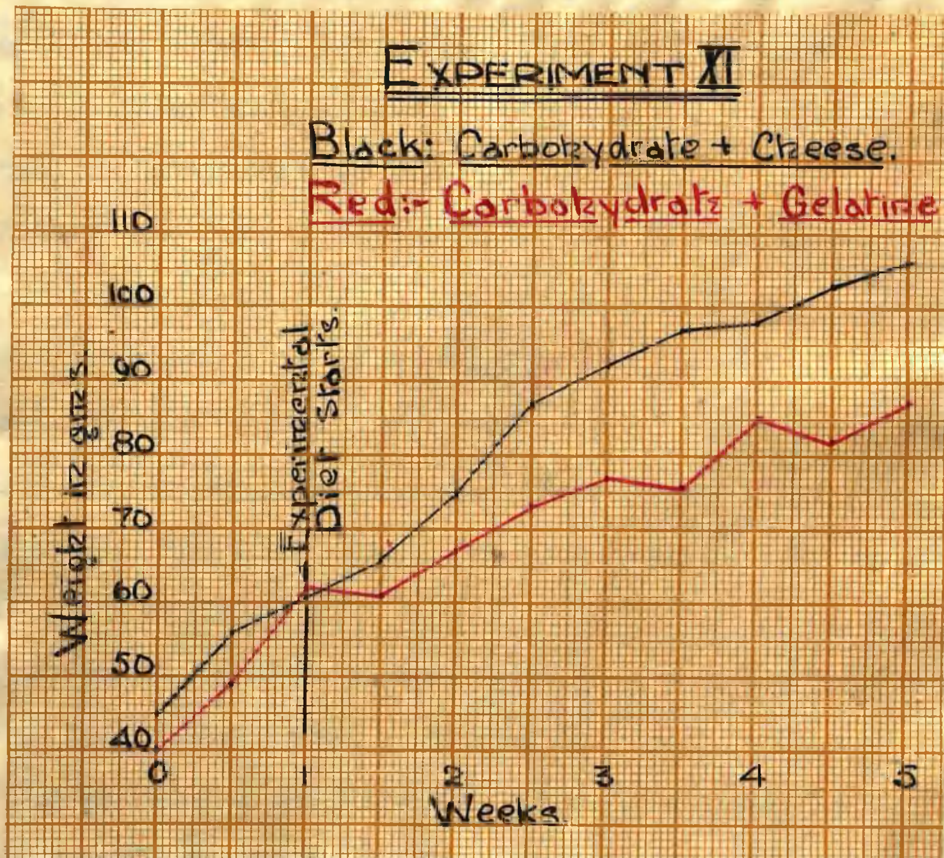
The effect of meat therefore was to improve the rate of growth and the nutritive condition of the animal and to cause the appearance of colloid in the thyroid. The other diets which may be regarded as defective in one way or another were, on the other hand, associated with a poorer condition of the animal and a colloid-free state of the thyroid. It is conceivable, therefore, that the colloid/


EXPERIMENT VI
Black:- Meat Diet.
Red:- Carbohydrate. Diet.
Weight in gms.
Experimental Diet Starts.
150
140
130
120
110
100
90
0
1
2
3
4
Weeks.

Graph 3.


EXPERIMENT VIII.
Black:– Carbohydrate + Meat.
Red:– " + Gelatine.
Weight in gms.
Experimental Diet Starts.
140
130
120
110
100
90
80
70
60
0
1
2
3
4
Weeks.

Graph 4.


EXPERIMENT XI
Black: Carbohydrate + Cheese.
Red:- Carbohydrate + Gelatine
Weight in gms.
110
100
90
80
70
60
50
40
Experimental Diet Starts.
0
1
2
3
4
5
Weeks

Graph 5.

colloid content of the thyroid gland is influenced by diet only so far as that affects the general nutritive condition of the animal.

