THE INFLUENCE OF THE AUTONOMIC NERVOUS SYSTEM ON THE FUNCTION OF THE THYROID GLAND.*

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Plate 1.

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The mechanism of the control of the function of the thyroid gland is a subject of great interest both from a physiological point of view and also because of the part it may play in the pathological changes which so frequently take place in this gland. The studies of Andersson (1), Berkley (2), and Rhinehart (3) on the anatomy of the thyroid have shown that nerves pass to the gland from the superior and recurrent laryngeal nerves and also from the cervical sympathetic cord by way of the thyroid arteries. It is still doubtful whether the fibers in the laryngeal nerves are of vagal or sympathetic origin. Within the gland many of the fibers end on the terminal vessels but others are distributed to the walls of the vesicles where they end at the base of the gland cells. The fact that nerve fibers pass to the secretory cells has led to the supposition that the thyroid like other glands, such as the salivary glands, is under nerve control. Attempts have been made to settle this question by histological, physiological, and chemical studies.

Histological Evidence.—Changes in the gland cells supposed to indicate active secretion have been described by some investigators as following the injection of pilocarpine (4, 5) and adrenalin (6), while others obtained negative results in similar experiments (6, 7). Division of the laryngeal nerves (8) or of the cervical sympathetic (9) has been reported to cause degeneration of the gland. This action in the case of the nerves first mentioned has been also attributed to hyperemia (10) or to section of sympathetic fibers (11). Stimulation of the cervical sympathetic from time to time over a period of 1 to 2½ months has been

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stated to cause changes indicative of increased activity, while the opposite effect followed excision of the cervical sympathetic (12, 13). Later workers have been unable to confirm this (14). Stimulation of the superior cervical ganglion or injecting *B. bronchisepticus* into it has been reported as producing either thyroid hyperactivity or exhaustion according to the duration of the stimulus (15), and degenerative lesions have been described in the cervical ganglia in cases of exophthalmic goiter (16). No histological difference could be detected between a graft of thyroid in which nerve control was absent and the part of the gland which remained after partial thyroidectomy (17–19).

Physiological Evidence.—Stimulation of the laryngeal nerves or injection of thyroid extract has been described as causing an increase in the activity of the depressor nerve (20), the splanchnic nerve (21), and the vagus (22), and an increased response to injections of adrenalin (20, 23). Other investigators have been unable to confirm these findings (24–26). Stimulation of the cervical sympathetic has been stated to cause an increased response to adrenalin after a latent period of an hour. Previous thyroidectomy prevented the action but adrenalectomy did not alter the response (27). However, an increased excitability of the autonomic nervous system in thyroidectomised animals was found by others to follow repeated injections of a constant dose of adrenalin (28). Cannon and Smith described an increase in rate of the denervated heart after massage of the thyroid or stimulation of the cervical sympathetic, which they considered in both instances was due to the discharge of thyroid secretion (29). A difference of electric potential in the gland has been shown after stimulation of the cervical sympathetic, the injection of adrenalin, or splanchnic stimulation, which was absent when other nerves were stimulated or anemia of the gland produced (30, 31). Cannon, Binger, and Fitz (32) utilising a method of phrenic-cervical sympathetic anastomosis devised by Langley and Anderson (33) reported that this caused symptoms resembling exophthalmic goiter. This work has not since been confirmed by the workers mentioned or by others (34–36). Marine found no change in the functioning capacity of the gland after sectioning the nerves running in the adventitial coats of the vessels and encasing the gland in paraffin (37).

Chemical Evidence.—Removal of the inferior cervical ganglion has been reported to cause a loss of weight and reduction of the iodine content while section of the vagus was without effect (11). Stimulation of the superior thyroid vessels with their accompanying nerves or the vagosympathetic trunk has been said to cause a similar effect (38, 39), but van Dyke could find no greater variations than those normally present (40).

In the present series of experiments the influence on the histology of the thyroid gland of section or stimulation either singly or combined of the various nerves which pass to the thyroid gland has been investigated. As a preliminary we studied the histology of the thyroid in normal animals and as in every case the microscopic appearance of the

two lobes in the same animal was found to be identical it was decided to use one lobe for the experiment and the other as a control.

