EFFECT OF PROLONGED ADMINISTRATION OF ACID EXTRACT OF ANTERIOR PITUITARY ON THE THYROID GLAND OF GUINEA-PIGS

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(With Special Plate)

Aron,¹ Loeb and Friedman,² and other investigators^{3,4} have shown that the initial hyperplasia of the thyroid gland produced in animals by the administration of the thyrotropic hormone of the anterior pituitary regresses and involutes to the colloid state in spite of continued treatment. Similarly, the basal metabolism, after an initial rise to a maximum on the seventh to the fourteenth day, falls to normal or below normal (Siebert and Smith,⁵ Anderson and Collip,⁶ Friedgood⁷). The mechanism whereby this regression in thyroid activity is brought about is unknown. It has been suggested by Friedgood⁷ and by Hertz and Kranes⁸ that it may be the result of thyroid exhaustion. The present experiments were undertaken to verify previous observations and to determine whether any explanation for the thyroid involution could be found.

Experimental Procedure

The extract of anterior pituitary was prepared after the method of Loeb and Bassett.⁹ Under sterile conditions 5 grams of Armour's undegreased anterior pituitary powder were extracted with 100 c.cm. of 0.5 per cent. acetic acid for twenty-four hours at 0° C. The mixture was then centrifuged, the supernatant fluid poured off, neutralized to pH 7.8 (phenol red), and again centrifuged. The extract was prepared weekly, and, when stored at a temperature of 0° C., could be safely injected without causing sepsis. Intraperitoneal injections in daily doses of 1 c.cm. (equivalent to approximately 40 units of thyrotropic hormone) into guinea-pigs weighing about 300 grams produced in three days thyroids which were considerably enlarged, very vascular, and markedly hyperplastic (Fig. 2).

Preliminary experiments on rabbits showed that they are much less reactive to the thyrotropic hormone than guinea-pigs. Four prepubertal rabbits, each weighing about 1,500 grams and maintained on a non-goitrogenic diet of whole oats, alfalfa hay, and distilled water, received subcutaneously 4 c.cm. of the extract daily for six days. On the seventh day the thyroids were inspected surgically, and were found to be slightly enlarged and moderately vascular. The injections were continued for a further nine days and the thyroids again inspected. The glands had now diminished in size and their vascularity had considerably decreased.

Since a greater thyroid response may be obtained in guinea-pigs we chose these as experimental animals. Six males and eight females, each weighing between 300 and 400 grams, and receiving a non-goitrogenic diet containing fresh lettuce three times weekly, were given daily intraperitoneal injections of 1 c.cm., and later 2 c.cm., of the extract for varying periods up to ninety-five days. Three males and three females of approximately the same age and weight as the experimental animals were used

as controls. The guinea-pigs which received thyrotropic hormone were weighed daily, the controls twice weekly.

Results

All the animals which were injected showed an initial loss of weight. It usually fell rapidly during the first week, and then returned to the original level by the end of the second week. Thereafter it rose steadily throughout the experiment. The control animals showed a progressive increase (see Chart). Two animals, however, lost weight progressively and died, one on the twelfth day and the other on the seventeenth. On the twelfth day the latter developed exophthalmos. At necropsy no cause for death could be found. In the one which died on the twelfth day the thyroid was extremely hyperplastic and the heart dilated. In the other, which was exophthalmic, the thyroid was slightly enlarged, but pale and translucent; microscopically there was slight hyperplasia, and colloid was beginning to accumulate in the vesicles. In both the suprarenals were enlarged and very hyperaemic.

Showing the weight curve of a representative guinea-pig receiving daily injections of anterior pituitary extract.

Exophthalmos also developed in two other animals, on the fourteenth and twenty-first days respectively, and persisted throughout the experiment (Figs. 5 and 6).

