THE RELATIONSHIP OF DELAYED HYPERSENSITIVITY AND CIRCULATING ANTIBODY TO EXPERIMENTAL ALLERGIC THYROIDITIS IN INBRED GUINEA PIGS

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PLATES 68 AND 69

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During the last 50 years many investigators have studied the immune response to thyroid antigens (1–6). In an extensive re-investigation of this problem, Witebsky and his coworkers first demonstrated that immunization with extracts of heterologous (7), isologous, or even autologous (8, 9) thyroid tissue produced allergic thyroiditis in rabbits, dogs, and guinea pigs.

In two other auto-immune diseases, allergic aspermatogenesis (10, 11) and allergic encephalomyelitis (12–14), a correlation between the presence of disease and delayed hypersensitivity has been found. The production of allergic encephalomyelitis in previously normal animals by injection of washed lymph node cells (15) from immunized donors and by the technique of parabiosis (16) supported the hypothesis that delayed hypersensitivity was involved in the etiology of this disease.

The relationship of delayed hypersensitivity and of antibody to the etiology and pathogenesis of allergic thyroiditis is not understood. Consequently, the work to be reported in this paper was undertaken to throw light on this subject by comparing the appearance and development of thyroiditis in inbred guinea pigs (*a*) with delayed hypersensitivity but no demonstrable antibody, (*b*) without delayed hypersensitivity but with antibody, and (*c*) with both delayed hypersensitivity and antibody.

Materials and Methods

Animals.—Guinea pigs, 400 to 600 gm. in body weight were obtained from the National Institutes of Health, Animal Production Section, Bethesda. Strain 13, inbred, histocompatible, immunologically homogeneous guinea pigs (17) were used exclusively as recipients of the various antigens. This and other strains of guinea pigs were employed for the various procedures described below.

Preparation of Various Antigens.—The thyroid glands of chloroform-killed guinea pigs were excised, trimmed of fat, and cut into small pieces. After the addition of saline, the mixture was ground in a glass or teflon tissue grinder at 0°C. and centrifuged for 2 hours at 2000 R.P.M. at 5°C. Following the removal of fat from the upper layer the fluid was decanted, centrifuged as before, fat again removed, and the remaining fluid decanted and frozen and stored at −20°C. Thyroid extracts, to be used expressly for immunization or antibody titration, were made by adding 5 ml. of saline per gm. of tissue, whereas other thyroid extracts, to be employed for skin testing, were made by adding 1.5 ml. of saline per gm. of gland. The thyroid extracts for immunization of all animals were obtained from the glands of Hartley, National Institutes of Health general purpose and strain 2 (17) guinea pigs with the exception of one experiment. In this one test the guinea pigs of group D were immunized with thyroid extract derived only from strain 13 histocompatible (17) guinea pigs. Only the glands of this strain were used to make thyroid extracts for skin tests and antibody titrations throughout this work.

Several other antigens were also employed including a 50 per cent guinea pig testicular extract in saline, prepared for immunization by the method of Freund *et al.* (10), a chloroform-purified guinea pig testicular extract, lot 83[1] (11), for skin testing, and guinea pig spermatozoa for antibody titration (10). Besides these a 1 per cent solution of egg albumin[2] in saline, and a 1 per cent solution of bovine gamma globulin–diazoarsanilic acid in buffered saline were used for immunization, skin testing, and antibody titration. The latter material, prepared by adding diazotized arsanilic acid to a saline solution of bovine gamma globulin,[3] in a molar ratio of 112 to 1, was adjusted to pH 9 with NaOH and held overnight at 4°C. It was then dialyzed against repeated changes of buffered saline, 0.137 M NaCl, and 0.0152 M phosphate buffer, at pH 7.6.

Antigen-Adjuvant Mixtures.—To make water-in-oil emulsions of antigens and Freund's adjuvants, a suspension or solution of antigen was added, one or two drops at a time to the adjuvants to be described presently. After each addition, the antigen-adjuvant mixture was sucked into and expressed from a 10 ml. syringe several times. The aqueous antigen phase was added to the oil phase until the ratio was 3 parts aqueous phase to 1 part oil phase. Complete adjuvant composed of 20 per cent arlacel A[4] (especially purified vacuum stripped lot 7B), bayol F 80 per cent, and *Mycobacterium tuberculosis* H37Rv 4 mg./ml. was used for all groups except Group C, which was immunized with antigen and complete adjuvant containing *M. tuberculosis* H37Rv 6 mg./ml. Incomplete adjuvant differed only by lacking the mycobacteria. All animals immunized with these water in oil emulsions were injected intradermally with a total of 1 ml. distributed at multiple sites in the foot-pads, over the proximal surfaces of the hind legs, and over the rump.

Antibody Titration.—The anti-thyroid, anti-egg albumin, and anti-bovine gamma globulin–diazoarsanilic acid antibodies were titrated by the bentonite flocculation test of Bozicevich *et al.* (18). Serum was separated from clotted blood, obtained by cardiac puncture of ether-anesthetized animals. All sera and antigens used in the bentonite flocculation test were first heated at 56°C. for 30 minutes and then centrifuged at 3000 R.P.M. for 15 minutes to obtain clear material for the test. For titration, serial 2-fold dilutions of sera were made with saline containing 2 per cent normal strain 13 serum, and a fresh chemically clean pipette was used to make each successive dilution.

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[1] Kindly supplied by Dr. Sanford Stone, Laboratory of Immunology, National Institutes of Health, Bethesda.

[2] Nutritional Biochemicals Corporation.

[3] Armour and Company.

[4] Atlas Powder Company.

