THE COURSE OF EXPERIMENTAL AUTOALLERGIC THYROIDITIS IN INBRED GUINEA PIGS

The Pathologic Changes and Their Relationship to the Immune Response over a 2 Year Period

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Interest in autoimmune diseases of humans has prompted extensive investigations of comparable phenomena in laboratory animals. The most clearly defined experimental models are allergic encephalomyelitis (1–7), aspermatogenesis (8, 9), and thyroiditis (10–13). In these diseases, specific organ damage has been established as result of actual autoimmunization (14). Witebsky and coworkers first produced experimental allergic thyroiditis and demonstrated its autoimmune nature by immunizing rabbits with portions of their own thyroid glands (11, 13). This has provided a system in which a well defined disease of the thyroid can be produced by immunization with crude or purified preparations of the same organ.

Many of the attempts to define the role of the components of the immune reaction in the three abovementioned autoallergic diseases have shown a greater correlation of the disease with delayed sensitivity than with circulating antibody. The relationship of delayed sensitivity and of circulating antithyroid antibody to allergic thyroiditis has been studied in strain 13, inbred, histocompatible guinea pigs (15–17). The immunologic homogeneity of these animals offers several advantages, including the use of their thyroid extracts for determination of circulating and fixed tissue antibodies without interference by heterologous antigens. This report describes the development and entire course of allergic thyroiditis in strain 13 guinea pigs, following a single immunization with thyroid antigen, and compares the pathologic changes at several intervals with parameters of the immune response over a period of more than 2 years.

Materials and Methods

Animals.—Strain 13 guinea pigs (18) weighing 400 to 600 gm, and strain 2, Hartley, and National Institutes of Health general purpose guinea pigs weighing 400 to 800 gm were used.

Thyroid Antigens.—Guinea pigs were killed with chloroform or ether, the thyroids excised, trimmed of fat, cooled to 4°C, and cut into small pieces. The tissue was ground with 0.85 per cent NaCl in a TenBroeck grinder, centrifuged at 3000 RPM at 4°C for 1 hour, the sediment was discarded, and the saline extract was centrifuged again at 3000 RPM for 1 hour to eliminate any fat and further precipitate.

Antigens for antibody titration and for skin testing were made exclusively from thyroids of strain 13 guinea pigs. The extracts for antibody titration were made to a concentration of 1 gm of tissue per 5 ml of saline; the extracts for skin testing were made to a concentration of 1 gm of tissue per 2.5 ml of saline.

Antigens for immunization were made from pooled thyroids of Hartley, National Institutes of Health general purpose, and strain 2 guinea pigs because of the paucity of strain 13 animals. The extracts for immunization were made to a concentration of 1 gm of tissue per 5 ml of saline.

Antisera from strain 13 guinea pigs immunized with these pooled extracts gave only one precipitin line in double diffusion agar gel when tested against strain 13 thyroid antigen.

Immunization.—Complete Freund's adjuvant was composed of 20 per cent arlacel A (especially purified vacuum-stripped lot 7B),[1] 80 per cent bayol F, and 4 mg/ml *Mycobacterium tuberculosis* H37Rv. Water-in-oil emulsions of antigens and adjuvant were prepared by adding a suspension or solution of the antigen, one or two drops at a time, to the adjuvant. After each addition, the antigen-adjuvant mixture was drawn 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.

This procedure was followed for immunizations with the 1:5 saline extract of thyroid, and for control immunizations with 1 per cent ovalbumin[2] in buffered saline, 1 per cent bovine gamma globulin–diazoarsanilic acid in buffered saline, and 50 per cent saline extract of guinea pig testis.

All animals received 1 ml of antigen-adjuvant emulsions intradermally *via* a 27 gauge needle, distributed in approximately 30 sites in the hindfoot-pads, the hind legs, and the rump.

Bentonite Flocculation Test for Antithyroid Antibody.—Antithyroid antibody, as well as antibodies to certain other antigens used in these experiments, were determined and titrated by a modification of the bentonite flocculation test of Bozicevich *et al.* (19).

To obtain clear serum for the test, blood was drawn by cardiac puncture from ether-anesthetized animals, allowed to clot, the serum was separated by two centrifugations, and inactivated at 56°C for ½ hour. Any precipitate was discarded and the fluid was stored at −20°C. For use it was thawed and centrifuged once more at 3000 RPM for ½ hour at 4°C, to eliminate any particles which could interfere with the test.

Serum samples were diluted twofold with 0.85 per cent NaCl containing 2 per cent normal strain 13 guinea pig serum, pretreated as above. Titrations began with undiluted serum or with a 1:10 dilution, depending on the level of antibody. A fresh chemically clean pipette was used to make each dilution and to place 0.1 ml of the dilution within one of the 9 wax rings on a 3 × 4 inch glass slide.

The thyroid antigen for antibody titration was prepared as noted above, made to a concentration of approximately 0.2 gm of crude tissue per ml before extraction. The final extract was inactivated at 56°C for ½ hour, and centrifuged at 3000 RPM for ½ hour to eliminate any precipitate.

Other antigens used as controls were dissolved in saline to a concentration of 10 mg/ml,

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[1] Atlas Powder Co., Wilmington.

[2] Nutritional Biochemicals Corporation, Cleveland.

inactivated, and centrifuged as above. The pH was adjusted close to neutrality with NaOH before heating to prevent precipitation of some antigens.

The stock solution of bentonite particles was prepared according to the procedure of Bozicevich *et al.* (19), and stored no longer than 1 week at 4°C. 10 ml of the stock bentonite suspension was centrifuged at 3000 RPM for 15 minutes, the supernatant distilled water discarded, and the particles were resuspended by tapping the bottom of the tube vigorously. 0.5 ml of thyroid extract or 1 ml of other antigen solution was added, and the tube gently swirled. 20 minutes later, the tube was centrifuged at 1200 RPM for 10 minutes, the liquid decanted, the particles suspended by tapping the tube as above, and 1 ml of normal strain 13 serum, inactivated as above, was added and mixed with the particles. Excess antigen was then removed by centrifugation at 1500 RPM for 15 minutes and resuspension of the mixture in 10 ml of distilled water. This washing was done three times, and the final suspension of the particles in each tube was made up to 5 ml in distilled water.

For the titration, one drop of the coated washed bentonite suspension was added to 0.1 ml of the antiserum dilutions, previously placed, as noted above, within a wax ring on a glass slide. The slide was then rotated at 90 RPM on a Boerner rotator for 22 to 25 minutes and read with a microscope at × 100. Aggregation of the particles was considered a positive reaction, and the percentage of particles aggregated was estimated (19). When the particles remained freely suspended the reaction was considered negative.