The remaining animals were killed at varying intervals. The thyroid glands of the controls ranged from 37 to 92 mg. in weight, and were histologically normal (Fig. 1). Of the twelve experimental animals the thyroid weights varied from 54 to 320 mg., and differed considerably in macroscopical and microscopical appearances. In four the thyroids were extremely hyperaemic and hyperplastic, and showed proliferative budding into the lumen of the vesicles (Fig. 3); in two the hyperplasia was less marked, and pale-staining colloid was accumulating in the vesicles. In the remaining six the glands were either pale and translucent or only slightly hyperaemic; histologically they had the characteristics of colloid glands, the vesicles being filled with well-staining colloid and the epithelium cubical or flattened (Fig. 4 and Table). In none of the thyroids was there evidence of exhaustion. The degree of thyroid involution appeared to bear no relation to the duration of treatment, neither was there any difference in the clinical appearance or behaviour of the animals which would make it possible to distinguish which animals still had hyperplastic glands. All were gaining in weight after the first week at a rate comparable with the controls.

The suprarenal glands showed marked changes. In the controls they were pale; in all the animals which received anterior pituitary extract they were enlarged and hyper-

aemic. The enlargement was greatest in those whose thyroid glands had involuted to the colloid state (see Table).

Effect of Prolonged Administration of Acid Extract of Anterior Pituitary on the Thyroid and Suprarenal Glands of Guinea-pigs

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No. of Animal	Sex	Initial Weight (grams)	Duration of Experiment (days)	Final Weight (grams)	Condition of Thyroid		Weight of Suprarenals (mg.)
					Weight (mg.)	Histology	
A.—Animals receiving anterior pituitary extract							
30	F	343	94	597	115	Colloid—normal	571
31	F	370	93	407	224	Moderate — marked hyperplasia	456
32*	F	345	17 (died)	260	81	Early—moderate hyperplasia	422
33	F	395	12 (died)	224	121	Marked hyperplasia	299
34*	F	365	95	630	60	Colloid—normal	480
35	F	390	93	700	88	Colloid—normal	515
36*	M	372	87	515	320	Marked hyperplasia	413
37	M	377	41	370	54	Colloid—normal	681
38	M	373	87	490	230	Marked hyperplasia	345
39	M	397	87	556	170	Early—moderate hyperplasia	519
40	M	335	86	460	75	Colloid	725
41	M	370	87	515	280	Moderate — marked hyperplasia	346
69	F	276	73	335	80	Early—moderate hyperplasia	455
70	F	336	73	490	140	Colloid—normal	290
B.—Controls receiving no extract							
42	M	315	92	625	92	Normal	374
44	M	340	80	550	80	Normal	300
45	F	285	62	553	62	Normal	406
46	F	356	60	690	60	Normal	260
71	M	330	37	500	37	? Early hyperplasia	211
72	F	325	60	520	60	Normal	314

* 32: Exophthalmos on the 15th day. 34: Exophthalmos on the 14th day. 36: Exophthalmos on the 21st day.

Discussion

Our results show that although the majority of the thyroids of animals receiving thyrotropic hormone over a prolonged period involute to the colloid phase, a certain percentage do not, but continue to be hyperplastic. It is unlikely that these colloid glands failed to become hyperplastic in the first place, since 100 per cent. of 150 of our guinea-pigs which have received treatment for various purposes with anterior pituitary extracts have shown marked thyroid hyperplasia.

It has been suggested, notably by Friedgood⁷ and by Hertz and Krane,⁸ that the regression of the hyperplasia is evidence of an exhaustion phenomenon in the thyroid as a result of the prolonged administration of the thyrotropic hormone. It must be stressed here that a colloid gland is not evidence of exhaustion, but is, as Marine¹⁰ has repeatedly pointed out, the nearest approach to a normal gland that a thyroid which has been previously hyperplastic can attain. Involution to the colloid phase is evidence that the thyroid is no longer working under strain. On the other hand,

"exhaustion is believed to result from sustained hyperactivity without periods of physical rest. . . . Microscopically the colloid is practically absent. The epithelial cells are in the early stages high columnar, with infoldings and plications as in the active hyperplasia. Later the cells lose their uniform and regular shape and size. . . . There is often some piling up of the epithelial cells. The nuclei are in general large, often hyperchromatic, sometimes small and pyknotic, and very irregular in size and shape. . . . The stroma is relatively, perhaps absolutely, increased, and as the follicles become smaller, due to the loss of their secreting epithelium, the fibrous bands become wider, and give to the gland the appearance of a diffuse fibrosis or cirrhosis, in

which lie the nests of distorted and degenerated cells of the former follicles" (Marine¹¹).

