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THYROID STUDIES

II. CHANGES IN THE THYROID GLAND PRODUCED BY FECAL EXTRACTS

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Remedi (1), using separately diphtheria and tetanus toxin injections directly into the thyroid gland, demonstrated changes in its structure. Farrant (2) injected various toxins intraperitoneally including both tetanus and diphtheria toxins. He found that after administering diphtheria toxin a hyperplasia always resulted. After further investigation (3), including the histological examination of 700 thyroid glands, part of them derived from humans and part from experimental animals, he has classified a great number of diseases as to the degree to which they affect the thyroid. As a result of experimental work he also concludes that it is the toxin from the microörganism and not the microörganisms themselves which produce this change. Rogers and Garnier (4) obtained changes in the thyroid following infections. McCarrison (5) reported his exhaustive work showing that the feeding of fecal extracts and residues to rats produced thyroid changes. He (6) further states that he found a strong tendency for the enlargement of the thyroid in animals which drank largely of water contaminated by feces. This work was carried out on rats, goats and humans by the administration of the sediment of water that contained excreta and waste material from goiter districts. These enlargements so produced disappeared if the water containing these sediments was boiled. Intestinal antiseptics also proved of value in treating goiter in these regions, according to this author. Later McCarrison (7) found that rheumatism, rheumatic arthritis and malaria are repeatedly accompanied by thyroid changes. Burget (8) produced thyroid hyperplasia in rats kept under unhygienic conditions. Marine and Lenhart (9) found goiter in fish which were kept in contaminated water. The condition automatically disappeared after proper sanitary procedure. Bircher (10) fed feces with cooked rice to rats and obtained definite thyroid changes. Hart and Steenbock (11) fed normal sows a diet contain-

ing no roughage, thereby producing poorer elimination and increasing intestinal putrefaction. Congenital goiter appeared in all of the offspring.

On the other hand Basinger (12), repeating the work of Remedi, found no evidence from which to infer that diphtheria or tetanus toxins produced thyroid changes. Burget (8), using cats and giving the emulsion of feces from both goitrous and non-goitrous individuals by stomach tube, found no change in the thyroid. Whipple and Dragstedt (14), (15) both have shown that very little, if any, fecal toxin is absorbed from the normal digestive tract. Kalkus (16) showed that when no iodine deficiency was present, goiter never developed in his animals. Marine, Kimball, Lenhardt and Rogoff (17) have prevented the occurrence of goiter in school children by administering minimal amounts of iodine. Thus it is seen that the conflicting evidence so far reported leads to no definite conclusion.

In the light of such an existing controversy, it seemed to us that perhaps some additional information might be obtained by the further use of certain toxins.

In these experiments rabbits were used as the experimental animals. They were kept in clean cages. Both experimental and control animals were kept at the same room temperature since Mills has shown that extreme limits may affect the thyroid. The amount of light was also roughly controlled (13). All animals had practically the same diet and were governed according to ordinary laboratory methods. Lastly, we were very careful to keep all iodine from the room in which the animals were housed.

The toxin used was extracted from rabbit feces with physiological saline. This fecal extract was prepared fresh daily. Fresh feces were used in order to keep the concentration of the extract as constant as possible. A known amount was taken and ground in a small meat grinder. Four times this quantity of normal saline was added and the mixture allowed to macerate twenty-four hours. It was then run through gauze, filter paper and lastly the Berkefeld filter to insure a bacteria-free preparation. Absolute sterility was not maintained throughout as we found that by using fresh solutions and thoroughly washing the final container each day no infections resulted. An extract so prepared produced death in rabbits when 10 to 12 cc. per pound of body weight were injected intraperitoneally. The possibility that death might have been produced by shock was eliminated by a similar administration of the same quantity of normal saline in control rabbits.

Most animals used in series I and II were females of approximately the same weight. In the first and second series of animals a partial thyroidectomy (one lobe) was performed before any toxin was administered. After weighing, the tissue was fixed in Orth's fluid. Sufficient time was always

allowed for recovery of the animal before any experimental procedure was imposed. Each rabbit was weighed twice a week. The belly of each was shaved and injections made six days a week. The first injection was 10 cc. of toxin, this being increased by 10 cc. on each succeeding day until the dosage reached 40 cc., which dose was maintained. This amount was established as the approximate maximal sublethal dose, by previous experiments. An autopsy was performed on the animals after death. The remaining lobe of the thyroid was removed, weighed and placed in