Skin Tests for Hypersensitivity.—To test for immediate and delayed hypersensitivity, 0.1 ml. of antigen solution was injected intradermally on a pigment-free area of the flank. As a routine the response was then measured at 1/2, 1, 2, 5, and 24 hours, and occasionally at 48, 72, and 96 hours. At 2 hours immediate reactions were graded as 1+ when the average of the maximal and minimal diameters of soft swelling was 1.5 to 3 times greater than in the normal controls. When this average was more than 3 times greater than the normal controls the reaction was graded 2+.

Twenty-four hours after the start of such skin tests, delayed reactions and the delayed components of mixed reactions were graded by measuring the maximal and minimal diameters of the erythematous area about the site of injection. When the average of these diameters was 15 to 25 mm. the reaction was graded as 1+, and when this average was greater than 25 mm. the reaction was graded as 2+.

Histologic Techniques.—Specimens of the skin and the right and left lobes of the thyroid glands of ether-killed guinea pigs were separately fixed in buffered formalin and paraffin sections cut at 6 microns in the usual manner. The skin sections were stained either with hematoxylin and eosin or with Giemsa. To study the pathological changes in the thyroid glands, serial sections were cut through the long axis of each lobe until 0.1 to 0.4 mm. of the tissue had been sectioned. Successive ribbons of 12 sections each were stained with hematoxylin and eosin, Giemsa, and periodic acid–Schiff.

The degree of thyroiditis was graded according to the amount of leukocytic infiltrate: 1+ if one or several foci of leukocytes appeared in excess of the normal control, and 2+ if multiple, distinct, or confluent foci of leukocytes appeared to occupy less than ¼ of the gland. When leukocytes or leukocytes and fibrosis occupied approximately ¼ of the gland the lesion was graded 3+, and if they occupied approximately ½ of the gland it was graded 4+.

RESULTS

For this work 223 strain 13 histocompatible (17) guinea pigs were used, of which 104 were held as controls and 119 were immunized with thyroid extract emulsified with either incomplete or complete Freund's adjuvants, as described above. The immunized animals were divided into groups (A to L), each of which was immunized with a separate lot of antigen. Individuals from these various groups were then bled and sacrificed at different intervals after immunization. In this way the findings were not dependent upon any one lot of antigen or adjuvant.

Findings 3 Days after Immunization.—3 days after immunization 6 guinea pigs of group A, immunized with thyroid extract in complete adjuvant, and 6 from group B, similarly immunized, were bled and sacrificed. None showed thyroiditis, nor was antibody detected in their sera.

Findings 5 Days after Immunization.—32 guinea pigs, divided into five groups (group A, 8 animals; group C, 2 animals; group D, 2 animals; group E, 10 animals; and group F, 10 animals—See Table I), each immunized with separate lots of thyroid extract and complete adjuvant, were bled and sacrificed 5 days later. Five of these 32 guinea pigs exhibited mild thyroiditis, of grade 1+. Anti-thyroid antibody was not detected in any of the 32 sera. 4 of the animals in group E and 5 in group F were skin tested with thyroid extract 4 days after immunization. Although none developed an immediate reaction, 7 of the 9 animals exhibited delayed reactions 24 hours later. 5 control animals had negative skin reactions.

TABLE I

Anti-Thyroid Antibody Titers, Skin Tests, and Thyroiditis at 5 Days

Guinea Pigs Immunized with Antigen and Complete Adjuvant

Group	Animal No.	Titer	Immediate reaction* 2 hrs. after injection	Delayed reaction‡ 24 hrs. after injection	Degree of thyroiditis
A	7	<1	—	—	1+
	8	<1	—	—	0
	9	<1	—	—	0
	10	<1	—	—	0
	11	<1	—	—	0
	12	<1	—	—	0
	13	<1	—	—	0
	14	<1	—	—	0
C	1	<1	—	—	1+
	2	<1	—	—	0
D	1	<1	—	—	1+
	2	<1	—	—	0
E	1	<1	—	—	0
	2	<1	0	1+	0
	3	<1	0	1+	0
	4	<1	0	2+	0
	5	<1	0	0	0
	6	<1	—	—	0
	7	<1	—	—	0
	8	<1	—	—	0
	9	<1	—	—	0
	10	<1	—	—	1+
F	1	<1	—	—	0
	2	<1	—	—	0
	3	<1	—	—	0
	4	<1	—	—	0
	5	<1	—	—	0
	6	<1	0	1+	1+
	7	<1	0	1+	0
	8	<1	0	0	0
	9	<1	0	1+	0
	10	<1	0	1+	0

0, negative result; —, not tested.

* Skin test read at 4 days.

‡ Skin test read at 5 days.

Findings 16 Days after Immunization.—16 guinea pigs of Groups A, C, D, and G, were bled and sacrificed 16 days after immunization with thyroid extract and complete adjuvant. All showed thyroiditis graded from 1+ to 4+, and all had antibody titers which ranged from 1:20 to 1:80. 2 other guinea pigs in group C were immunized with thyroid extract in incomplete adjuvant. Although they did not show thyroiditis,

TABLE II

Anti-Thyroid Antibody Titers, Skin Tests, and Thyroiditis at 7 Weeks

Guinea Pigs Immunized with Antigen and Complete Adjuvant

Group	Animal No.	Titer	Immediate reaction 2 hrs. after injection	Delayed reaction 24 hrs. after injection	Degree of thyroiditis
H	11	1/80	1+	2+	2+
	12	1/40	0	2+	2½+
	13	1/40	1+	2+	3½+
	14	1/80	1+	2+	3+
	15	1/10	1+	2+	2+
	16	1/60	0	2+	3+
	17	1/80	0	2+	4+
	18	1/160	0	2+	3½+
	19	1/320	1+	2+	3½+
I	14	1/10	1+	—	1½+
	15	1/80	1+	—	2½+
	16	1/80	1+	—	2½+
	17	1/80	1+	2+	2+
	18	1/20	2+	2+	1+
	19	1/40	1+	—	2+
	20	1/40	—	—	4+
	21	1/40	1+	2+	2+
	22	1/20	2+	—	1+
	23	1/40	1+	—	3+
	24	1/80	—	—	3+

0, negative result; —, not tested.

their sera contained slight amounts of antibody, positive only in the undiluted serum of 1 animal, and at a dilution of 1:2 in the other. These animals were not skin-tested.