Only male rabbits were used and for a short period before the experiment was commenced they were given a diet consisting of raw cabbage which was continued throughout the time the experiment was in progress. In the survival experiments the preparatory technique at each operation was the same. The animal was anesthetised with ether, the neck shaved, and alcohol used as an antiseptic. Iodine was strictly avoided during the operation and we carefully guarded against the possibility of the animal obtaining any iodine supply while under observation. The animals were killed at varying intervals of time. When the time came it was anesthetised with chloretone and exsanguinated. We had previously compared the histology of normal animals so treated with that of similar animals which had been killed by a blow on the occiput, and were unable to detect any alterations due to this anesthetic. In the experiments in which the various nerves were stimulated the animals were similarly anesthetised with chloretone. The nerves were isolated, placed on platinum electrodes, and kept moist throughout the experiment. Instantaneous faradic stimuli were applied to the nerve at the rate of 20 per minute by means of a metronome connected with an induction coil. The criterion of the efficacy of the stimulation in the case of the sympathetic was dilatation of the pupil following stimulation, while stimulation of the vagus gave rise to twitching of the larynx and pharynx on that side. In all the sympathetic experiments and many of the vagal experiments artificial respiration was employed. The tracheotomy tube was inserted low in the neck and damage to the thyroid veins was carefully avoided. The animal was exsanguinated after the experiment was completed. The tissues to be examined were immediately fixed with Müller's fluid to which had been added 5 per cent formalin and the sections were stained with Weigert's iron-hematoxylin and eosin. Occasionally neither lobe was of uniform structure and in these cases we compared average fields from both lobes. Infection was rare in these experiments but when it occurred the result was discarded.

The Normal Thyroid of the Rabbit.

A number of experiments were performed to study the normal histology of the gland in the rabbit under conditions similar to those which prevailed during our experiments. The striking feature of these observations was the marked differences which were seen in different rabbits although in all the animals the histological picture was the same in both lobes. All these observations were made at the same period of the year so that a seasonal variation could not explain the differences. No detailed protocols are given as the histological

descriptions given in the tables of the experiments correspond to the pictures seen in the normal thyroid. The figures shown also illustrate well the type of variation which was found. In some animals the acini were large and filled with colloid which stained well (Fig. 1), while in others the acini were very small and contained much less colloid (Fig. 2). There was often a good deal of irregularity both in the size and shape of the vesicles, and the staining power of the colloid also varied. The cells in the largest acini were usually flat, while in the others they were cuboidal, but cylindrical cells were never seen. Some thyroids showed very marked vacuolation of the colloid, whereas in others this was completely absent. Epithelial cells were often seen in small clumps in the intervesicular tissue. This was present so frequently that it seems to be part of the normal picture in the thyroid of the rabbit. In a few instances small cellular invo ⟋ uns into the lumen of some of the vesicles were seen. Intermediate pictures between these two extremes were seen most frequently (Fig. 3).

The Effect of Drugs.

Before we fully realized the marked individual differences in the thyroid of normal rabbits we endeavored to study the function of the gland by repeated injections of adrenalin, pilocarpine, and strychnine. The pictures which were obtained varied and were within the range of those seen in the normal gland. It was seen that this method of studying the problem could lead to no conclusive results and that the only method by which results could be obtained was to carry out the experiment on one lobe and use the other as a control.

The Effect of Nerve Division.

Three experiments were performed in which the right vagus was divided above the level at which the superior laryngeal nerve is given off, and three in which the right sympathetic cord was divided as low in the neck as possible. In two other experiments the right sympathetic cord, the right recurrent laryngeal nerve, and the depressor nerve were cut, as it was thought that some sympathetic fibers might gain access to the thyroid by way of the recurrent laryngeal. Finally one experiment was carried out in which the right vagus, the

superior laryngeal nerve, and the right sympathetic cord were divided. The animals were killed at intervals varying from 7 to 35 days. Protocols of the experiments and of the histological pictures seen in the two lobes are given in Table I. The results obtained from these experiments were entirely negative, as in every instance the histological appearance of the two lobes was the same.

The Effect of Nerve Stimulation.