TABLE 1

GROUP	RABBIT	SEX	DAYS	AMOUNT OF TOXIN	DOSES	AVERAGE DOSE	WEIGHT		WEIGHT THYROID		GENERAL APPEARANCE OF GLAND
							Lost	Gain	First lobe	Second lobe	
				cc.		*cc.*	*lbs.*	*lbs.*	*mgm.*	*mgm.*	
I	61	F	2	10	1	10	¼		95	100	Mild hyperplasia
	71	F	3	30	2	15	¾		145	170	Mild hyperplasia
	66	F	4	60	3	20	1		340	275	Mild hyperplasia
	69	F	30	Control rabbit			0	0	50	55	Normal
II	68	F	8	180	6	30	¾		175	270	Hyperplasia
	60	F	8	180	6	30	½		?	?	Hyperplasia
	65	F	16	500	14	35	1¼		105	105	Marked hyperplasia
	67	F	30	1020	26	39	2¼		150	140	Marked hyperplasia
	34	F	30	Control rabbit				½	60	75	Normal
III	1	F	77	308	46	6	¼				Mild hyperplasia
	3	F	75	333	46	7	¾				Mild hyperplasia
	4	M	72	420	46	9	0	0			Mild hyperplasia
	2	F	77	Control rabbit*			1¾				Mild hyperplasia
IV	44	F	57	842	35	24	1¾				Degeneration
	45	F	71	769	45	17		½			Marked hyperplasia
	46	F	71	593	39	15		½			Marked hyperplasia
	55	F	71	523	45	11		1½			Hyperplasia

* Control rabbit 2 became pregnant during the experiment.

the fixing fluid. The tissue was then embedded and sections, 5 microns in thickness, were cut and stained with hematoxylin and erythrosin.

In the third series, the preliminary partial thyroidectomy included but one-half of one lobe. An average dose of 7 cc. of toxin was given daily, with now and then periods of a few days of rest. At the end of the experiment the animals were killed and autopsied. The remaining thryoid tissue was prepared and sectioned.

In the fourth series unoperated animals were injected with moderate doses ranging from 11 to 24 cc. daily, a few rest days being given to insure

complete absorption. After six to eight weeks of experimentation a partial thyroidectomy was performed on each rabbit of this group. But little toxin was given after this operation and the animals were killed within four to six weeks. The thyroid tissues from operation and also from autopsy were prepared for histological examination. Table 1 gives a summary of the experimental conditions.

The animals showed characteristic symptoms following each injection. These were marked if the injections were large and mild or absent if the injections were small. Abdominal distress seemed present. Respirations invariably became greatly increased in rate for a time. Within fifteen to thirty minutes the animals would begin to show signs of depression, appearing to be in a state of shock. Muscular weakness developed, the rabbits being unable to sustain their weight on their legs. The respiratory rate slowed and became subnormal. The animals almost always defecated

TABLE 2

HOUR	TEMPERATURE	
	Sub lethal dose	Lethal dose
1	102.2	103.0
2	99.5	99.8
3	100.1	98.5
4	100.3	97.0
5	99.5	Died
6	97.5	
7	97.6	
8	97.9	
9	98.2	
10	99.0	
24	102.0 Recovery	

and urinated shortly after the injections. The temperature became subnormal following each injection and all animals showed an extremely low temperature before death if a lethal dose was given. (No. 65 had a terminal peritonitis and temperature of 105.4, which was caused by intestinal perforation from hypodermic puncture.) Table 2 shows a characteristic temperature reaction, the normal temperature of rabbits being approximately 102.5°. Three control rabbits (nos. 81, 83, 84), which were given the same amounts of normal saline intraperitoneally, showed none of the above symptoms. All animals except the controls lost weight.

The normal rabbit thyroid is quite uniformly constant in its histological appearance. The cells vary from a flat to a low cuboidal. The nuclei are slightly angular or round, very distinct, and stain moderately deep. The colloid content is moderate in amount and stains rather deeply, and is for the most part uniform. The follicles vary in size from small to

medium, the shape being round or ovoid and the edges are quite constantly regular. Projections into the follicles by epithelial cells are not observed. The intrafollicular cells are not of great quantity. Masses of solid epithelial cells are uncommon but not lacking. The stroma is scant and vacuolization of the colloid is sometimes observed, but not in any large degree.

The specimens of thyroid removed at autopsy from series I showed marked absorption of colloid. The remaining colloid stained poorly, was intensely vacuolated, and somewhat granular in appearance. The acini were unequal in size, some being completely empty. The epithelium was high cuboidal to columnar. The intervesicular tissue was not increased. The blood vessels showed dilatation. Two types of epithelial cells were seen; those with large, clear, granular nuclei and clear cytoplasm, and those with smaller, dark nuclei and granular cytoplasm, staining as normal cells. Processes from the cells lining each acinus seemed to extend into the colloid so that the cell boundaries were indistinguishable in places. A few acini showed definite desquamation of epithelium into the lumen.