Findings 7 Weeks after Immunization with Thyroid Extract and Complete Freund's Adjuvant.—20 guinea pigs (group H, 9 animals; group I, 11 animals) which had been immunized with thyroid extract in complete adjuvant, were bled, most of them skin tested, and all sacrificed 7 weeks after immunization. 17 of these 20 had moderate to severe thyroiditis (2+ to 4+), while the remaining 3 had thyroiditis graded 1+. The antibody titers varied from 1:10 to 1:320. 18 of these 20 animals were skin-tested with thyroid extract, and 14 responded with immediate reactions. 12 of these, allowed to survive for 24 hours, all showed delayed reactions (Table II). In addition to these 20 animals, 2 guinea pigs of group A were sacrificed 7 weeks after immunization. They

showed severe thyroiditis (3+ and 4+), and had serum antibody titers of 1:40 and 1:80 respectively. Furthermore, 2 guinea pigs in group D, which were immunized with an emulsion of complete adjuvant and a thyroid extract derived from strain 13 histocompatible guinea pigs, showed thyroiditis of 4+ degree and antibody titers of 1:20 and 1:40.

TABLE III

Anti-Thyroid Antibody Titers, Skin Tests, and Thyroiditis at 7 Weeks

Guinea Pigs Immunized with Antigen and Incomplete Adjuvant

Group	Animal No.	Titer	Immediate reaction 2 hrs. after injection	Delayed reaction 24 hrs. after injection	Degree of thyroiditis
H	1	1/2	—	—	0
	2	1/2	0	0	0
	3	1/4	0	0	0
	4	1/1	—	—	0
	5	1/4	1+	0	0
	6	1/2	0	0	0
	7	1/2	0	0	0
	8	<1	0	0	0
	9	1/4	—	—	1+
	10	1/1	0	0	0
I	1	1/2	1+	—	0
	2	1/1	1+	—	0
	3	1/2	1+	—	0
	4	1/1	0	0	0
	5	1/2	0	0	0
	6	1/2	0	0	0
	7	1/4	0	—	0
	8	1/2	1+	—	0
	9	1/8	1+	—	0
	10	1/2	—	—	0
	11	1/2	—	—	0
	12	1/2	—	—	0
	13	1/2	—	—	0

0, negative result; —, not tested.

Findings 7 Weeks after Immunization with Thyroid Extract and Incomplete Freund's Adjuvant.—7 weeks after immunization with thyroid extract and incomplete adjuvant, 23 guinea pigs (group H, 10 animals; group I, 13 animals) were bled, 16 skin-tested, and all sacrificed. 1 animal, number 9 of group H, showed thyroiditis of slight, or 1+ degree, a single chronic inflammatory focus, comparable to that seen in the earliest 5-day lesions. No others showed lesions. The sera of all but 1 of these animals had anti-thyroid titers which ranged from positive in undiluted serum to a dilution of 1:8 (Table III). 6 of the 16 skin-tested animals developed immediate reactions, but none of the 12 which were allowed to survive for 24 hours had delayed reactions.

Hypersensitivity reactions did not occur in the 20 control animals tested concurrently with groups H and I.

Additional Control Tests.—5 days after immunization with egg albumin in complete adjuvant, 10 guinea pigs were bled and sacrificed (group J). They did not show thyroiditis, nor did they have demonstrable anti-egg albumin antibodies in their sera.

Seven weeks after immunization with testicular extract and complete adjuvant, 10 guinea pigs (group K) were bled, skin-tested, and then sacrificed. The thyroids of 9 of these animals were normal. One showed slight (1+) thyroiditis. The sera of all contained sperm immobilization antibodies, ranging in titer from 1:20 to 1:80, and all

TABLE IV

Antibody Titers, Skin Tests, and Thyroiditis in Control Guinea Pigs

Immunizing agent (and duration)	Antibody titers against:				Skin test reactions for:				Thyroiditis
	Thyroid	Bovine γ-globulin D-AA	Guinea pig testis	Egg albumin	Thyroid	Bovine γ-globulin D-AA	Guinea pig testis	Egg albumin	
Bovine γ-globulin–diazoarsanilic acid (7 wks.)	0/9	9/9*	—	—	—	9/9‡	—	—	1/9
Guinea pig testis (8 wks.)	0/10	—	10/10§	—	—	—	10/10‡	—	1/9
Egg albumin (5 days)	0/10	—	—	0/10	—	—	—	—	0/10
None—no treatment	0/40	—	—	—	—	—	—	—	0/10‖
None—skin-tested with thyroid extract (3 days)	—	—	—	—	—	—	—	—	0/10

Numbers indicate No. of animals positive/total number tested.

* Titers ranged from 1:160 to 1:1280.

‡ Immediate and delayed reactions were 2+.

§ Titers ranged from 1:20 to 1:80.

‖ 10 animals examined histologically.

