

From the Allergy Research Laboratory, Department of Dermatology,
Karolinska Sjukhuset, Stockholm 60.

THE INFLUENCE OF THE THYROID GLAND ON THE HISTAMINE AND ANAPHYLACTIC REACTIONS IN ANIMALS

1. *The Influence of Thyroidectomy on the Sensitivity of Guinea-Pigs to Aerosols of Histamine and Acetylcholine*

By

ÅKE NILZÉN, M.D.,
Stockholm

With the technical assistance of Mrs. Gun Lie and Mrs. B. Widjer

For a great many years the opinion has been maintained that among the organs of internal secretion the thyroid gland governs anaphylactic and allergic states. In 1920 Pistocchi observed that experimental anaphylactic shock in animals could be prevented by removal of the thyroid. His results were corroborated by Képinow, who also found that attempts at producing passive anaphylaxis failed if the donor was thyroidectomized. The thyroid was necessary for successful sensitization. In sensitized animals, thyroidectomy did not interfere with the anaphylactic reaction. Several workers, *inter alia* Doerr, Haag and Karrenberg, Kämmerer, confirmed Pistocchi's findings, and Eickhoff demonstrated that both systemic and topical anaphylaxis were inhibited by thyroidectomy. In addition, as shown by Eickhoff, the inflammatory cellular reaction, which is found in the organs following injection of large doses of antigen, was absent in thyroid-

ectomized animals. Eickhoff also pointed out that the formation of antibodies was hampered by thyroidectomy.

Several workers—in the first place Blom and Eickhoff—have stated that in animals the anaphylactic reaction is enhanced by thyroid preparations (thyroxin, extract prepared from the thyroid of patients with exophthalmic goitre). The latter investigated the question of whether the thyrotropic hormone affects anaphylaxis in thyroidectomized animals, but failed to demonstrate such an influence.

Most of the authors already quoted also studied a possible action of thyroxin or thyroid preparations on anaphylactic conditions, and found a certain increase in anaphylactic reactivity when the treatment had been given for some time previously. On the other hand, Stietzel was unable to demonstrate any enhancement of the anaphylactic reaction in guinea-pigs treated with thyroid hormone. More recently Long & Miles and Nilzén found that in animals tuberculin allergy and epidermal allergy were enhanced if thyroxin was administered systemically.

Thus it seems clear that the thyroid gland is of importance to anaphylactic and other allergic reactions; however, in the available literature I have not found satisfactory explanations for the very conspicuous change of anaphylactic reactions after thyroidectomy and administration of thyroid preparations respectively. In the earlier literature the theory that the thyroid acts upon the autonomic nervous system, has been largely favoured, and none but Eickhoff has in a short series of qualitative experiments studied antibody formation in normal and thyroidectomized animals.

In modern research on anaphylaxis a long enzymatic reaction chain is postulated after the antigen-antibody reaction has taken place (see Ungar). Theoretically, the thyroid may interfere with, on the one hand, antibody formation, and on the other the separate links of the reaction chain. In the present and following studies I have made it my object to investigate whether thyroidectomized animals present an altered reactivity not only to shock doses of antigen but to hista-

mine and acetylcholine; whether there is a quantitative change in the histamine and histaminase contents of the pulmonary tissues; whether passive anaphylaxis and different qualities of the anaphylactic reaction are modified; and whether the amount of antibody is changed.

In this paper I shall deal with the results of experiments pertaining to histamine and acetylcholine.

MATERIALS AND METHODS

Male guinea-pigs of from 200 to 600 Gm. were used in the experiments. They were fed with hay, Swedish turnips or carrots, and oats.

The thyroidectomy was performed with local and general anaesthesia. As local anaesthetic was used a 2 per cent solution of Xylocain injected both superficially into the skin covering the thyroid and more deeply on either side of the trachea and larynx. The general anaesthetic used was pentobarbital sodium given in a dosage just falling short of producing deep anaesthesia.

The lobes of the thyroid were removed in a one-stage operation, and the site was cauterized with a platinum loop. Before closing the wound with sutures, sulfathiazole powder was introduced into the cavity.

In a proportion of the animals the thyroid was destroyed by injecting the isotope, I^{131} , of which 1 ml. containing 1 mC. was given in each instance.

That nothing was left of the thyroid was checked partly by histological examination after the killing of the animals, and partly by doses of tracer iodine during life. The latter procedure consisted in intraperitoneal injection of tracer iodine with subsequent recording of the numbers of impulses, starting at the snout of the animal and continuing downwards at intervals of about 1 cm.

Sensitivity to histamine and acetylcholine was estimated in a glass cylinder into which the substance was introduced in the form of an aerosol. A normal animal was invariably tested together with a thyroidectomized one in the aerosol.