The four glands in the present series which remained hyperplastic would be the most likely to show these changes, but in none of these were there any signs of exhaustion atrophy.

It can be safely concluded, therefore, that the gain in weight after the initial fall which was seen in our animals and the decrease in basal metabolism following the preliminary rise, which has been observed by other investigators to run more or less parallel with the thyroid involution, cannot be attributed to thyroid exhaustion. Some other explanation has to be sought.

Upper: showing the thyroid enlargement (weight 280 mg.) in guinea-pig No. 41, which received anterior pituitary extract daily for eighty-seven days. Lower: Thyroid gland of normal control.

Marine and Baumann^{12, 13} showed that a sufficient but sublethal injury to the suprarenal glands of rabbits produced an increase in basal metabolism, which began from the third to the sixth day, and lasted from a week to several months. This was accompanied by definite hyperplasia of the thyroid.¹³ The diminution in the size of the suprarenal cortex which occurs in infants shortly after birth was shown to be associated with a rise in metabolism.¹⁴ It is significant that in the present experiment all the animals receiving anterior pituitary extract showed marked hyperaemia and enlargement of the suprarenal glands, and that this enlargement was greatest in those animals whose thyroids had involuted. It seems probable that the suprarenals exert some control over thyroid function, since their enlargement is associated with a regression, whereas their partial removal causes an increase of thyroid activity.

The suprarenal glands appear to be dependent on the anterior lobe of the pituitary. Removal of the pituitary results in atrophy of the cortex.¹⁵ Evans *et al.*,¹⁶ and later Collip, Anderson, and Thomson,¹⁷ have shown, that anterior pituitary extracts which contain the thyrotropic hormone also contain an adrenotropic principle. This adrenotropic factor was in all probability partly responsible for the suprarenal hypertrophy seen in our experiments. In addition, however, the suprarenals may also have been stimulated by an adrenotropic factor elaborated by the animal's own pituitary in response to the increased thyroid secretion produced by the administration of the thyrotropic hormone. Lastly, it is possible that an excess of thyroid secretion may directly cause suprarenal hypertrophy. This question is not yet settled; experiments are being conducted on these lines.

The demonstration by Collip and Anderson¹⁸ of the presence of an antithyrotropic principle in the serum of animals treated for a prolonged period with anterior pituitary extract containing the thyrotropic hormone is interesting. We have recently confirmed this. A rabbit, aged 8 months, was given subcutaneously 8 c.cm. of anterior pituitary extract daily for six weeks. At the

end of this period the daily subcutaneous injection of 2 c.cm. of its serum prevented the occurrence of thyroid hyperplasia in guinea-pigs receiving at the same time daily intraperitoneal injections of 1 c.cm. of anterior pituitary extract. The formation of antithyrotropic principle is probably responsible for the regression of thyroid hyperplasia in animals which have received prolonged treatment with thyrotropic hormone. It is inconceivable that an antibody be formed to a hormone, as this would render it quite useless to the organism. The finding suggests that there exists normally a balance between thyrotropic and antithyrotropic principles. If large doses of the one be given, the other is elaborated in an endeavour to bring about hormonal equilibrium. Whether this antithyrotropic principle is produced by the suprarenal cortex in response to stimulation by adrenotropic hormone also contained in the extract is as yet unknown, but, if this be so, then the adenotropic hormone may be of value in the treatment of Graves's disease.

Summary

1. Thyroid hyperplasia, exophthalmos, and suprarenal enlargement have been produced in guinea-pigs by the prolonged administration of acid extract of anterior pituitary substance.

2. Although the majority of the thyroid glands had involuted to the colloid state during treatment, a certain percentage remained hyperplastic even after ninety-three days.