Skin Tests for Hypersensitivity.—0.1 ml of antigen as prepared for skin testing was injected intradermally on a pigment-free area of the flank. The response was measured in most cases at 1, 2, 3, 4, 6, and 24 hours, and occasionally at later intervals.

Immediate hypersensitivity: Immediate reactions were graded at 2 hours as 1+ when the average of the maximal and minimal diameters of soft swelling was 1.5 to 3 times greater than in the non-immunized controls. When this average was more than 3 times greater than the controls the reaction was graded 2+.

Delayed hypersensitivity: At 24 hours, 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+.

"Intermediate" reaction: A previously unrecorded type of reaction was observed in some long term animals; *i.e.*, those which had been maintained for 6 months or longer after immunization. This was an erythematous reaction which appeared later than an immediate, and disappeared earlier than a delayed, reaction. Such erythematous reactions were generally noted at 6 hours after skin testing, but not at 1 or 2 hours, and had faded or disappeared at 24 hours. This erythema often followed by a few hours an immediate reaction of soft swelling.

Histologic Technique and Grading of Thyroiditis.—The right and left lobes of the thyroid glands of ether-killed guinea pigs were separately fixed in neutral buffered formalin and embedded in paraffin. Serial sections were cut at 6 microns through the long axis of each lobe of the thyroid until 0.1 to 0.4 mm of the tissue had been sectioned, and were stained with hematoxylin and eosin, buffered Giemsa, and periodic acid–Schiff stains.

The diagnosis and grading of thyroiditis were restricted to the presence and extent of actual inflammatory cellular reaction. The colloid and cellular content of thyroid follicles, alterations in size or shape of follicles, and other architectural changes were not considered in the determination of thyroiditis.

Lesions were graded 1+ if one or several foci of leukocytes appeared in excess of the normal control (most of the controls showed no inflammatory changes, although some contained rare minute foci of very few leukocytes). Lesions were graded 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 to ½ of the gland, the lesions were graded 3+. If they occupied approximately ½ or more of the gland, lesions were graded 4+.

RESULTS

For this work a total of 226 strain 13 guinea pigs (18) was used. 147 animals were immunized only once with thyroid extract emulsified with complete Freund's adjuvant, and sacrificed at intervals from 3 days to 26 months after immunization. For comparison, 79 animals served as controls, and were immunized with various antigens other than thyroid, or were not immunized.

TABLE I

Thyroiditis in Guinea Pigs Immunized with Thyroid Antigen

Degree of disease	Time after immunization										
	3 days	5 days	7–12 days	16 days	3–5 wks.	6–7 wks.	10–14 wks.	6 mos.	13 mos.	18 mos.	26 mos.
0	12*	27	7							1	
1+		5	3	9	2	2		4	7	6	2
2+				4	3	7	2	4	3	2	1
3+				2	1	9	6	1		1	
4+				1		8	4	1			
Total positive/total tested	0/12	5/32	3/10	16/16	6/6	26/26	12/12	10/10	10/10	9/10	3/3

* Numbers indicate No. of animals.

Histologic Findings.—

Animals Immunized with Thyroid Antigen (Summarized in Table I).—

3 days after immunization: Of 12 guinea pigs from 2 separate groups, none showed thyroiditis.

5 days after immunization: Of 32 guinea pigs from 5 separate groups, 5 showed mild, grade 1+ thyroiditis. The lesions were small and focal, and they were either single or multiple.

In the thyroids showing single focal lesions the foci contained more than 100 inflammatory cells; in those with multiple foci there were one or more collections of 50 to 100 cells accompanied by one or more aggregates of 25 to 50 cells, and other smaller foci of 10 to 25 cells. The cellular infiltrate was predominantly lymphocytic, with varying numbers of accompanying macrophages and plasma cells (Fig. 2). Eosinophilic and neutrophilic polymorphonuclear leukocytes were present in varying proportions, and occasionally either was the predominant cell type. This feature was noted only in the early stages (5 to 12 days), where the composition of the cellular infiltrate was much more variable than at later stages. In the early developing lesions, the perivascular distribution of the in-

filtrate was obvious, a feature which generally became obscured as the disease progressed to a more extensive involvement. In general, there was little or no destruction of follicles, distortion of glandular architecture, or fibrosis at this stage (Figs. 1 and 2).

Rarely glands from control animals 3 to 5 months old contained occasional minute foci of chronic inflammatory cells. These foci usually numbered one or two, never in excess of three or four; they usually contained 5 to 10, and occasionally 10 to 15 cells. Such thyroids were considered normal. Structural changes in the thyroid such as alterations in size and shape of follicles, follicular epithelium, and in colloid and cellular content of follicles, were seen in both untreated and immunized animals. However, such structural changes were far more frequently found accompanying inflammatory lesions, and appeared to be a sequel of these.

7 to 12 days after immunization: Of 10 animals sacrificed during this period, 3 showed mild, grade 1+ thyroiditis; none of 2 at 7 days, 1 of 4 at 9 days, and 2 of 4 at 12 days. The lesions were generally comparable to those described at the 5 day stage, although there were a few more foci at 9 and 12 days.

16 days after immunization: All of 16 immunized animals from four separate groups showed thyroiditis. The degree of severity was generally greater than that seen earlier, ranging from mild grade 1+ to the most severe grade 4+ (Table I). The grade 1+ lesions (Figs. 3 and 4) were more extensive than the 5-day lesions and the perivascular distribution of the infiltrate remained obvious. This perivascular distribution was not evident in the more severe grade 3+ lesions (Figs. 5 and 6).

3 to 5 weeks after immunization: All of 6 immunized animals exhibited thyroiditis; 2 each at 3, 4, and 5 weeks. The degree of severity ranged from 1+ to 3+.

6 to 7 weeks after immunization: All of 26 immunized animals from five separate groups had thyroiditis during this period. The lesions in most animals were quite well advanced, with many showing grade 4+ and only 2 showing grade 1+ thyroiditis (Table I).

The histologic appearance of the thyroid at the 6 to 7 week stage was considered to represent the fully developed disease (Figs. 7 and 8), although relatively severe thyroiditis had been seen at some of the earlier stages, in much lower incidence. The inflammatory cellular infiltrate was predominantly chronic, consisting mainly of lymphocytes, macrophages, and plasma cells. Eosinophilic leukocytes in varying numbers and fewer numbers of neutrophilic leukocytes were frequently seen accompanying the round cell infiltrate. The inflammatory reaction was generally severe or moderate, and usually involved the entire gland in diffuse and/or focal distribution. The cellular infiltrate was found between follicles, and replacement or destruction of follicles by the infiltrate was common. A variable amount of follicular destruction was seen in

and about the cellular infiltrates. Phagocytosis of cellular debris within follicles or remnants of follicles was also noted. Fibrosis of slight to moderate degree was occasionally present in the areas of most intense infiltration and destruction.