The histamine solution used was prepared by dissolving 0.1 Gm. histamine hydrochloride in 10 Gm. glycerine and 40 Gm. distilled water.

The acetylcholine solution, viz. 5 mg. crystalline acetylcholine in 1 ml. normal saline, was freshly prepared for each experiment.

The amount of histamine in the pulmonary tissues was estimated with Code's technique, and the histaminolytic ac-

tivity with a method previously described (Granroth and Nilzén).

Thyroxin was given in amounts of 100 μ g. per Kg. body weight. This dosage was selected because according to earlier experience (Nilzén and Hussey) it did not seem to produce systemic impairment.

RESULTS

Among 70 guinea-pigs thyroidectomized by extirpation, 60 per cent displayed a definite resistance to histamine. By means of histological examination and/or tracer iodine it was possible to establish that the thyroid had been completely removed in this 60 per cent of the animals used in the experiments. In the remaining 40 per cent, where the resistance had not increased, some residue of the thyroid was usually found. Table 1 shows a series of experiments taken at random from the above 60 per cent. As will be seen *inter alia*, this resistance to histamine remains for a considerable period (more than 2 months). That the animals do not become habituated to histamine is disclosed by Table 3, where one and the same control animal was used. This animal was also subsequently used for several months without any signs of habituation appearing. As a rule the experiments were discontinued after 6 minutes, as a guinea-pig by that time not developing shock symptoms from breathing in histamine would be able to remain in the histamine aerosol for a considerably longer period. Several guinea-pigs were in this manner exposed to histamine for as much as 15 minutes without suffering shock. Some thyroidectomized animals developed mild dyspnoea, became restless, scratched their snouts; others presented signs of serious intoxication with passing of urine and stools, yet they were able to stay in the aerosol without succumbing to shock. Still other guinea-pigs remained completely unaffected in the aerosol.

In Table 2 is listed a series of experiments in which acetylcholine served as the shock-producing agent. In this series the

TABLE I
Histamine Aerosol Experiments with Thyroidectomized Guinea-Pigs.

Animal	Weight of op. animals in gm.	Weight of control ani- mals in gm.	Date of operation	Date of histamine exposure	Time until shock in op. animals	Time until shock in control animals
6	320	310	1953 June 5	1953 June 6	5 min. 53 sec.	2 min. 2 sec.
	390	330		9	6 53	1 47
	350	430		15	6 0	1 51
12	300	300	June 6	June 9	5 10	2 57
	270	430		15	6 0	1 51
24 a	410	360	Sept. 20	Sept. 22	2 20	1 20
	470	360		26	2 30	3 10
	470	380		Oct. 17	6 10	2 5
	470	340		Nov. 20	5 20	1 20
33	380	460	Aug. 21	Aug. 25	4 7	2 27
	440	520		Sept. 16	5 45	3 25
	450	250		29	3 40	1 58
	530	360		Nov. 6	6 0	1 10
52	310	480	Aug. 31	Sept. 1	8 20	1 45
	310	280		12	8 52	1 43

TABLE 2
Acetylcholine Aerosol Experiments with Thyroidectomized Guinea-Pigs.

Animal	Weight of thyroidectomized animals in gm.	Weight of control animals in gm.	Date of operation	Date of choline exposure	Time until shock in op. animals	Time until shock in control animals
1	330	260	1953 June 5	1953 June 17	5 min. 5 sec.	1 min. 55 sec.
6	320	360	1	17	4 45	30
12	260	530	6	17	4 50	1 11
13	290	260	6	17	5 5	1 55

TABLE 3
Experiments with Histamine and Acetylcholine Aerosols; Formalin-Treated Guinea-Pigs
 (2 ml. 10% formalin intramuscularly).

Animal	Type of aerosol	Date and hour of formalin injection	Date and hour of exposure	Weight of formalin-treated animals in gm.	Weight of control animals in gm.	Time until shock in formalin-treated animals	Time until shock in control animals
24	histamine	1953 June 23 10 a.m.	1953 June 23 1 p.m.	670	540	9 min. 42 sec.	1 min. 59 sec.
			3 p.m.	670	540	10 10	2 45
			June 24 10 a.m.	620	490	9 35	2 13
			June 26 10 a.m.	600	500	8 54	2 30
			June 27 10 a.m.	630	430	8 40	2 35
			June 29 10 a.m.	610	480	4 15	2 28
25	acetylcholine	June 17 10.30 a.m.	June 17 2 a.m.	520	420	7 17	1 44
			June 18	540	380	7 4	1 41
			June 22	570	530	2 27	1 41

TABLE 4
Histamine Aerosol Experiments with Guinea-Pigs Operated Upon.