In four experiments the right stellate ganglion was exposed posteriorly by Sherrington's rib resection method. The sympathetic cord below the ganglion and the cardiac branches from the ganglion were divided and stimulation was applied to the cord immediately above the ganglion. Three experiments were carried out in which the right sympathetic cord was cut as low in the neck as possible and stimulated at this point. In two instances the right vagus was divided above the superior laryngeal nerve and again below the level at which the recurrent laryngeal nerve is given off. The electrodes were applied to the nerve at the junction of the superior laryngeal and the main trunk. Two further experiments were performed in which the vagus was divided above and below the origin of the superior laryngeal nerve while the recurrent laryngeal nerve was also divided. Both nerves were then stimulated. Stimulation was maintained for periods varying from 1 to 6 hours. No alteration in the microscopic appearance could be observed between the lobe which had been stimulated and the control lobe (Table II).

DISCUSSION.

In the experiments which have been carried out we have been unable to find any histological difference in the thyroid gland either from cutting off the nerve supply or from nerve stimulation. We fully realize that histological study is not the ideal method for investigating the functions of an organ, but we think that this method is more useful in the case of the thyroid gland than with most organs of the body because very slight morphological changes can be detected and, as Marine and his coworkers (41, 42) have shown, there is a close relation between the histological structure and the iodine con-

TABLE I.

Experiment No.	Time killed after operation.	Weight before operation.	Weight when killed.	Left lobe.							Right lobe.
				Vesicles.		Colloid.		Vacuolation.	Cells.	Cellular involution.	
				Size.	Shape.	Amount.	Staining.				
Right vagus divided above superior laryngeal.											
	days	*gm.*	*gm.*								
1	7	2080		Medium and regular.	Regular.	Moderate.	Good.	Marked.	Cuboidal.	Absent.	No difference.
2	28	1960	2025	Irregular.	Irregular.	"	"	Slight.	"	"	" "
3	28	1560	1520	Medium and regular.	Fairly regular.	Fairly large.	"	"	"	"	" "
Right sympathetic cord divided low in neck.											
4	35	1995		Small and some elongated scattered throughout.	Regular; elongated vesicles irregular.	Small.	Faint.	Marked.	Cuboidal.	Absent.	No difference.
5	28	2350	2220	Irregular.	Regular.	Varied greatly.	Rather faint.	Slight.	"	"	Contained rather more colloid.
6	7	2150	2120	Very irregular; some very large.	Very irregular.	Varied considerably.	Varied.	Marked.	"	"	No difference

Right sympathetic cord, right depressor, and right recurrent laryngeal divided.

7	7	1730	1820	Medium; some very large.	Most regular; large irregular.	Large vesicles well filled; others had much less.	Good.	Slight.	Large vesicles flat; others cuboidal.	Absent.	No difference.
8	28	1870	1500	Medium and regular.	Regular.	Moderate.	"	Marked.	Cuboidal.	"	" "

Right vagus, right superior laryngeal, and right sympathetic cord divided.

9	35	2000	1460	Medium and regular with a few larger scattered irregularly.	Regular.	Moderate.	Good.	Moderate.	Cuboidal.	Absent.	No difference.

TABLE II.

Experiment No.	Weight.	Duration of stimulation.	Left lobe.							Right lobe.
			Vesicles.		Colloid.		Vacuolation.	Cells.	Cellular involution.	
			Size.	Shape.	Amount.	Staining.				
Right stellate ganglion stimulated.										
	gm.	*hrs.*								
10	1950	6	Large and irregular.	Fairly regular.	Large.	Faint.	Absent.	Cuboidal.	Absent.	No difference.
11	1900	1	Medium and regular.	Regular.	Small.	"	"	"	"	" "
12	2000	6	Small and regular.	"	"	Good.	Slight.	"	"	" "
13	2100	1	Mostly small; some large.	"	Large well filled; small contained little.	"	Absent.	"	"	" "
Right sympathetic cord stimulated low in neck.										
14	1250	5	Medium and regular.	Regular.	Moderate.	Good.	Absent.	Cuboidal.	Absent.	No difference.
15	1600	1	Irregular, some being large—some small.	Fairly regular.	Varied.	"	"	"	"	" "
16	1250	1	Small and regular.	Regular.	Small.	Faint.	"	"	"	" "