10 to 14 weeks after immunization: All of 12 animals sacrificed at this period showed thyroiditis; 7 at 10 weeks and 5 at 14 weeks. The lesions at this stage ranged from 2+ to 4+ severity, and were quite similar to those described at the 6 to 7 weeks stage, with slightly more prominent fibrosis and occasional hemorrhage into follicles.

6 months after immunization: All of 10 animals exhibited grade 1+ to 4+ thyroiditis. One showed more severe thyroiditis than had been seen previously, in that there was considerable destruction of glandular architecture and fibrosis, as well as extensive inflammatory cellular infiltration. Two animals showed minimal single lesions (Fig. 9), the first time such slight lesions were seen since the 12 day stage. The remaining 7 animals had lesions intermediate between these extremes.

13 months after immunization: All of 10 animals showed grade 1+ to 2+ thyroiditis (Table I), a marked decrease in the overall extent of disease compared with the lesions seen throughout the 6 weeks to 6 months periods. Many of these milder lesions consisted of single or multiple small focal collections of chronic inflammatory cells, with macrophages somewhat more frequent than had been observed earlier, although lymphocytes were predominant and some plasma cells were also present. Two thyroids showed germinal follicle formation (Fig. 10).

18 months after immunization: 9 of 10 animals showed grade 1+ to 3+ thyroiditis (Table I); this was the first period since the 12 day stage that 100 100 per cent incidence of this disease was not seen. The histologic picture was in general similar to that seen at 13 months. The degree of severity was somewhat comparable to the extent of disease seen at 13 months, except that 1 animal showed more severe disease at this 18 month stage. Germinal follicle formation was seen in one lesion.

26 months after immunization: The 3 animals remaining in this series were sacrificed at 26 months, and all showed grade 1+ to 2+ thyroiditis. The histologic picture was in general similar to that seen in grade 1+ to 2+ thyroiditis at 13 and at 18 months. One thyroid showed germinal follicle formation.

Control Animals (Summarized, in Table II).—All immunizing control antigens were emulsified with complete Freund's adjuvant. Of the immunized control animals, 1 of 9 given bovine gamma globulin–diazoarsanilic acid and 1 of 10 given testicular extract had minimal thyroiditis 7 to 8 weeks after immunization. These lesions were single small foci, unlike the extensive thyroiditis seen at 7 to 8 weeks after immunization with thyroid antigen. A series of 32 guinea pigs immunized with crystalline ovalbumin were sacrificed at intervals over 26 months. From 5 days to 16 months, none of 25 had thyroiditis. However, 3 of 5

at 18 months, and 1 of 2 at 26 months, had small focal lesions which were primarily lymphocytic.

Of 20 non-immunized or skin-tested animals, 3 to 5 months of age, none had lesions. Of 8 old non-immunized guinea pigs, 18 to 26 months of age, 2 had minimal lesions, similar to those seen in the albumin immunized controls. The incidence of lesions in control animals increased with time after immunization or with age.

Moreover, after immunization with ovalbumin and complete adjuvant, the thyroids of 4 of 8 guinea pigs at 16 months and 1 of 5 at 18 months contained occasional small focal collections of histiocytes. The cellular composition of these minute histiocytic foci differed completely from the lesions in the thyroid immunized animals, and from the lesions in the control animals described above.

TABLE II

Thyroiditis in Control Guinea Pigs

Immunizing antigen	Time after immunization	Thyroiditis
Ovalbumin	5 days	0/10*
"	2 mos.	0/2
"	6 "	0/5
"	16 "	0/8
"	18 "	3/5
"	26 "	1/2
Guinea pig testis	7 wks.	1/10
Bovine gamma globulin–diazoarsanilic acid	8 "	1/9
Untreated	3–5 mos.‡	0/10
Untreated	26 " ‡	1/4
None: skin-tested with thyroid extract	3–5 " ‡	0/10
None: skin-tested with thyroid extract and P. P. D.	18 " ‡	1/4
Total		8/79

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

‡ Indicates age. Animals not immunized.

The qualitative distinction between experimental animals with thyroiditis and control animals totally devoid of inflammatory cells, made in nearly all cases up to and including the 13 month stage, became more quantitative at 16 months and later stages.

Of 79 control guinea pigs which had received various types of immunizing stimuli, or no treatment, at stages ranging from 5 days to 26 months, a total of 8 showed lesions of thyroiditis. Most of these lesions were seen in the older animals (Table II).

Immunological Findings.—

Skin Test Reactions in Guinea Pigs Immunized with Thyroid Antigen.—Immediate type skin reactions did not develop at 4 to 5 days after immunization, but were seen in many animals at 7 weeks, the next time interval studied (Table III). They were found in high incidence at 6 and 13 months, in low incidence at 18 months, and had disappeared at 26 months.

Delayed type skin reactions , by contrast, developed in most animals 5 days after immunization, and were present in all animals at 7 weeks. At 6 months, these reactions were found in only 4 of 10 animals, and did not appear at later stages.

At 6 months after immunization, 6 of 10 guinea pigs showed an unusual type of skin reaction. All showed mild edema 1 hour after skin test. Erythema was

TABLE III

Skin Test Reactions in Guinea Pigs Immunized with Thyroid Antigen

Type of Reaction	Time after immunization					
	5 days	7 wks.	6 mos.	13 mos.	18 mos.	26 mos.
Immediate	0/9*	13/18	10/10	10/10	3/10	0/3
Delayed	7/9	12/12	4/10	0/10	0/10	0/3
Intermediate	0/9	0/18	6/10	7/10	8/10	2/3

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

absent at 1 hour, but appeared in 1 animal at 3 hours, and in all 10 at 6 hours; however, in 6 of these 10 guinea pigs the erythematous reaction had faded at 24 hours. This erythema often followed, or became superimposed upon, the slight soft swelling of a weak immediate reaction. The erythematous area was centrally located about the injection site, was circular with a smooth edge, and varied from faint pink to red in individual animals. In these 6 guinea pigs, the appearance of erythema was later than in the usual immediate reaction, and its disappearance was much earlier than in the usual delayed reaction. This was provisionally designated an "intermediate" reaction (see Table III).

At 13 months after immunization, all of 10 guinea pigs showed edema 2 hours after skin test. Again, none showed erythema at 2 hours. At 4 hours 6 of 10, and at 6 hours 7 of 10, showed erythema, and at 24 hours the erythematous reaction had faded in all animals. At 18 months after immunization, 3 of 10 animals showed edema at 1 hour. Erythema was absent at 1 hour, but did appear in 5 of 10 at 3 hours, and was present in 8 of 10 at 6 hours, and this reaction had faded in all animals at 24 hours. The erythematous reactions seen at 13 and 18 months were similar to those noted above for 6 months.