10 animals responded with 2+ immediate and 2+ delayed skin test reactions to chloroform-purified guinea pig testicular extract.

Nine other guinea pigs (group L), immunized with bovine gamma globulin–diazoarsanilic acid in complete adjuvant, were bled, skin-tested, and then sacrificed 8 weeks after immunization. 8 of these did not show thyroiditis, but 1 had slight (1+) thyroiditis. In the sera of all these animals, antibody titers of 1:160 to 1:1280 to bovine gamma globulin–diazoarsanilic acid were present. When skin tested with this antigen they all responded with 2+ immediate and 2+ delayed reactions, (Table IV).

Anti-thyroid antibody was not detected in the sera of any control animal immunized with antigens other than thyroid extract, or in the sera of an additional 40 normal untreated animals. Furthermore, the sera of immunized animals did not react with bentonite coated with pooled strain 13 serum. Finally, 10 animals immunized with thyroid extract in complete adjuvant failed to react when skin tested with pooled strain 13 serum.

Gross and Microscopic Appearance of Skin Reactions: Immediate Reactions.—The immediate reactions observed in immunized guinea pigs were characterized by soft swellings at the site of intradermal injection which were much greater than those seen in non-immunized control animals. The swellings reached a maximum within 2 hours and had nearly disappeared by 24 hours, leaving only small papules up to 6 mm. in diameter. Occasionally the swellings contained dark, central erythematous areas which did not increase in color or size after 5 hours. Sections of these immediate reactions, from animals in group I, showed much larger numbers of eosinophils and neutrophils located both within and without the vessels than were seen in the controls. These cells were more numerous at 2 and 5 hours than at 24 hours.

Delayed Reactions.—The delayed reactions observed in immunized guinea pigs consisted of erythematous, slightly indurated areas which were firm to palpation and located about the site of injection. Such areas were seen in the controls. By their lighter color these delayed reactions were easily distinguished from the dark, central erythema sometimes seen in the immediate reaction. The delayed reactions did not begin to appear until at least 8 hours after the intradermal injections; they reached a maximum at 24 hours or later, and persisted for 3 to 4 days. From animals of group I, skin sections of delayed reactions, 24 hours old, showed marked perivascular infiltrates composed of lymphocytes, mononuclear macrophages, and plasma cells, as well as intravascular and extravascular eosinophils and neutrophils. In the absence of delayed hypersensitivity, as judged by gross appearance, a mononuclear infiltrate did not appear, regardless of the age of the skin test at the time the animal was sacrificed.

Pathologic Changes in the Thyroid Glands.—The thyroid glands of the immunized animals, as well as those of the control animals, showed no gross changes. The histologic appearance of the fully developed thyroiditis seen at 7 weeks in immunized guinea pigs was a moderate to severe inflammatory infiltration, which was either focal or diffuse, and usually involved the entire lobe (Fig. 1.). The cellular infiltrate was distributed between follicles, and replaced considerable numbers of follicles. Characteristically, the infiltrate consisted of lymphocytes, mononuclear macrophages, and plasma cells, in no constant proportions. Varying numbers of eosinophilic leukocytes were frequently present, as were lesser numbers of neutrophilic leukocytes. Slight to moderate fibrosis sometimes accompanied the infiltrate. Partial destruction of follicles adjacent to areas of marked inflammatory reaction, and some phagocytosis of cellular debris was occasionally observed.

The appearance of the thyroiditis at periods earlier than 6 weeks was generally less severe. At 16 days, lesions ranged in degree of severity from that seen at 7 weeks to the slight, or 1+, degree.

At 5 days, the earliest time at which thyroiditis was detected, all lesions were of slight, or 1+ degree, and were considered minimal to trace thyroiditis. These lesions were smaller, and the cellular composition was more variable, than those seen at later stages. Occasionally they contained greater proportions of eosinophils or neutrophils. They were composed of either a single focus of more than 100 cells (Fig. 2), or one or more foci of 50 to 100 cells accompanied by one or more foci of 25 to 50 cells, and other smaller foci of 10 to 25 cells. Any inflammatory involvement of lesser degree than the above was not considered positive thyroiditis.

Most of the normal thyroid glands from untreated and from skin-tested control

strain 13 guinea pigs showed no inflammatory infiltrates. Some contained a few minute foci of chronic inflammatory cells, usually one or two, and not in excess of three or four. These foci usually contained 5 to 10, and occasionally 10 to 15 cells. These were considered normal. Alterations in sizes of follicles and colloid and cellular content of follicles were seen in both untreated and immunized animals, and were not considered in the grading of thyroiditis.

With the exception of a lobe of one thyroid which contained a focus of approximately 40 leukocytes, all the thyroids of guinea pigs given thyroid extract and incomplete adjuvant (Fig. 3) resembled the thyroids of untreated controls (Fig. 4).

DISCUSSION

The immune response to thyroid antigens is not confined to experimental animals, since it also occurs in man (19, 20). For example, anti-thyroid antibodies accompany many human thyroid disorders, including Hashimoto's disease in which leukocytic infiltrates of the thyroid are also present. Although the appearance of the cellular reaction in Hashimoto's disease differs from that in experimental allergic thyroiditis, both diseases possess in common anti-thyroid antibody, skin hypersensitivity, and leukocytic infiltrates of the thyroid gland (8, 19–22).