Douglas (37) was of the opinion that variation in the appearance of the thyroid in animals receiving similar diets and kept under similar conditions was due to the state of nutrition. Chang came to similar conclusions and considered that the lack of colloid in the thyroid described by Cramer in tryptophane deficiency was due to inanition, An enormous proportion of stray dogs, which one may assume to be in a poor state of nutrition, were found by Marine (48) to have thyroids deficient in colloid. Hammet (40), however, believed that the improved nutritive condition was due to a more efficient thyroid.

The experiments in this series seem to present evidence in favour of the former view. It has been seen that cod liver oil may produce a colloid-rich thyroid without improving the rate of growth of the animal. A meat diet on the other hand stimulates growth and is also associated with a colloid-rich gland. One finds, therefore, that by assisting the formation of colloid by administering cod liver oil which appears to have a specific action in this connection, one cannot improve the state of nutrition as reckoned by the rate of growth. However, when an adequate diet is given the nutritional state of the animal is improved/

improved and incidentally the thyroid assumes the normal appearance.

Much work has been done to show that the thyroid possesses some action in stimulating growth. Vincent and Jolly (69) found that thyroidectomy interfered very strikingly with growth, and this has been confirmed by Bassinger (23). Simpson (61) states that age is an important factor with regard to the effects of removal of the thyroid. The stunted growth of the cretin, supposed by McCarrison (54) to be due to the influence of toxic agents on the developing thyroid of the unborn child, would seem to demonstrate the result of deficient thyroid secretion. Further evidence that the thyroid plays some part in stimulating growth is seen from the work of Carrel (31) who found that tissues grown in vitro increased several times as rapidly in the presence of thyroid substance than in its absence.

Nevertheless, in these experiments it appears that an adequate diet affording a good nutritional state and a steady gain in weight is associated with a thyroid in a more or less normal condition, while the converse does not seem to hold. It may not be legitimate to argue from an histological standpoint on such a question, but if we may, then it would appear that these experiments do not bear out the contention that the thyroid stimulates growth./

growth in animals. As is well known the thyroid gland exercises a profound influence on metabolism, and it may be that thyroidectomy interferes with growth by causing an upset of that process.

Conclusions.

1. Meat appesrs to have the effect of increasing the amount of colloid in the thyroid and of stimulating the rate of growth.
2. The addition of tryptophane to a diet deficient in that substance such as Diet B does not cause any change in the appearance of the thyroid or in the rate of growth.
3. The influence of meat does not seem to be due to its content either of tryptophane or of its extractives independently.
4. It seems possible that the thyroid is influenced by diet only so far as the latter affects the nutrition of the animal.
5. The reported action of the thyroid in stimulating growth is questioned, and the possibility presents itself that the interference with growth following thyroidectomy may be due to metabolic disturbance.

--------oOo---------

the thyroid showed a normal appearance, many were associated with a hyperplastic state of the gland. This hyperplasia was in some cases acute or "complete", in others chronic or colloid. Lane (73) stated that in chronic intestinal stasis the thyroid was at first hypertrophied and later became atrophied.

In his work on experimental goitre in rats, McCarrison(75) found that keeping animals in dirty, infected cages produced an acute hyperplastic condition of the thyroid. He observed the same appearances after subcutaneous injection of bacterial and other toxins. He has more recently (76) confirmed the former observation in pigeons, and found that the hyperplasia could be prevented by scrupulous cleanliness.

Sorour (84) described the thyroids of rats which he had fed on various diets which were supposed to cause rickets. He found that some, particularly those taken from rats kept in darkness, showed hypertrophy and microscopical appearances similar to those seen in exophthalmic goitre.

In 1923 I (78) published a report on the condition of the thyroid in young dogs suffering from experimental rickets. A brief resume of this work, which seems to bear out the findings of the above mentioned workers, is given below.

Description of Experiments.