3. The suprarenal enlargement was greatest in those animals whose thyroids had involuted.

4. No evidence of thyroid exhaustion was observed.

5. The production by Collip and Anderson of an antithyrotropic factor in the serum of an animal treated for a prolonged period with thyrotropic hormone has been confirmed.

6. The mechanism responsible for the thyroid involution is discussed.

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The professorial chairs which the French Ministry of Education recently had to abolish on the grounds of economy include those of pathology and medico-surgical anatomy in Paris, therapeutics and external pathology at Lyons, medical physics at Nancy, operative medicine, physiology, and clinical obstetrics at Toulouse, physiology and clinical surgery at Bordeaux, pharmacy and operative medicine at Lille, and natural history and parasitology at Montpellier.

A NOTE ON THE X-RAY EXAMINATION OF EMPYEMA CAVITIES

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(With Special Plate)

One of the main difficulties in the treatment of empyema is to determine the proper time at which to cease draining the cavity. At present the indications usually given for this turn upon two factors. The first is the period which has elapsed since drainage started. The time advised for drainage varies with different authors; a very widespread tradition puts it at three weeks, but it is often amazingly short, of the order of a week or so. The second indication is the character of the discharge, the tube being removed when it becomes scanty, watery in consistence, or sterile upon bacteriological examination.

Now it is to be noted that neither of these indications takes any account of the physical conditions, the size of the cavity, and the firmness of its walls. There is nothing in them to ensure against the closing up of a considerable hollow filled with varying proportions of gas and fluid. If this should occur, as it not infrequently does, the contents may fail to absorb, the pleura thickens and stiffens in its characteristic fashion, and the final obliteration of the cavity becomes extremely difficult. A persisting empyema is nearly always one in which drainage has been stopped too early and this thickening process allowed to occur.

I came to the conclusion some time ago¹ that the process of cure in all empyemata was the growing together of the walls of the abscess, with the complete permanent obliteration of the part of the pleural cavity concerned (an interesting contrast with the frequent dissolving of apparently firm adhesions of the peritoneum). In consequence, the only logical indication for ceasing drainage appeared to me to be completion of this process; otherwise it was impossible to foretell a result controlled by the unknown factors of the size of the cavity, the thickness of its walls, the nature of its contents, and the absorptive power (never very high) of the pleura. To work in this way means, of course, a renunciation of impressively quick cures, those in which drainage is successfully stopped in a week or so. I have had my share of these, but I have found their absence a small price to pay for freedom from secondary operations on those cases in which the accumulation of pus persisted.

Procedure Adopted

My first method of judging the process of healing was the crude but quite effective one of measuring the amount of fluid that the cavity would hold, running it in through the intercostal de Pezzer catheter which I employed for drainage. To the many who did not agree with me, however, this was not at all convincing, so that I sought for some more demonstrable evidence. One of the most obvious means was x rays, but their employment to define the cavity was not easy. Barium paste is difficult to get in and more difficult to get out; lipiodol is expensive, and, being a very viscous oil, breaks when in thin layers into globules that look like a charge of shot instead of giving a clean outline; and sodium iodide is liable to cause poisoning if used on an absorptive granulating surface.

In this difficulty I turned, as often before, to the head pharmacologist at the Hospital for Sick Children, Mr. Wycliffe Peck. After many experiments he succeeded in producing a cheap, sterile, stable, and very fluid

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FIG. 1.—Photomicrograph of section of normal thyroid (guinea-pig).

FIG. 2.—Thyroid hyperplasia produced by daily injection of acid extract of anterior pituitary for 3 days (guinea-pig).

FIG. 3.—Marked hyperplasia of the thyroid of a guinea-pig which received daily injections of anterior pituitary extract for 87 days.

FIG. 4.—Involution of the thyroid to the colloid state in a guinea-pig which received daily injections of anterior pituitary extract for 86 days.

FIGS. 5 AND 6.—Exophthalmos produced by anterior pituitary extract.

FIGS. 7 AND 8.—Normal control guinea-pigs.