The specimens taken from series II presented a similar but more advanced picture. There was a greater degree of intervesicular cell growth and formation of new acini. Epithelial projections into the larger alveoli were abundant. The epithelium was columnar with a more granular and reddish cytoplasm. Both types of nuclei were in abundance, many of the darker staining nuclei having the appearance of mitotic figures. An occasional typical mitotic figure was seen. Polychromatic granules were present in the cytoplasm of many of the lining cells of the acini at the poles of the cells nearest the lumen. Other signs of hyperplasia described in the thyroids of series I were present. An occasional alveolus was seen in which the colloid was completely absorbed, leaving only scattering fragments of cells. Cytolysis was apparent in some of the epithelial cells. Mononuclear phagocytes were seen in the intervesicular spaces.

In the final specimens of thyroid removed from series III, these animals being subjected to small doses over a long period of time, variations from the normal were also observed. The cells appeared more cuboidal, although some flat cells were seen. The colloid seemed to be in less amount and the follicles showed a slight irregularity. They varied in size from small to medium large and in some cases quite a large size was reached. Vacuolization of the colloid was not common. Hyperplasia of the parenchyma was moderate. The interfollicular cells were more prominent than in the normal and in places masses of epithelium could be seen. Slight hypertrophy of the cells was noted. The stroma appeared normal in amount. All changes were moderate in degree.

The first specimens of thyroid tissue examined from series IV were those removed during the height of toxin administration. These sections showed the colloid to be decreased in amount. Vacuolization was common

and in some sections quite pronounced. The follicles varied in size from small to large and were very irregular in appearance. Projections of cells were noted in the acini. The epithelium varied from high cuboidal to columnar showing area of some hyperplasia and in some cases hypertrophy. Areas of masses of cells were seen quite frequently. The interfollicular tissue appeared to be increased. The nuclei for the most part were of one type but in some sections the two types previously described were seen. Examination of the sections made from the tissue removed at autopsy showed a different picture. The colloid was more abundant in amount and vacuolization, while not absent, was decidedly less. The follicles were more regular in appearance and did not vary as much in size. There were but few projections noted in the acini. The cells were cuboidal, varying from high to low. Areas of masses of cells were seen but these were infrequent. Hyperplasia was present in some sections but not pronounced. The interfollicular tissue was increased above the normal amount but less so than in the preceding sections. The nuclei were all of one type, being clear and staining deeply.

The results of these experiments would indicate that thyroid tissue reacts to fecal extract injections. The first reaction seems to be an increased activity. This is manifest in the more acute condition, as is shown in series I and II, by a pronounced colloid absorption with a beginning hyperplasia and in some cases a mild hypertrophy. The process progresses if the intoxication continues or becomes more severe. This is evidenced by the small amount of colloid and the more pronounced hyperplasia shown in the rabbits whose injections covered a longer period of time. The greater the amount of fecal extract given, the greater were the changes observed in the thyroid, providing quantities closely approaching the lethal dose were not given. The results obtained from series IV are quite conclusive. In these animals the thyroid showed decidedly more change during the height of fecal extract injections than they did at the

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Fig. 1. I: Section removed from rabbit 45 during the period of injections. Note colloid absorption, moderate hypertrophy and hyperplasia.

II: Section removed from rabbit 45 about three weeks following section in I. Note decrease in colloid, marked hypertrophy of cells and variation of follicles.

III: Normal thyroid section from rabbit 71.

IV: Section from rabbit 71 after toxin injections. Duration, three days. Note vacuolization of colloid and two types of nuclei.

V: Normal thyroid section from rabbit 4.

VI: Section from rabbit 4 after toxin injections. This animal had small amounts over a long period of time. Note the increase in cells and the irregularity of follicles. Colloid absorption is noted in one location.

VII: Section from rabbit 67 which was taken following the administration of large doses of toxin over a period of one month. Note degenerative changes present.

Note: The magnification of I, II and VII is the same, of III and IV the same and of V and VI the same.