The following experiments were carried out by Noël Paton and Watson in an investigation into the aetiology of rickets, and most of them have been published (Noël Paton, Findlay and Watson (80): Noël Paton and Watson (81,82). I was afforded the opportunity of examining the thyroids of all the dogs used in these experiments. The results together with a report on the condition of the thyroids are summarised in Table VIII.

The object of Experiments I and II was to demonstrate the effect of confinement in the laboratory in contrast to controls of the same litters kept in the country. The latter were normal, while the others developed rickets, although those pups which were allowed to run free in the laboratory were affected in a milder degree than those confined in cages.

Experiments VII and VIII were performed to discover what effect a diet poor in milk fat had on the occurrence of rickets. Only one of the series showed any sign of a rachitic condition, and that to a very mild degree.

Experiment XVII was carried out to discover whether olive oil played any part in the production of experimental rickets, while the influence of subdued light was investigated in Experiment XIX. In these experiments three pups developed rickets. Two of these received cod liver oil

and/

TABLE VIII.

Experiment.	Dog.	Thyroid.	Condition of pup.	Remarks.
I	D2	Normal	Normal	Kept in country.
	D3	Acute hyperplasia.	Rickets.	Confined in cage in laboratory.
	B1	Normal	Normal	Kept in country.
	B2	Commencing hyperplasia.	"	Kept in country for fortnight, then in laboratory for 3 weeks.
	B3	Acute hyperplasia.	Rickets.	Confined in cage in laboratory.
	B4	" (slight)	"	Running free in laboratory.
	B5	Acute hyperplasia.	"	Confined in cage in laboratory.
II	D1	Normal	Normal	Kept in country.
	D2	Acute hyperplasia	Rickets.	Confined in cage in laboratory.
	B1	"	"	" " " "
	B2	Normal	Normal	Kept in country.
	B4	Acute hyperplasia.	Rickets.	Confined in cage in laboratory.
	B6	" (slight)	"	Running free in laboratory.
	B7	ditto.	"	" " " "
VII.	1	Normal	Normal	-
	2	"	"	-
	3	Normal	Slight rickets.	-
	4	"	Normal	-
	5	"	"	-
	7	"	"	-
VIII.	2	"	"	-
	3	"	"	-
	4	"	"	-
	5	"	"	-
	6	"	"	-
XVI	1	"	"	-
XVI	5	"	Rickets (healing)	Diet included olive oil.
	6	"	Rickets (healed)	Diet included olive oil, then cod liver oil.
	8	"	Normal	Diet included olive oil.
XVIII.	1	Chronic hyperplasia.	"	-
	2	"	Rickets from Ca starvation.	Ca deficient diet.
	3	"	Normal	-
	4	"	Rickets from Ca starvation.	Ca deficient diet.
XIX	1	Normal	Rickets (healed)	Subdued light. Cod liver oil after rickets developed.
	4	"	Normal	Subdued light.
XX	1	Chronic hyperplasia.	Rickets.	-
	2	"	"	Olive oil added to diet.
	3	Acute hyperplasia.	"	" " " " "
XXI.	1	"	"	Ca free diet.
	2	"	"	Calcium lactate added to diet.
	3	"	"	Calcium sulphate added to diet.
	4	"	"	Ca free diet.
	5	"	"	Calcium carbonate added to diet.

and recovered completely while the other improved spontaneously to such an extent that from the histological report on the costo-chondral junctions it could not be stated definitely that rickets was present. Experiment XX was a repetition of Experiment XVII, but here all the pups showed signs of rickets, although pups 1 and 2 remained healthy till almost the end of the experiment, while pup 3 developed the condition early, and was so ill that it had to be killed three weeks before the others.

The effect of calcium starvation was investigated in Experiments XVIII and XXI. Two pups in the former experiment on a Ca-deficient diet developed a rachitic condition, which, however, should perhaps not be looked upon as true rickets. In accordance with Korenchevsky's (72) suggestion, this condition has been noted in Table VIII as "rickets from Ca Starvation." In Experiment XXI all the pups became very rickety, although three of them were receiving calcium salts in their diet. Their condition may be regarded as true experimental rickets which abundance of calcium was unable to prevent.