At 26 months, none of 3 animals showed any skin reaction at 1 hour, whereas 2 of 3 showed mild edema and erythema at 4, 10, and 13 hours, which reactions had disappeared by 24 hours.

All control animals immunized with antigens other than thyroid extract gave mixed skin reactions with immediate plus delayed components, when tested with the corresponding antigen 7 weeks to 18 months after immunization.

Circulating Antibody in Guinea Pigs Immunized with Thyroid Antigen.— Antibody was not found at 3 and 5 days after immunization (Table IV). It was first detected at 7 days in relatively low titer, and was present in all animals from 7 days to 10 weeks after immunization. Levels were relatively low at the early stages, increased to a maximum at 7 weeks, and began to decrease at 14

TABLE IV

Circulating Antithyroid Antibody in Guinea Pigs Immunized with Thyroid Antigen

Reciprocal of antibody titers	Time after immunization										
	3 days	5 days	7–12 days	16 days	3–5 wks.	6–7 wks.	10–14 wks.	6 mos.	13 mos.	18 mos.	26 mos.
Neg.	12*	32					2			1	
1			3					1	3	4	2
2								3	1	2	
4			2						3	1	1
10			3			2		3	2	2	
20			2	4		3		1	1		
40				9	3	10	2	1			
80				3	2	8	1				
160					1	2	3				
320						1					
Total pos./total tested	0/12	0/32	10/10	16/16	6/6	26/26	6/8	9/9	10/10	9/10	3/3

* Numbers indicate No. of animals.

weeks. Antibody persisted, although in low titers, in nearly all animals at the later stages, and was still detectable more than 2 years after a single immunization with thyroid antigen.

Sera of control animals, immunized with antigens other than thyroid extract, never gave a positive test for antithyroid antibody. Those control sera tested with the antigen used to immunize reacted in high titer, at all periods later than 5 days after immunization, and relatively high titers of antialbumin antibodies persisted for more than 2 years after immunization.

DISCUSSION

Experimental allergic thyroiditis was produced in strain 13 histocompatible guinea pigs by immunization with a single dose of guinea pig thyroid extract emulsified in complete Freund's adjuvant. Lesions appeared as early as 5 days

after immunization, the shortest time interval recorded for their induction. The disease persisted for at least 2 years, the longest reported duration of experimental allergic thyroiditis. The extent and severity of the disease progressed rapidly, and reached a maximum at 6 to 8 weeks. Relatively severe thyroiditis continued as long as 6 months after immunization. However, at this time a few animals showed small, focal lesions. At 13 months and later, most animals showed much less severe disease. The slight lesions at the 6 month stage were interpreted as a regression of the severe disease observed earlier, rather than a failure of the disease process to develop, because nearly all animals studied throughout the 3 week to 3½ month stage had relatively severe thyroiditis. The findings at later stages were consistent with this view.

Thyroiditis had developed in all animals examined from 16 days to 13 months after immunization with thyroid extract. At 18 months, 90 per cent still had thyroiditis, and at 26 months all of 3 showed thyroiditis. However, at 18 months, some guinea pigs immunized with ovalbumin and 1 non-immunized animal showed mild lesions of thyroiditis indistinguishable from the disease in animals immunized with thyroid extract. At 26 months, some albumin immunized controls, as well as untreated animals of the same age also had lesions, although in lower incidence. The occurrence of thyroiditis in the various types of control animals at 18 and 26 months of age made the distinction between these and the thyroid antigen–induced lesions more difficult. Nevertheless, there were unequivocal differences in the occurrence and severity of the lesions between the control and experimental groups at these late stages.

Previously, the disparity between the short duration of experimental thyroiditis in some species and the long duration of Hashimoto's disease in man has caused most investigators to refrain from drawing comparisons between the two. The demonstration that the disease can persist in experimental animals for 26 months, nearly half the life span of the guinea pig, once more invites further speculation upon a similar pathogenetic mechanism being operative in these two diseases. The chronic, primarily lymphocytic, thyroiditis described here is not unlike some cases of chronic thyroiditis in humans classified as Hashimoto's thyroiditis. However, the relatively limited types of histologic response which can occur may give similar or even identical pathologic patterns resulting from entirely different stimuli. Conversely, the histologic response of the two species to a given stimulus may conceivably be widely different. The short course of the disease in the rat as opposed to the guinea pig may be due to species differences. In like fashion, this may account for the differences in the appearance and course of the disease in the human and the guinea pig.

The prolonged duration of thyroiditis in these strain 13 guinea pigs is in marked contrast to the reports of early resolution of the disease in guinea pigs (20), rats (21), and other species. Several factors other than species or strain may account for this difference: the use of a single immunization; the proportion

of oil-to-water phase in the immunizing emulsion giving it a very high viscosity; the specially purified arlacel used in the adjuvant; the use of *Mycobacterium tuberculosis* rather than *Mycobacterium butyricum* in the adjuvant; the large amount of antigen employed; an the great number of intradermal injections given at one time.

The restriction to a single immunization appears to be critical, for, as Janković has shown (22, 23), and as we have observed, *vide infra*, repeated antigenic stimulation may reduce the incidence and severity of the disease. The viscosity of the emulsion is a possible factor in our results. It was very much higher than that customarily employed; however, on standing, it tended to break down more rapidly. Its rapid breakdown is a possible cause of the more rapid decline in skin tests and antibody titers to thyroid extract than was seen in the albumin immunized controls, which were injected with a more stable emulsion.

Skin test reactions and the levels of circulating antibody of these strain 13 guinea pigs were measured at intervals during the course of the disease. These measurements, made using thyroid antigen from the same histocompatible strain, provided a system free of extraneous antigens (15–17) which might interfere The skin responses and antibody titers reported here are therefore considered to be specifically reactive to the antigenic material from the thyroid.

Immunization with thyroid extract in complete adjuvant consistently yielded delayed skin reactions which appeared before or simultaneously with the disease. Thereafter the two progressed in parallel fashion for several months, during which time all animals with thyroiditis that were skin-tested exhibited delayed sensitivity. Immunization with thyroid extract in incomplete adjuvant produced neither disease nor delayed sensitivity (15, 16). Apparently the presence of mycobacteria in the adjuvant leads not only to delayed sensitivity but to the development of the disease as well. Whether these two results of mycobacteria in the adjuvant are independent, or whether the delayed reaction causes the disease remains moot. On the basis of a general correlation between delayed sensitivity and the early disease, such a causal relationship has been suggested by the authors (15–17). This has been reaffirmed by others, who picrylated the thyroglobulin to reduce the antibody titer (24), compared immunized animals treated with 6-mercaptopurine with those not so treated (25), and reduced both the delayed reaction and the disease by injecting the animal with complete adjuvant before giving the usual immunization (23, 26). From these various approaches it appears reasonable to consider delayed sensitivity as one of the more important factors, if not the major factor, in the development of the disease.