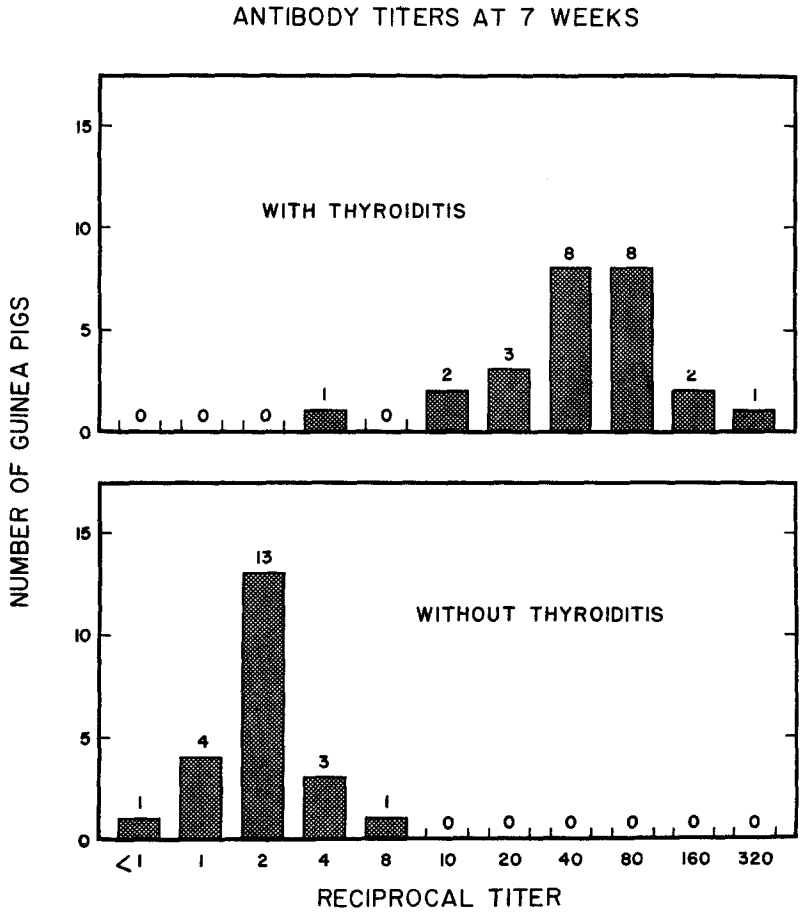
Just as human thyroid disorders arise from many causes, experimental thyroiditis may be produced in many ways. The intravenous injection of I^{131} (23), the intrathyroidal injection of a thyroid extract described by Kracht (24), and the subcutaneous implantation of pieces of the thyroid all cause leukocytic invasion of the thyroid gland (23).

To immunize animals, and yet to avoid direct mechanical trauma to the thyroid gland, Witebsky and his coworker injected antigens into the hind footpads (8). When these animals received thyroid extract in complete Freund's adjuvant they developed antibody, delayed hypersensitivity, and allergic thyroiditis. However, when extracts of other organs were substituted for the thyroid extract, no thyroiditis appeared. Furthermore, since Witebsky *et al.* had produced allergic thyroiditis in the guinea pig (9), and since this species responds well to skin testing, it was selected for this study of the relationship of delayed hypersensitivity and antibody to experimental allergic thyroiditis. The histocompatible strain 13 was used because it was found that thyroid extracts obtained from these animals were free from antigenic serum groups which might otherwise have interfered with our measurements of anti-thyroid antibody and delayed hypersensitivity.

Although allergic thyroiditis has been reported in guinea pigs after immunization with thyroid extract alone (25), we have used in addition, for the present work, Freund's incomplete adjuvant to intensify the formation of antibody to the antigen incorporated within it, and Freund's complete adjuvant to produce both antibody and delayed hypersensitivity. Although antibody of low titer

was present in 22 of 23 animals sacrificed 7 weeks after immunization with thyroid extract and incomplete adjuvant, thyroiditis was present in only 1.

The results of immunization with thyroid extract and complete adjuvant were very different. 5 days after such immunization some guinea pigs exhibited

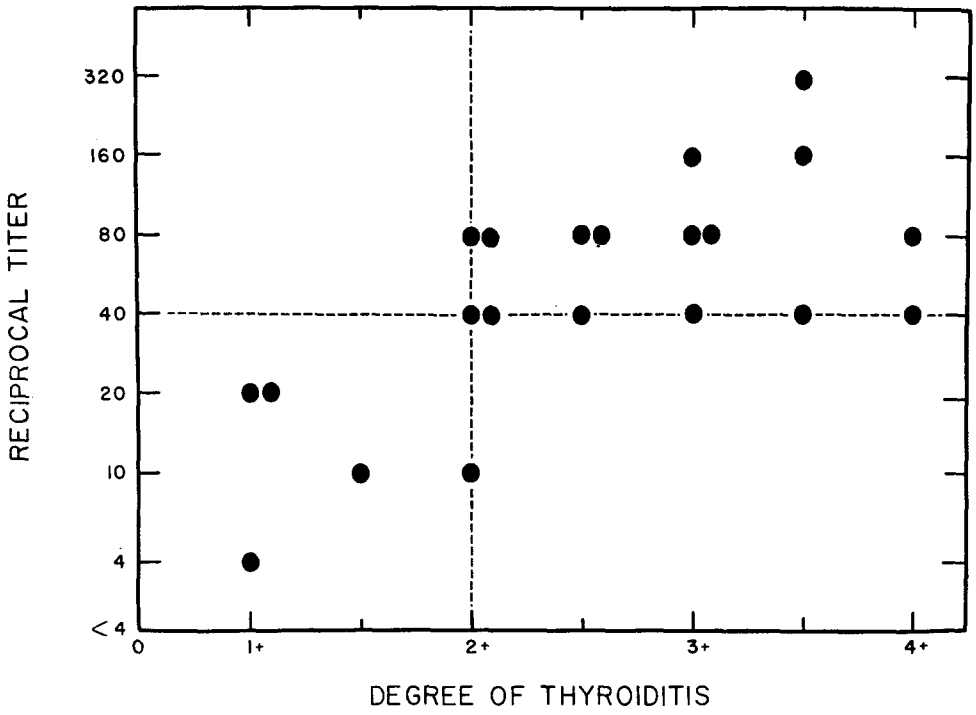


TEXT-FIG. 1. Serum anti-thyroid antibody titers in guinea pigs with and without thyroiditis 7 weeks after immunization, (groups H, I, A, D).