Animal	Weight of op. animals in gm.	Weight of control animals in gm.	Date of operation	Date of hist-amine exposure	Type of operation	Time until shock in op. animals	Time until shock in control animals
22	300	350	1953 June 13	1953 June 15	Incision over the thyroid region	1 min. 32 sec.	1 min. 32 sec.
	290	290		24			
	300	310		26			
	360	370		July 3			
26	460	390	June 23	June 24	Removal of one kidney	1 40 1 45 1 6	1 40 1 22 1 40
	390	330		26			
	480	300		July 6			
25	320	260	June 23	June 24	Removal of the spleen	2 12 1 25 1 15	1 45 1 22 1 15
	295	330		26			
	330	420		July 6			

results were largely analogous to those of the histamine experiments.

The thyroid gland being one of the most important organs of internal secretion, its removal must be expected to interfere rather seriously with the vital functions of any organism. In order to rule out—so far as this can be done—that already the operation in itself might make the animals resistant to histamine and acetylcholine aerosols, normal, not thyroidectomized guinea-pigs were subjected to various procedures. As will be seen from Table 3, formalin treatment of guinea-pigs does produce an appreciable rise in resistance to histamine and acetylcholine aerosols extending over the period during which the system is affected by the drug. In guinea-pigs treated with formalin, the resistance to histamine is not depressed by thyroxin. In a large number of animals an incision was made through the skin covering the thyroid, adipose tissue was removed, and the wound was cauterized in exactly the same manner as was done in the thyroidectomized animals. Since such an operation should be regarded as a fairly mild procedure, even major surgery, e.g. splenectomy, unilateral kidney extirpation, was performed. Table 4 shows that these procedures did not give rise to any increase whatever in histamine resistance.

For a couple of days following thyroidectomy, as well as after formalin treatment, the guinea-pigs fed more sparingly than prior to the operation, which also might be supposed to raise the resistance in analogy with the stress hypothesis already mentioned. For this reason two animals were left without food for four days, and their histamine-aerosol times were daily recorded. No difference emerged between fasting and normal animals.

To ascertain whether this resistance to histamine and acetylcholine was due to the loss of thyroid hormone, one guinea-pig was set apart which had been thyroidectomized and was very markedly resistant to histamine. Two guinea-pigs with intact thyroids were given the isotope, I^{131} . Thyroxin was then administered in the doses and at the times stated in Table 5.

TABLE 5
Histamine Aerosol Experiments with Thyroidectomized Guinea-Pigs Before and After Thyroxin Treatment.
 In guinea-pigs 1 d and 2 d the thyroid was destroyed with I^{131} , 1 mC, I^{131} /ml.
 Amount of thyroxin 100 γ /kg bdw.

Animal	Weight of thyroidectomized animals	Weight of control animals	Date of operation respectively injection of I^{131}	Date of histamine exposure	Date of thyroxin treatment	Time until shock in thyroidectomized animals	Time until shock in control animals
22 a	460	340	1953 Sept. 20	1953 Sept. 22	1953 Nov. 27.30 Dec. 2. 4 Dec. 16.18.21	1 min. 15 sec.	1 min. 40 sec.
	520	310		26			
	540	470		Nov. 17			
	520	290		Dec. 4			
	540	690		5			
	550	360		11			
	540	390		16			
	560	350		22			
1 d	430	260	Sept. 23	Sept. 28		2	0
	480	410		Oct. 12			
	550	310		22			

580	370	Nov. 2	2	45	1	25
580	510	13	6	0	1	40
590	370	20	6	0	1	10
490	370	Dec. 4	3	24	1	36
		Nov. 23.25				
		27.30				
510	400	Dec. 5	3	30	1	54
450	350	11	6	0	1	24
470	350	16	3	18	1	24
480	340	22	2	48	1	12
		Dec. 16.18.21				
2 d	410	Oct. 12	2 min. 30 sec.		2 min. 40 sec.	
450	310	22	2	30	1	20
480	370	Nov. 2	2	20	1	25
510	400	13	3	40	1	40
500	370	20	3	5	1	10
		Nov. 23.25				
		27.30				
460	370	Dec. 4	3	24	1	36
460	400	5	2	15	1	54
480	350	11	3	30	1	24
		Dec. 16.18				
		21				
490	340	22	2	48	1	12

Thyroxin administration was found to depress the histamine resistance of the animals.

Furthermore, Table 5 shows that in the guinea-pigs treated with I^{131} the resistance to histamine appeared when there was reason to suppose that the thyroid activity was markedly lowered, i.e. six weeks after the injection. To secure objective evidence as to the degree of thyroid activity, the tracer-iodine retention of the thyroid was repeatedly recorded. In this manner it was possible to demonstrate that the resistance to histamine culminated when retention was very low to none.

In thyroidectomized animals the amount of histamine present in the lungs did not differ from the rate for normal animals. Neither in thyroidectomized, nor in normal animals, was there any evidence of a histaminolytic activity of the pulmonary tissues.