Moreover, when the disease began to regress, *i.e.* at 6 months, the general level of delayed sensitivity was reduced, for at this time only some animals continued to give typical delayed reactions. Thus, changes in the delayed reaction

appeared to parallel changes in the disease. By 13 months the typical delayed reaction had disappeared, and did not reappear, while the severity of the disease had declined further. Nevertheless, the disease continued, and even outlasted the delayed reaction by as much as 18 months (Table V).

Those guinea pigs which did not give a typical delayed reaction at 6 months, as well as those tested later, gave a modified skin reaction, in that the time of appearance of erythema, superimposed on mild soft swelling, was that of a delayed reaction, but its disappearance was more rapid. The accelerated disap-

TABLE V

Thyroiditis, Circulating Antithyroid Antibody, and Skin Tests in Guinea Pigs Immunized with Thyroid Antigen

Time after immunization	Thyroiditis	Range	Antibody	Range	Skin test		
					Immediate	Delayed	Intermediate ¶
3 days	0/12*	—	0/12*	—			
5 "	3/32	0–1+‡	0/32	—	0/9*	7/9	0/9
7–12 "	3/10	0–1+	10/10	1–1:20§			
16 "	16/16	1+–4+	16/16	1:20–1:80			
3–5 wks.	6/6	1+–3+	6/6	1:40–1:160			
6–7 "	26/26	1+–4+	26/26	1:10–1:320	13/18	12/12	0/18
10–14 "	12/12	2+–4+	6/8	0–1:160			
6 mos.	10/10	1+–4+	9/9	1–1:40	10/10	4/10	6/10
13 "	10/10	1+–2+	10/10	1–1:20	10/10	0/10	7/10
18 "	9/10	0–3+	9/10	0–1:10	3/10	0/10	8/10
26 "	3/3	1+–2+	3/3	1–1:4	0/3	0/3	2/3
Total	100/147 (68.7%)		95/142 (66.9%)				

* No. of animals positive/total No. tested.

‡ Degree of severity.

§ Bentonite flocculation titer.

pearance became even more marked at 13 and 18 months, for there was no visible erythema 24 hours after skin test injection. This modified skin reaction was termed intermediate for descriptive rather than categorical purpose. If this were a distinct type of reaction, rather than an altered immediate or delayed, it would be masked by the erythema of the immediate or delayed as long as either reaction persisted, and only then appear, as did occur. It is not known whether this modified skin reaction represents a retarded immediate, an accelerated delayed, or a third type of sensitivity reaction intermediate between the first two. Whatever its nature, it persisted in many animals until the 26 month stage, and thus lasted as long as the disease.

Skin test reactions with an erythematous component which appeared late but disappeared by 24 hours have been noted by Chase in guinea pigs which had been passively sensitized with serum to picryl chloride (27). Certain of the skin reactions in guinea pigs reported by Kaplan and Dienes (28) bear some relation to these reactions described above. That repeated skin testing could result in an accelerated delayed reaction was noted by Freund (29) and Edwards and Magnus (30), and the former predicted that in an experimental autoimmune disease the delayed reaction would become accelerated as time after immunization increased.

In contrast to the delayed reaction, immediate sensitivity reactions and circulating antibody did not correlate with the early developing phase of the disease. In some cases the disease even appeared before either was detectable. At 7 to 8 weeks, however, a good correlation between the disease and the antibody titer did exist. Thereafter the antibody titer gradually fell, eventually reaching low levels, without any apparent relation between the titer and disease. However, antibody did persist long after the delayed reaction had disappeared, and continued as long as the disease. As might be expected from the antibody titers, the immediate skin reaction lasted through the 18 month stage (Table V).

Other attempts to correlate the disease with antibody were not successful. Immunization with incomplete adjuvant and thyroid extract led to low antibody titers but did not cause disease (15, 16). Such immunization, boosted by repeated skin testing with thyroid extract led to titers comparable to the highest seen in diseased animals, but still did not result in disease or delayed hypersensitivity. Furthermore, repeated skin testing of guinea pigs after immunization with thyroid extract and complete adjuvant resulted in generally high titers, but 9 of 10 animals had neither disease nor delayed reaction, while one had both (31). Janković (22) also noted a lack of correlation of antibody with disease following treatment similar to the above. The finding that 6-mercaptopurine also reduces the severity of the disease but not the antibody titer adds to the evidence that circulating antibody does not correlate with the disease. Failures to transfer the disease with serum antibody support the view that circulating antibody is not by itself capable of producing the disease. Whether it contributes to the severity of the disease or maintains it once established, remains to be determined.

SUMMARY

Experimental allergic thyroiditis produced in strain 13 histocompatible guinea pigs after a single immunization with thyroid extract and Freund's adjuvant was followed for more than 2 years. The disease appeared as early as 5 days and persisted for the entire period studied, although it regressed in the later stages.

Circulating antithyroid antibody was detected at low levels as early as 7

days after immunization, and increased to a peak at the time of most severe disease. Thereafter, antibody decreased, but was still detectable in most animals as late as 2 years. There was no correlation between antibody levels and extent of disease except at the 7 week stage.

Delayed sensitivity to thyroid antigen was found as early as 5 days after immunization, and appeared to precede the development of thyroiditis in many animals. It correlated closely with thyroiditis at 5 days and 7 weeks. At 6 months, the delayed skin reaction was decreased, and a modified type of reaction appeared which persisted as long as 26 months.

The time relationship of delayed sensitivity, thyroiditis, and circulating antibody continue to confirm the role of delayed sensitivity in the pathogenesis of this disease. The accumulated data demonstrating production of thyroiditis without antibody, and the converse, tend to strengthen this view.