delayed hypersensitivity, and a few already showed thyroiditis. 7 weeks after immunization with thyroid extract in complete adjuvant all animals had developed thyroiditis, and all of those skin-tested exhibited delayed hypersensitivity. By contrast, those animals skin-tested 7 weeks after immunization with thyroid extract and incomplete adjuvant did not respond with delayed reactions, nor had they developed thyroiditis. At this time the presence of delayed

hypersensitivity correlated with the presence of thyroiditis, and the absence of delayed hypersensitivity correlated with the absence of thyroiditis.

The relationship between serum antibody and thyroiditis was not so consistent. 7 weeks after immunization the anti-thyroid antibody titer in 24 of the 25 animals with thyroiditis ranged from 1:10 to 1:320, and in most was greater than 1:20. All 22 animals without thyroiditis had serum antibody titers less



TEXT-FIG. 2. Relationship of serum anti-thyroid antibody levels to degree of thyroiditis in guinea pigs 7 weeks after immunization, (groups H and I).

than 1:10, and most were less than 1:4 (Text-fig. 1). Furthermore, an analysis of the antibody titers of all the animals in groups H and I which had thyroiditis 7 weeks after immunization, showed a significant correlation between the incidence of an anti-thyroid antibody titer of 1:40 or greater and thyroiditis of 2+ degree or greater, as determined by the 4-fold chi square test ($p < 0.001$) (Text-fig. 2). However, this relationship was not present at certain other times after immunization. For example, antibody was not found in any of 32 guinea pigs sacrificed 5 days after immunization, and yet 5 of them already showed thyroiditis. Despite the presence of thyroiditis without detectable antibody in these guinea pigs at 5 days, 21 other animals with more antibody, although of

low titer, did not have thyroiditis when examined 7 weeks after immunization with thyroid extract and incomplete adjuvant.

Consequently, these experiments do not demonstrate a role of antibody in the etiology of allergic thyroiditis. However, they do indicate that delayed hypersensitivity is associated with experimental allergic thyroiditis.

SUMMARY

Strain 13 histocompatible guinea pigs developed allergic thyroiditis after immunization with thyroid extracts derived from the same strain or from other strains of guinea pigs. This thyroiditis appeared as early as 5 days after immunization, and by 7 weeks was uniformly present and generally severe. 7 weeks after immunization, the anti-thyroid antibody titer correlated with the presence and degree of thyroiditis. However, at certain other times after immunization, the titer did not correlate with the thyroiditis. By contrast, all animals with thyroiditis, which were skin-tested with thyroid extract, exhibited delayed hypersensitivity. Moreover, all those which failed to respond with delayed reactions, when skin-tested, had not developed thyroiditis. The present work correlates the presence of experimental allergic thyroiditis with delayed hypersensitivity.

BIBLIOGRAPHY

1. MacCallum, W. G., On the production of specific cytolytic sera for thyroid and parathyroid, with observations on the physiology and pathology of the parathyroid gland, especially in its relation to exophthalmic goiter, *Med. News, London*, 1903, **83,** 820.
2. Beebe, S. P., Preparation of a serum for the treatment of exophthalmic goiter, *J. Am. Med. Assn.*, 1906, **46,** 484.
3. Beebe, S. P., The serum treatment of hyperthyroidism, *J. Am. Med. Assn.*, 1915, **64,** 413.
4. Hektoen, L., and Schulhof, K., The precipitin reaction of thyroglobulin, *Proc. Nat. Acad. Sc.*, 1925, **11,** 481.
5. Hektoen, L., Fox, H., and Schulhof, K., Specificness in the precipitin reaction of thyroglobulin, *J. Infect. Dis.*, 1927, **40,** 641.
6. Morgan, J. E., and Ivy, A. C., Experimental production of "Cretinism" by thyrocytotoxin, *Proc. Soc. Exp. Biol. and Med.*, 1934, **31,** 1139.
7. Witebsky, E., and Rose, N. R., Studies on organ specificity. VII. Production of antibodies to rabbit thyroid by injection of foreign thyroid extracts, *J. Immunol.*, 1959, **83,** 41.
8. Rose, N. R., and Witebsky, E., Studies on organ specificity. V. Changes in the thyroid glands of rabbits following active immunization with rabbit thyroid extract, *J. Immunol.*, 1956, **76,** 417.
9. Witebsky, E., Rose, N. R., Terplan, K., Paine, J. R., and Egan, R. W., Chronic thyroiditis and autoimmunization, *J. Am. Med. Assn.*, 1957, **164,** 1439.
10. Freund, J., Lipton, M. M., and Thompson, G. E., Aspermatogenesis in the guinea pig induced by testicular tissue and adjuvants, *J. Exp. Med.*, 1953, **97,** 711.