DISCUSSION AND CONCLUSION

For the following reasons I have selected the aerosol technique for estimating the sensitivity to histamine and acetylcholine: (i) the method is very convenient for comprehensive observation of the exposed animals; (ii) the animals used in the experiments and the controls are treated uniformly as regards administration and dosage of the preparations; and (iii) the procedure is economical in animals.

Though the thyroid gland is fairly easy to remove in guinea-pigs, in some of the instances histological examination will show that small pieces of thyroid tissue have been left behind at operation. The test with tracer iodine gave the same result. In these cases there was generally no evidence of an increase in histamine resistance. In some guinea-pigs subjected to operation, or deprived of their thyroid activity by means of isotopes, retention was noted of a considerable amount of tracer iodine, but the animals were none the less able to withstand the histamine aerosol during the maximum time. This phenomenon might perhaps be explained by assuming that the thyroid tissue remaining and capable of retaining tracer iodine,

is functionally impaired or does not produce the amount of thyroxin required for the normal functions of the system. These observations will perhaps be discussed in another connexion.

Judging from the evidence secured by means of sham operations, the rise in histamine resistance would not seem to be due to the stress imposed by the operation. The results of the experiments in which the thyroid activity was suppressed with isotopes also support this opinion, as this cannot be regarded as a procedure which in itself gives rise to systemic impairment. In no instance where this examination was performed did the adrenals of the guinea-pigs show an increase in weight or any abnormality on histological examination. Histochemical estimations of the lipid content or quantitative determinations of ascorbic acid were not carried out. (The relationship between thyroid and adrenal cortex has been more closely considered in a preceding paper by Nilzén and Hussey.) On the other hand, in a number of thyroidectomized animals the cortisone content of the blood was estimated, and in association with Dr. Gemzell I shall report later on the results of this investigation. Whether the observed difference in histamine resistance between guinea-pigs operated upon (kidney extirpation or splenectomy) and those treated with formalin, is due to different degrees of stress (activation of the adrenal cortex) cannot be decided with confidence. The question arises whether an injection of formalin really is sufficient to activate the adrenal cortex to a greater extent than major surgery as e.g. a kidney extirpation. Perhaps formalin produces another, unknown, effect by which histamin shock is prevented. However, as other procedures interfere with the animal system, so formalin injections alleviate or suppress anaphylactic shocks—in Selye's opinion by starting an alarm reaction. Accordingly, the research work so far done would not seem to supply a satisfactory answer to the above question.

From the investigations recorded in the present paper the conclusion can be drawn that it is depressed thyroid activity which gives rise to increased resistance of the guinea-pigs to

histamine and acetylcholine. When thyroxin is administered, the resistance is lowered. In those instances where it was impossible to demonstrate a rise in resistance, the function of the thyroid was not completely suppressed. Sham operations disclosed that the phenomenon cannot be accounted for by the operation in itself. Powerful systemic impairment, due to e.g. formalin injections or serious illness of the guinea-pigs, raises the resistance to histamine and acetylcholine, but in these instances thyroxin fails to make normal the shock times.

It is naturally conceivable that the observed resistance to histamine and acetylcholine may explain the increased resistance to anaphylactic shock doses as demonstrated by other workers. This point will be more closely considered in subsequent papers.

SUMMARY

The present writer has demonstrated that the resistance of guinea-pigs to aerosols of histamine and acetylcholine is enhanced by thyroidectomy. The thyroid activity was depressed or suppressed by either extirpation or injection of the isotope, I^{131} . Tracer iodine was used to check the function of the gland.

Administration of thyroxin lowers the resistance.

Sham operations disclose that the results are not dependent upon the stress imposed by the operation.

The conclusion is drawn that the increase in resistance to histamine and acetylcholine is due to depressed thyroid activity.

REFERENCES

- Blom, T.*: Zeitschr. f. Immunitätsf. 83, 373, 1934.
Doerr, R.: Quoted by *Eickhoff*.
Eickhoff, W.: Virchows Arch. f. Path. Anat. 303, 481, 1939.
Granroth, T. and *Å. Nilzén*: Acta Physiol. Scand. 15, 188, 1948.
Haag, Fr. E. and *C. L. Karrenberg*: Quoted by *Eickhoff*.
Képinow, L.: C. r. Soc. Biol. 87, 494, 1922.
Kämmerer, H.: Quoted by *Eickhoff*.

Long, D. A. and A. M. Miles: Lancet 258, 492, 1950.

Nilzén, Å. and F. Hussey: To be published in J. Invest. Derm.

Pistocchi: Quoted by *Blom*.

Stietzel, H. W.: Zeitschr. f. Immunitätsf. 96, 352, 1939.

Ungar, G.: Intern. Arch. of Allergy and Applied Immunology 4, 258, 1953.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.