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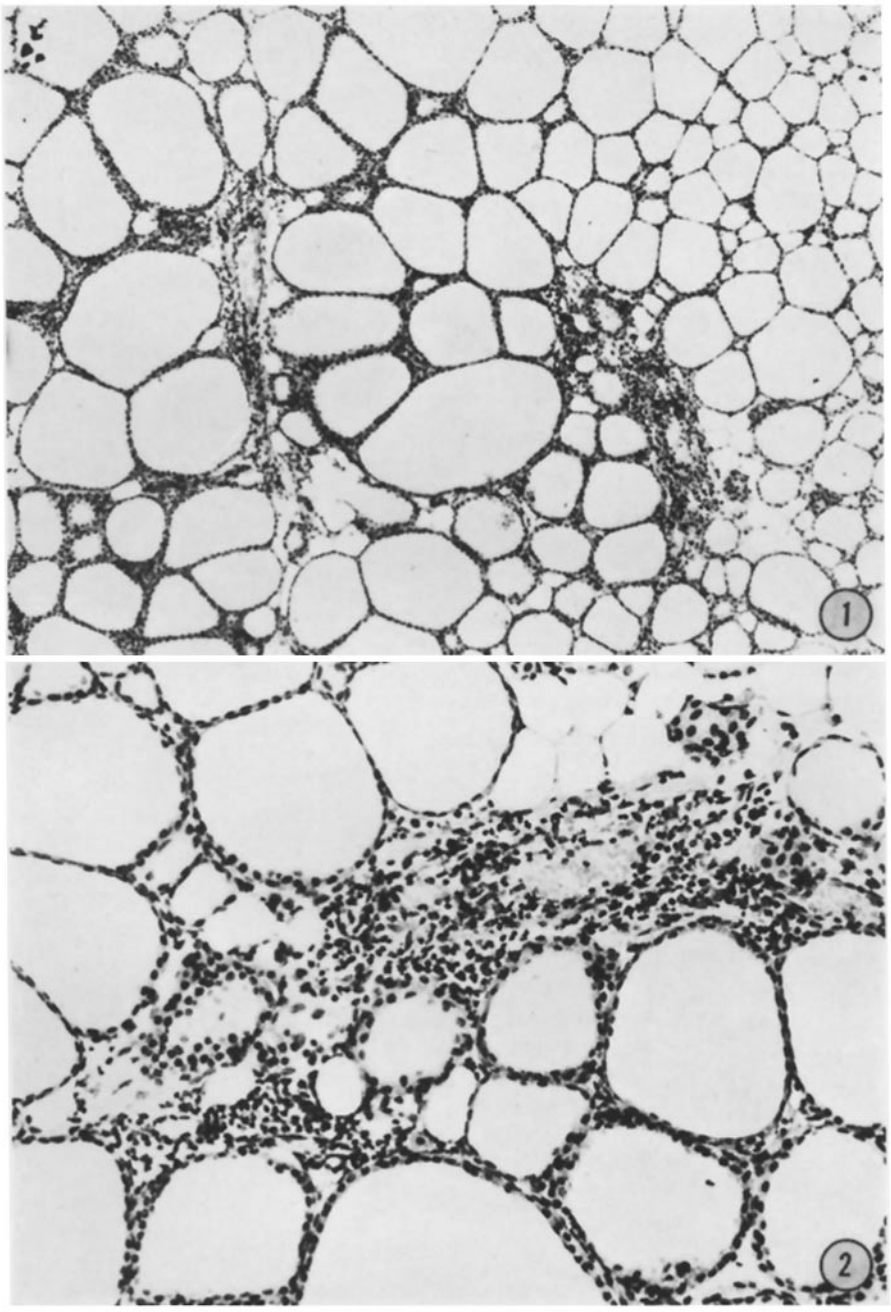
EXPLANATION OF PLATES

All photomicrographs were prepared from sections stained with hematoxylin and eosin. These were made on Kodak process panchromatic film, using a Kodak wratten filter No. 25 (A), with a transmission of 600 to 700 mμ, and with a dominant wavelength of 617.2 mμ. As result, nearly all colloid in thyroid follicles and nearly all red blood cells were not apparent.

PLATE 32

FIG. 1. 5-day-old lesion. Two small inflammatory foci. Perivascular distribution of infiltrate is apparent in one. Remainder of gland appears normal. Thyroiditis grade 1+. × 55.

FIG. 2. Greater magnification of Fig. 1. Small focus of chronic inflammatory cells, predominantly lymphocytes. No destruction of follicles. × 155.

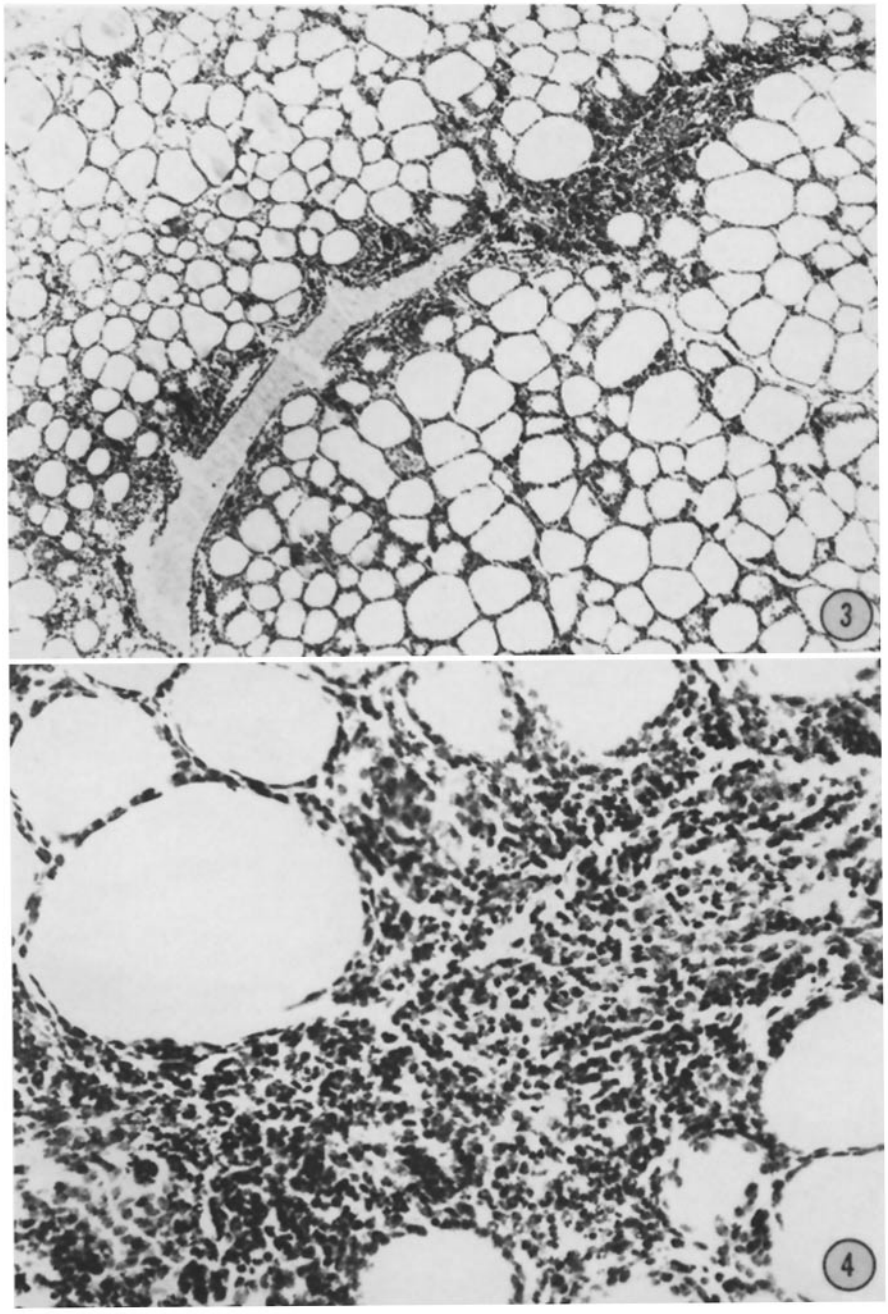


(Lerner *et al.*: Autoallergic thyroiditis)

PLATE 33

FIG. 3. 16-day-old lesion. Small proportion of gland is involved. Perivascular location of infiltrate is prominent. Thyroiditis grade 1+. × 55.