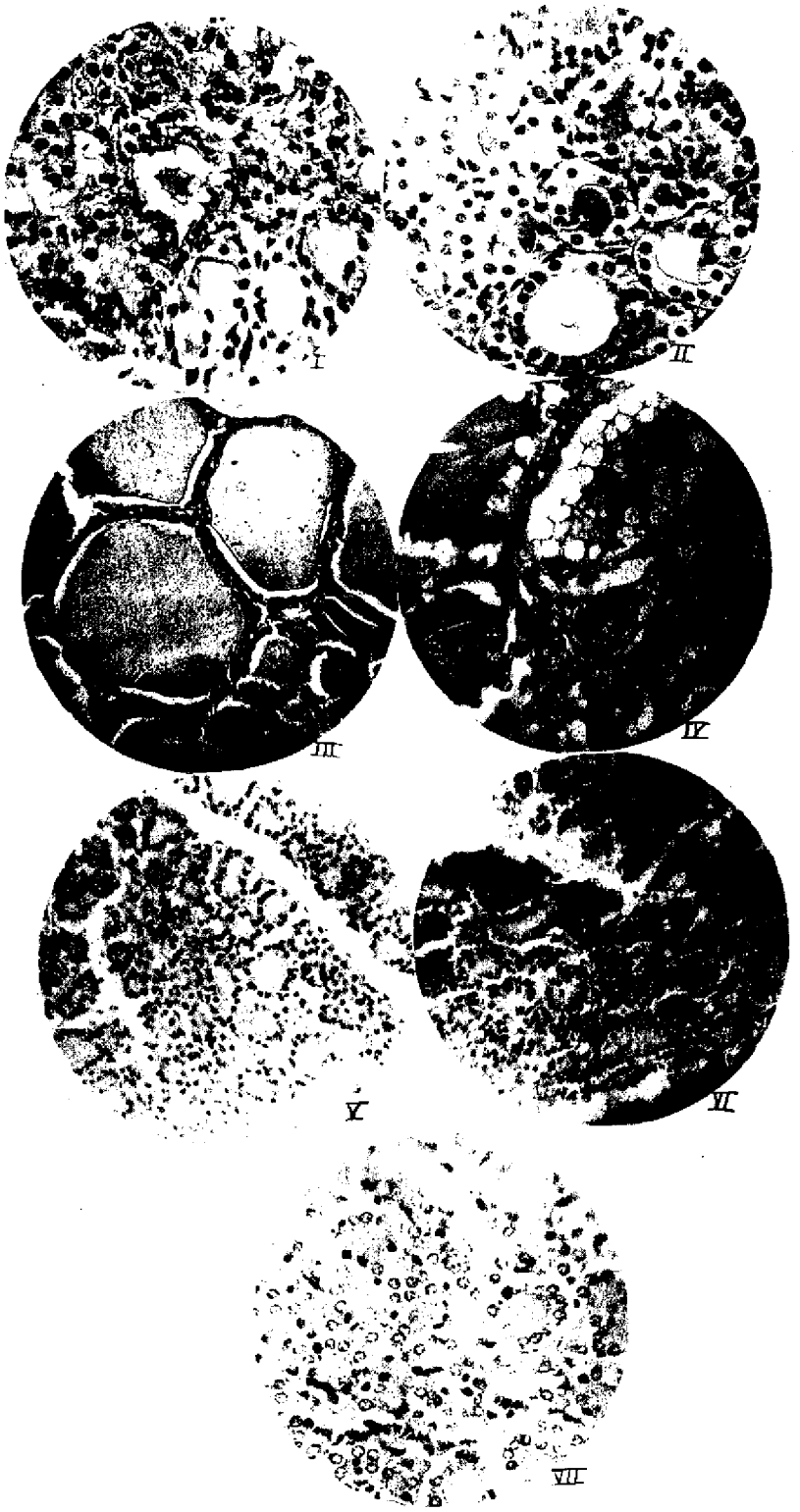

Fig. 1

end of the experiment when the amount of toxic material which they were receiving was much less. Therefore it would seem as though some intoxications, at least, call forth an increased thyroid activity. Whether this increased activity denotes an increased thyroid output or a detoxication function for the thyroid we are not prepared to say, but experiments now being conducted appear to favor the former idea.

All rabbits used in these experiments did not show the typical increased activity described but some (67–44) gave a picture of degeneration. While these two animals are not sufficient from which to draw conclusions, it would seem that a heavy dosage, one nearly approaching the lethal dose, could not be tolerated long by the rabbits. The thyroid activity was apparently unable to meet the demand and the thyroid succumbed to the intoxication which had been produced. Degenerative changes were seen in these glands which were characterized by the disappearance of colloid, cytolysis of the epithelial cells, parenchymatous degeneration, destruction of nuclei and evidences of inflammatory reaction.

CONCLUSIONS

1. Fecal extract when injected intraperitoneally into rabbits causes an increased activity of the thyroid gland as evidenced by colloid absorption, hyperplasia and hypertrophy.

2. The increase in activity of the gland seems to be roughly comparable to the amount of toxin given.

3. It would seem as though the thyroid breaks under the strain of large doses of toxin and undergoes degenerative changes as evidenced by cytolysis and varying degrees of inflammatory changes.

4. This response so far has shown itself to be merely a physiological increase in the gland.

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