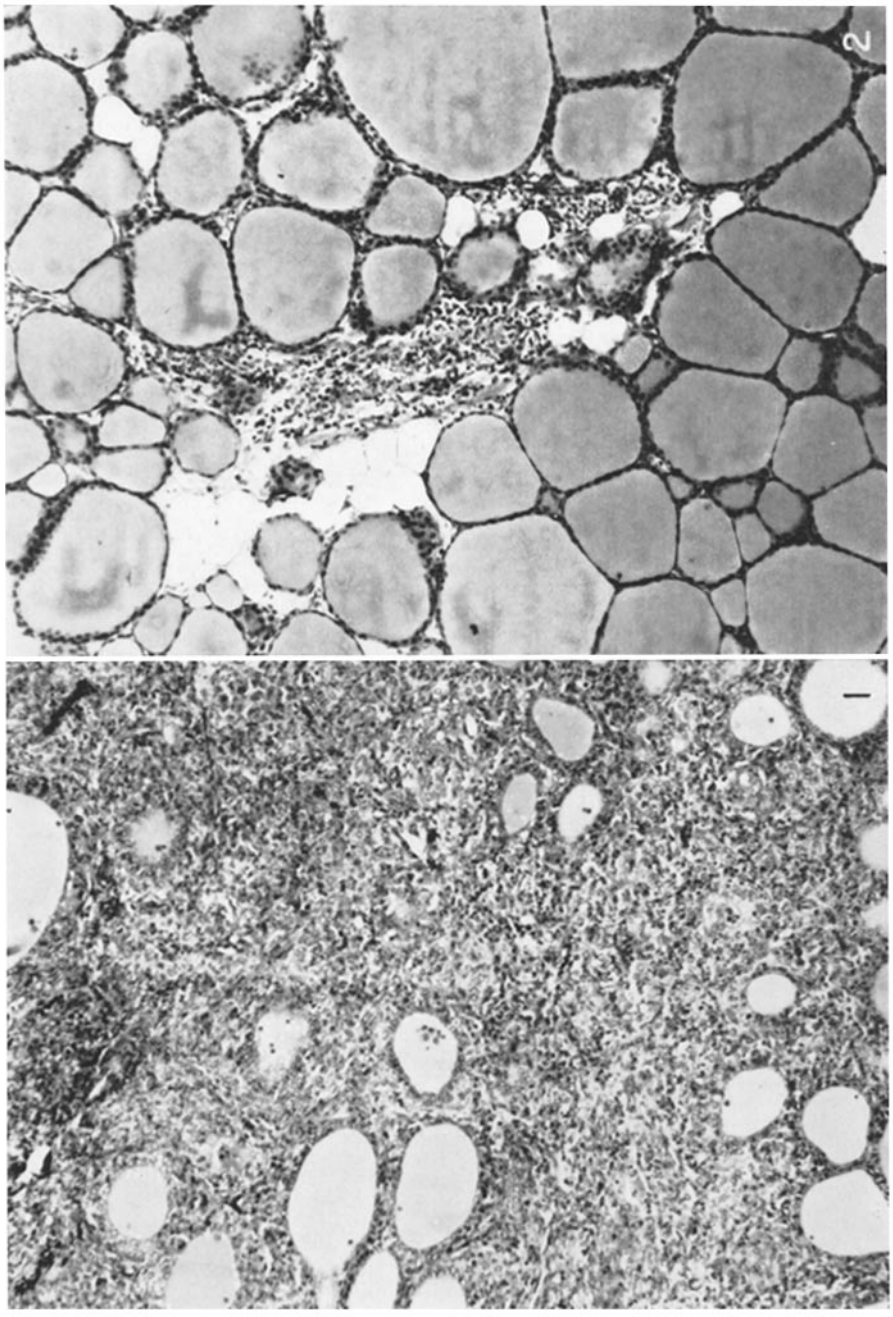
11. Freund, J., Thompson, G. E., and Lipton, M. M., Aspermatogenesis, anaphylaxis, and cutaneous sensitization induced in the guinea pig by homologous testicular extract, *J. Exp. Med.*, 1955, **101,** 591.
12. Freund, J., Stern, E. R., and Pisani, T. M., Isoallergic encephalomyelitis and radiculitis in guinea pigs after one injection of brain and mycobacteria in water-in-oil emulsion, *J. Immunol.*, 1947, **57,** 179.
13. Lipton, M. M., and Freund, J., The efficacy of the intracutaneous route of injection and susceptibility of the Hartley strain of guinea pigs in experimental allergic encephalitis, *J. Immunol.*, 1953, **70,** 326.
14. Waksman, B. H., and Morrison, L. R., Tuberculin type sensitivity to spinal cord antigen in rabbits with isoallergic encephalomyelitis, *J. Immunol.*, 1951, **66,** 421.
15. Paterson, P. Y., Transfer of allergic encephalomyelitis in rats by means of lymph node cells, *J. Exp. Med.*, 1960, **111,** 119.
16. Lipton, M. M., and Freund, J., The transfer of experimental allergic encephalomyelitis in the rat by means of parabiosis, *J. Immunol.*, 1953, **71,** 380.
17. Bauer, J. A., Jr., Histocompatibility in inbred guinea pigs, *Ann. New York Acad. Sc.*, 1958, **73,** 792.
18. Bozicevich, J., Tobie, J. E., Thomas, E. H., Hoyem, H. M., and Ward, S. B., A rapid flocculation test for the diagnosis of trichinosis, *Pub. Health Rep., U.S.P.H.S.*, 1951, **66,** 806.
19. Roitt, I. M., Doniach, D., Campbell, P. N., and Hudson, R. V., Auto-antibodies in Hashimoto's disease (lymphadenoid goitre), *Lancet*, 1956, **2,** 820.
20. Roitt, I. M., and Doniach, D., Thyroid auto-immunity, *Brit. Med. Bull.*, 1960, **16,** 152.
21. Buchanan, W. W., Anderson, J. R., Goudie, R. B., and Gray, K. G., A skin test in thyroid disease, *Lancet*, 1958, **2,** 928.
22. Witebsky, E., Rose, N. R., and Shulman, S., Studies of normal and malignant tissue antigens, *Cancer Research*, 1956, **16,** 831.
23. Hellwig, C. A., and Wilkinson, P. N., Experimental production of thyroiditis, *Arch. Path.*, 1956, **62,** 23.
24. Kracht, J., Thyrotrophin and experimental thyroiditis, *Acta Endocrinol.*, 1955, **18,** 437.
25. Terplan, K. L., Witebsky, E., Rose, N. R., Paine, J. R., and Egan, R. W., Experimental thyroiditis in rabbits, guinea pigs, and dogs, following immunization with thyroid extracts of their own and of heterologous species, *Am. J. Path.*, 1960, **36,** 213.