Right vagus stimulated above superior laryngeal.											
17	2080	5½	Irregular, some large, some small.	Fairly regular.	Large.	Faint.	Moderate.	Cuboidal.	Absent.	No difference.	
18	2300	1	Small and regular.	Regular.	Small.	Fairly good.	Marked.	"	"	"	"
Right superior laryngeal and right recurrent laryngeal stimulated.											
19	2360	1	Many large, many small.	Irregular.	Large well filled; small contained less.	Good.	Very slight.	Cuboidal in smaller vesicles; flatter in large.	At some points in both lobes small cell accumulations were seen which projected to a slight extent into the vesicle.	No difference.	
20	1950	6	Medium and regular; some large.	Regular; large irregular.	Large well filled; medium contained moderate amount.	"	Absent.	Cuboidal.	Absent.	"	"

tent of the gland. Our experiments have clearly demonstrated the marked variations which are present in the microscopic picture of the normal thyroid gland in different rabbits. This difference was seen mostly in the size and shape of the vesicles, the quantity and staining power of the colloid, and the amount of vacuolation. This individual variation in the microscopic appearance of the thyroid gland had been noted in 1888 by Hale White (43) in material he obtained from autopsies on patients who during life had shown no signs of thyroid disease, and other observers have reported similar findings in the course of experimental work. This variable histological picture seems to us to be of great importance in the interpretation of experimental results. The histological evidence which has been brought forward in support of the secretory innervation of the thyroid has been based principally on the structure of the particular lobe which was examined and not on a comparison of the two lobes of the same animal. In view of the marked individual differences which are present normally this evidence cannot be accepted as leading to any definite conclusions. This same criterion applies to experiments in which the gland was studied histologically after drug injection. The results of stimulation of the sympathetic over a period of months reported by Reinhard are quite at variance with his interpretation. He considered that they indicated hyperactivity, whereas the histological picture tended towards that of a colloid rather than a hyperactive gland. Excision of the cervical sympathetic has been frequently performed for the relief of exophthalmos in exophthalmic goiter but with few exceptions no change has been reported either in the exophthalmos or in the thyroid.

The evidence of an altered excitability of the autonomic nervous system following injection of thyroid extracts or stimulation of the cervical sympathetic also cannot be held as proof of an increased outpouring of thyroid secretion after nerve stimulation, as similar experiments by Sharpey-Schafer and Dryerre in which thyroid extracts were injected gave entirely negative results. The experiments of Lieb and Hyman showed a gradual increase in the excitability of autonomic nerves following repeated injection of a constant small dose of adrenalin after thyroidectomy and throw considerable doubt on the experiments in which adrenalin was used as a test of this ex-

citability. The parallelism which Cannon and Smith draw between the action on the denervated heart of massage of the thyroid and sympathetic stimulation does not necessarily prove that the nervous impulses caused an increased outpouring of secretion. Even if one grants that thyroid secretion can alter the rate of the denervated heart it seems possible that the effect of the intermittent stimulation of the thyroid was to produce an alternate contraction and dilatation of the vessels which was in reality a massage. Complete anemia of the gland for the period of the experiment could not be a suitable control for experiments when intermittent stimulation was used. This massaging action might explain also the alteration in the iodine content of a lobe following stimulation of the sympathetic although all authors do not agree that this difference exists. The experiments which have been carried out by Cannon and his coworkers on the production of "action currents" in the thyroid gland as indicating active secretion are interesting but it seems to us that the state of knowledge in regard to the physiological significance of "action currents" is so unsatisfactory that no definite conclusions can be drawn at present from experiments of this nature. The studies which have been made on the changes which take place in thyroid grafts in various parts of the body show conclusively that nerve control is not essential to the adequate functioning of the thyroid gland, therefore any influence which nerves can have is only of an accessory nature. At present one must decide that although the thyroid undoubtedly is supplied by nerves there is no conclusive evidence as to their function.

CONCLUSIONS.

1. There is a marked individual variation in the histological structure of the thyroid gland of rabbits examined at the same season under standard conditions. Although the microscopic appearance varies in different rabbits, the structure of the two lobes in the same animal does not differ. The variations are seen principally in the size and shape of the vesicles and in the quantity and character of the colloid.

2. With one lobe as a control no histological changes in the other lobe have been observed following section or stimulation of either the cervical sympathetic or the vagus and its branches.

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EXPLANATION OF PLATE 1.

FIG. 1. Experiment 19. Right vagus and right recurrent laryngeal nerves stimulated for 1 hour; 20 stimuli per minute.

FIG. 2. Experiment 4. Right sympathetic cord divided low in neck; killed 5 weeks later.

FIG. 3. Experiment 11. Right stellate ganglion stimulated for 1 hour; 20 stimuli per minute.

Right lobe. Left lobe.