FIG. 4. Greater magnification of Fig. 3. Large focus of chronic inflammatory cells, predominantly lymphocytes. Little destruction of follicles. × 205.

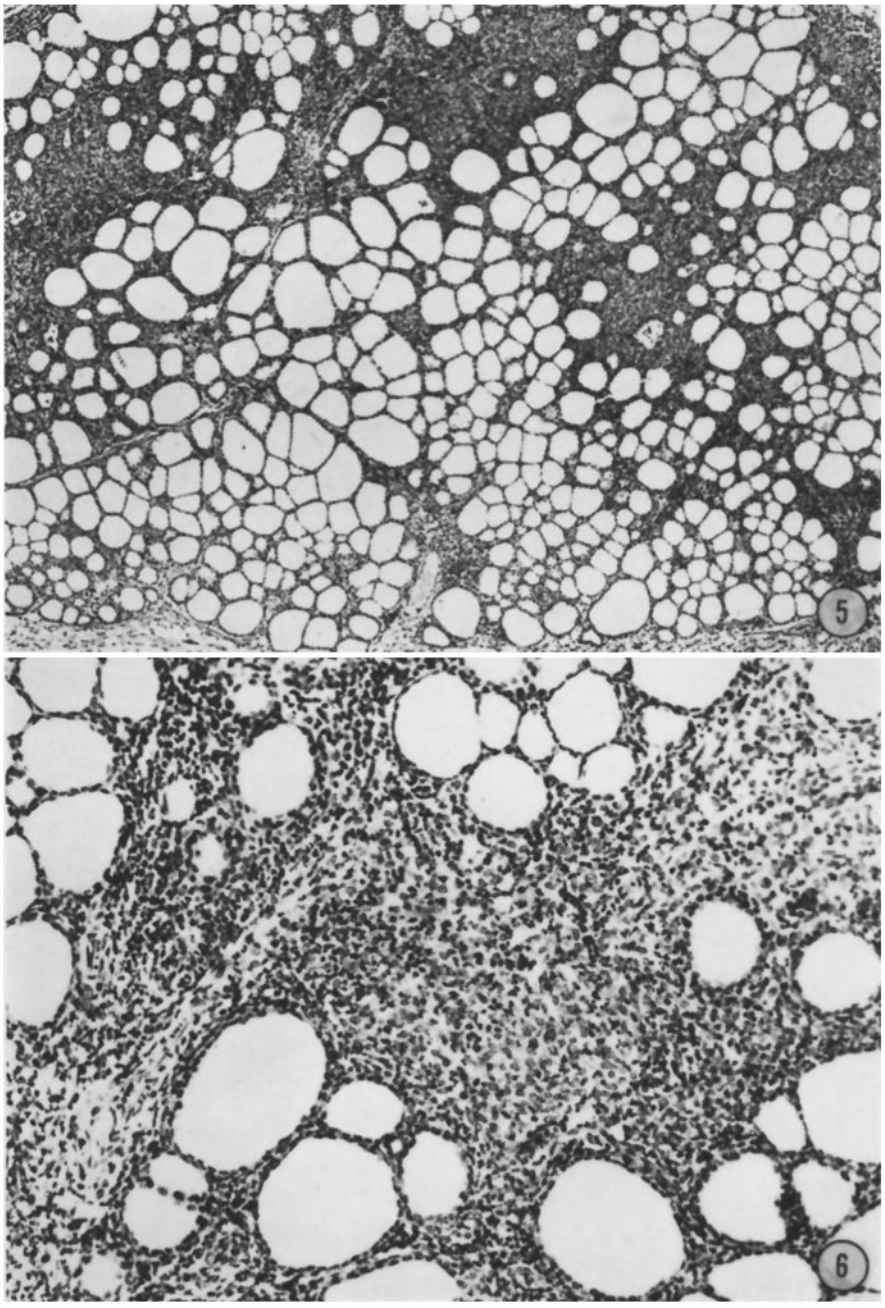


(Lerner *et al.*: Autoallergic thyroiditis)

PLATE 34

FIG. 5. 16-day-old lesion. Relatively large proportion of gland is involved by focal and diffuse inflammatory process. Some follicular destruction. Thyroiditis grade 3+. × 39.

FIG. 6. Greater magnification of Fig. 5. Considerable focal and some interstitial chronic inflammatory infiltrate. Many lymphocytes and moderate number of macrophages. Some replacement of follicles. × 120.