EXPLANATION OF PLATES

PLATE 68

FIG. 1. Guinea pig thyroid 7 weeks after immunization with antigen and complete adjuvant. Extensive chronic inflammatory infiltrate has replaced many follicles. Lesion graded 4+. Hematoxylin and eosin. × 100.

FIG. 2. Guinea pig thyroid 5 days after immunization with antigen and complete adjuvant. Small focal lesion, composed of chronic inflammatory cellular elements, with little or no destruction of follicles. Lesion graded 1+. Hematoxylin and eosin. × 100

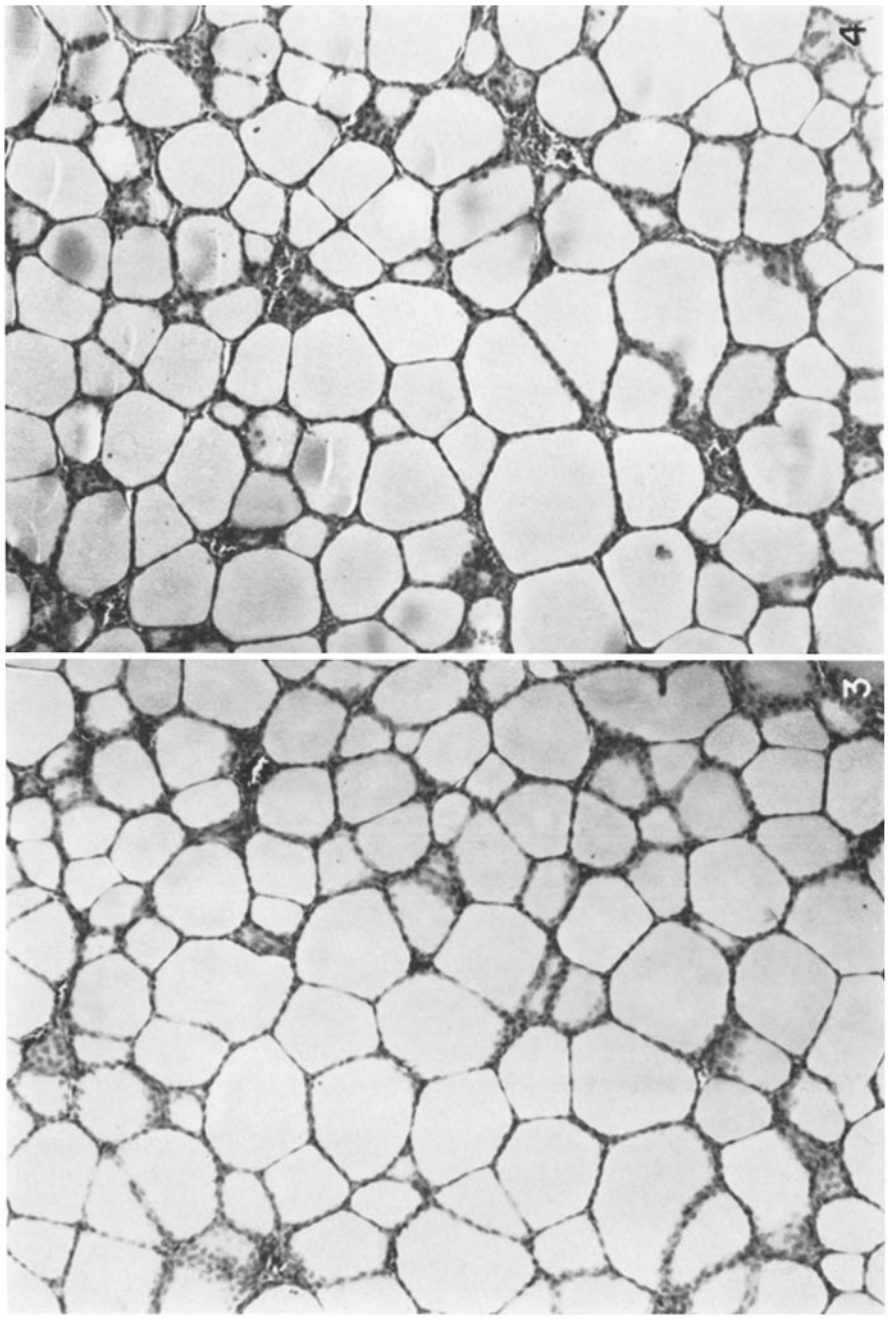


(McMaster *et al.*: Experimental allergic thyroiditis)

PLATE 69

FIG. 3. Guinea pig thyroid 7 weeks after immunization with antigen and incomplete adjuvant. No inflammatory lesions. Hematoxylin and eosin. × 100.

FIG. 4. Guinea pig thyroid from untreated animal. Hematoxylin and eosin. × 100.



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