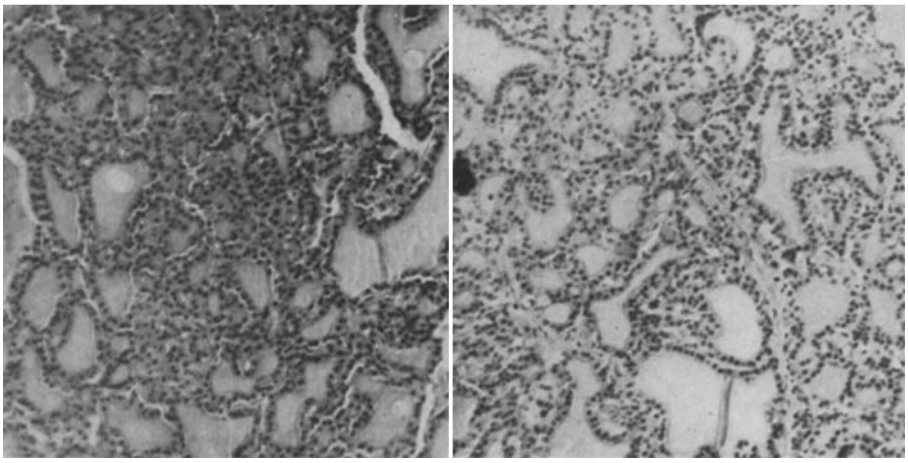

FIG. 1.

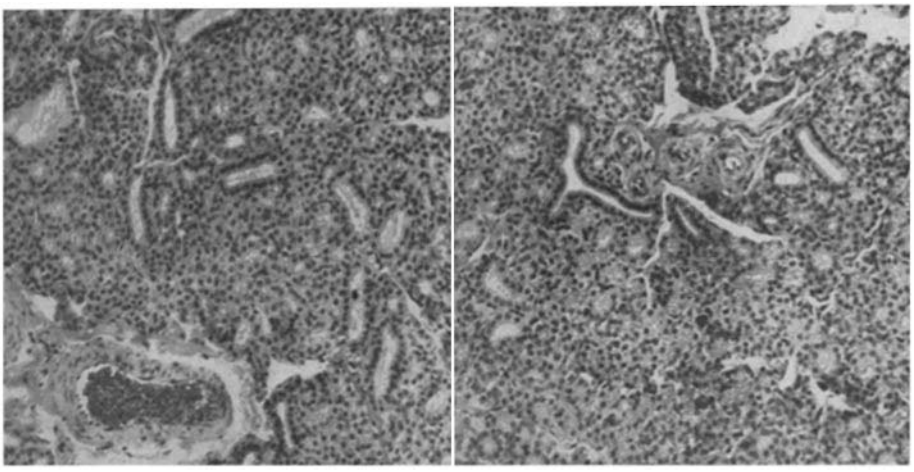

FIG. 2.

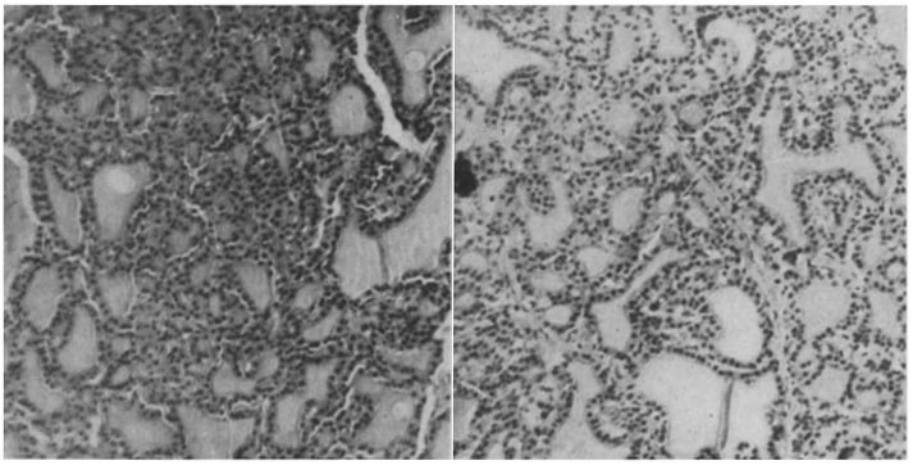

FIG. 3.

(Crawford and Hartley: Autonomic nervous system and thyroid gland.)