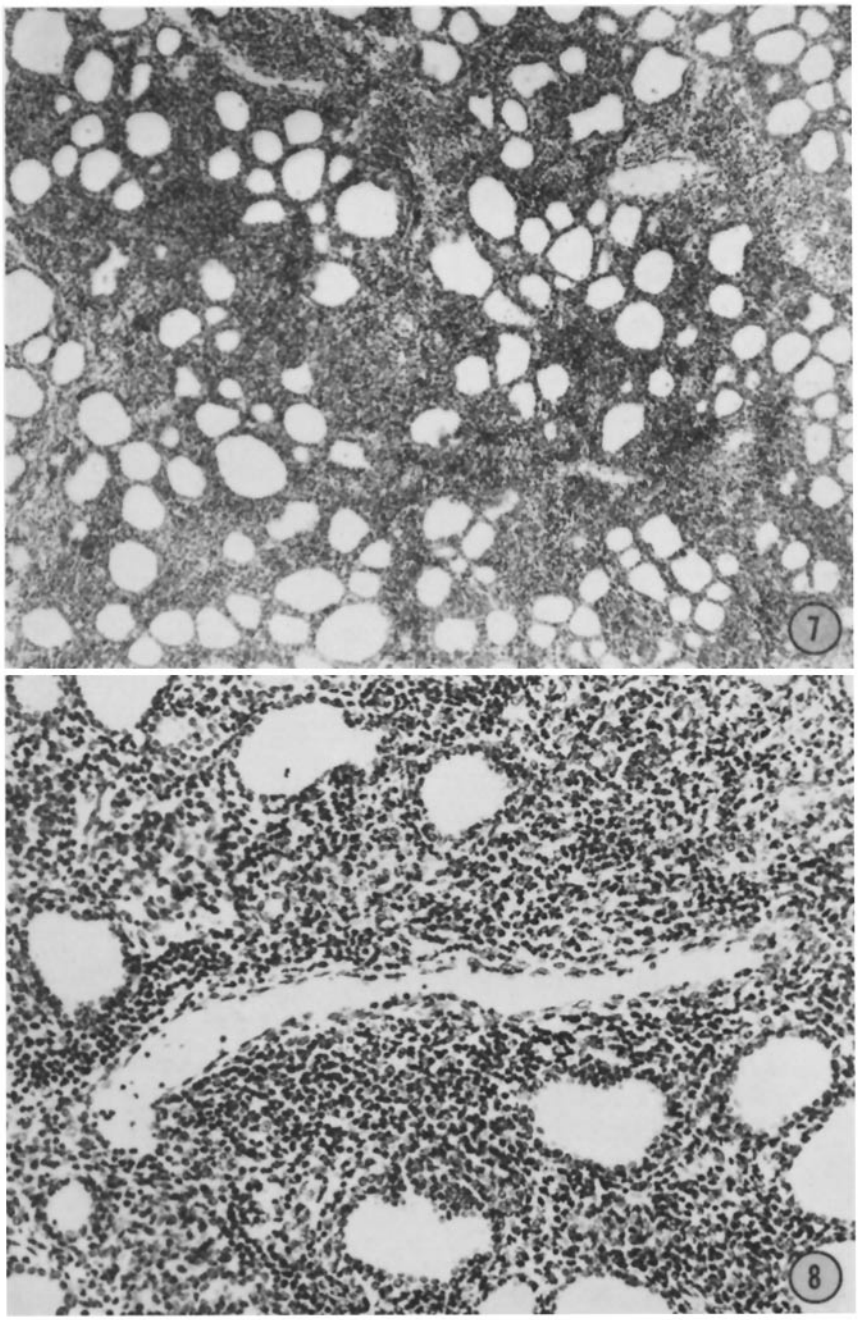


(Lerner *et al.*: Autoallergic thyroiditis)

PLATE 35

FIG. 7. 7-week-old lesion. Widespread, severe, focal and diffuse inflammatory process. Considerable destruction of follicles and obliteration of glandular structure. Blood vessels are apparent in areas of greatest reaction. Thyroiditis grade 4+. × 55.

FIG. 8. Greater magnification of Fig. 7. Extensive focal and diffuse inflammatory infiltrate, predominantly lymphocytes. Considerable destruction of follicles. Note prominent blood vessel in center of reaction. × 155.

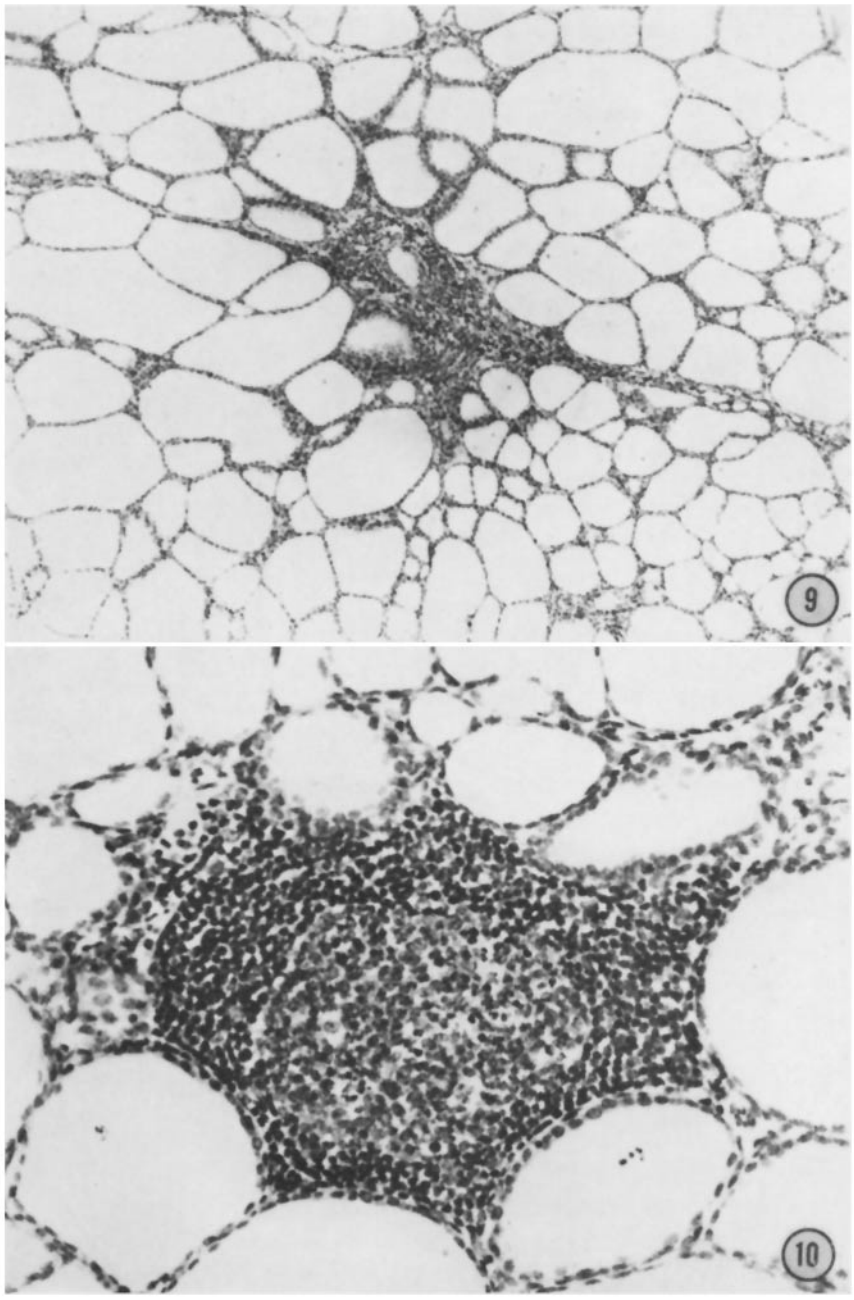


(Lerner *et al.*: Autoallergic thyroiditis)

PLATE 36

FIG. 9. 6-month-old lesion. Small focal area of inflammatory reaction. Minimal destruction of follicles in area of reaction. Remainder of gland appears essentially normal. Note resemblance to Fig. 1. Thyroiditis grade 1+. × 55.

FIG. 10. 13-month-old lesion. Germinal follicle formation in center of chronic inflammatory focus. Thyroiditis grade 1+. × 200.



(Lerner *et al.*: Autoallergic thyroiditis)