Histological Examination.

The thyroids from these experiments fall into three groups, according to their microscopical appearances:- normal, acute hyperplasia, and chronic hyperplasia. These terms have been adopted from Farrant's paper already quoted, as the appearances which he describes under these names agree closely with those found in the pups.

The normal glands were made up of vesicles filled with colloid and lined with low cubical epithelium. (Plate 17).

Acute or complete hyperplasia (Plate 18). Such vesicles as were discernible were empty of colloid and very irregular in outline. Between these was a mass of cells, formed probably of collapsed vesicles. Cells lining the vesicles were definitely columnar in shape.

Some thyroids showed changes similar to that of acute hyperplasia but to a milder degree. The vesicles were small and empty of colloid. The cells composing the walls were cubical (Plate 19). In Table VIII these glands have been described as showing "acute hyperplasia - slight." They were all taken from pups which were allowed to run free in the laboratory, and which, although showing signs of rickets, were less affected than those confined.

Chronic or colloid hyperplasia (Plate 20). The vesicles were well filled with colloid which, however, contained numerous vacuoles. Many of the vesicles were of irregular shape/

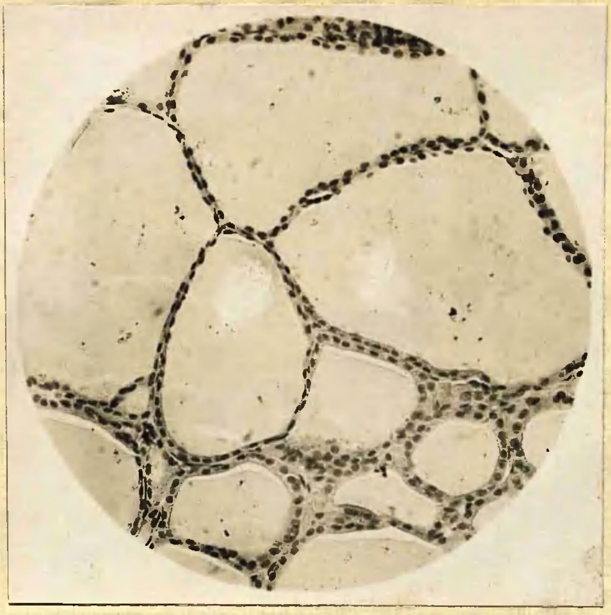


PLATE 17.

Normal thyroid of dog.
x 300.

PLATE 18.

Thyroid of dog, showing acute hyperplasia.
x 300.

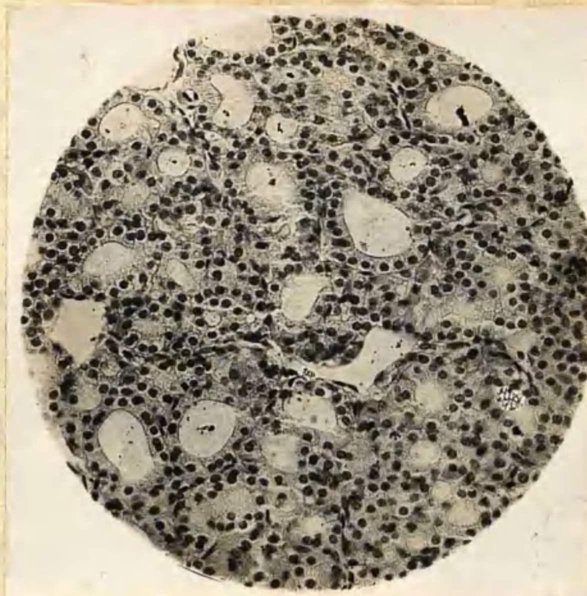


PLATE 19.

Thyroid of dog, showing acute
hyperplasia of a milder degree.
x 300.

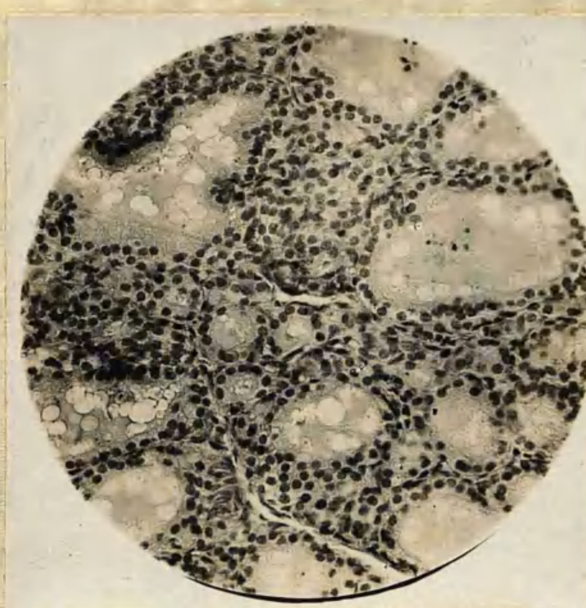


PLATE 20.

Thyroid of dog showing "colloid"
hyperplasia.
x 300.

shape. The walls appeared in parts to be made up of more than the normal single layer of cells, and showed numerous invaginations into the lumen. The cells were cubical in shape.

This condition of the thyroid would appear to be the mildest form of hyperplastic change. The glands of pups 1 and 2 of Experiment XX were in this state of colloid hyperplasia, and it is to be noted that these animals had very recently developed rickets. The other animal in this experiment had suffered from rickets for some time, and its thyroid showed acute hyperplasia.

Discussion of Results.

It will be seen from Table VIII that every pup which was suffering from rickets had a thyroid showing marked changes, while, in those animals which remained healthy, that gland remained normal. It appears, therefore, that in the diseased condition known as experimental rickets the thyroid gland presents an appearance markedly different from the normal being in a state of hyperplasia. This is confirmatory of the work of Farrant and the other workers mentioned above on the condition of that gland in other diseases. One may conclude, therefore, that in certain disease conditions of the body the thyroid becomes empty of colloid.

In/

In many diseases the rate of metabolism is greatly increased. It may be that the production of this increase is associated with a depleted condition of the thyroid, since, apparently, the secretion of that gland is closely related to the rate of metabolism.

It has been pointed out in a previous part of this paper that both iodine and cod liver oil appear to exert an influence on the thyroid, causing the formation of colloid in its vesicles. Now these substances are of great value in therapeutics and the possibility arises that they may bring about the beneficial action, which they are known to possess on various diseases, by assisting the thyroid gland to regain its normal state, and presumably to reinforce its functional activity.

Llewellyn (74) has stated that the administration of iodine, or thyroid extract, in cases of rheumatoid arthritis may help the condition greatly, and he suggested that its action was due to its power to relieve a state of subthyroidism which favoured the development of arthritis. Nott (79) successfully treated various various conditions with small doses of thyroid extract together with lavage of the colon with a solution of potassium permanganate. I have tried this treatment on children suffering from acute rickets. The rectal injection of the permanganate solution, however, proved an almost insuperable difficulty for most dispensary/

dispensary patients. In one case, however, when the instructions were carefully carried out, a child which had had acute rickets was greatly improved. After treatment for a fortnight the sweating ceased and the child who had been losing weight, gained one pound.

These facts seem to show that the condition of the patient suffering from certain diseases, apart from diseases of the thyroid gland itself, is improved by the administration of substances which are known to influence the thyroid in the formation of colloid. This bears out the view expressed above that iodine and cod liver oil may in some cases exert their therapeutic action through the medium of the thyroid gland.

Conclusions.

1. Experimental rickets in young dogs is associated with hyperplasia of the thyroid gland.
2. It seems possible that the beneficial action of iodine and cod liver oil in certain diseases may be effected through their influence on the thyroid gland.

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