Studies on the Pathogenesis of Experimental Immune Thyroiditis.* (26519)

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Immunization with homologous or autologous thyroglobulin is followed in a high proportion of experimental animals by a chronic inflammatory reaction in the thyroid gland, which bears some resemblance to human chronic lymphocytic thyroiditis(1,2). It has been established that thyroglobulin is an antigen capable of producing this experimental autoimmune disease. Whereas much investigation has been devoted to the question of the antigenicity of thyroglobulin, the serology of antithyroglobulin antibody and the course of this experimental immune thyroiditis, the pathogenetic mechanism has not been elucidated(3,4). Circulating antibody directed against thyroglobulin has been considered to be an important pathogenic factor; however, the correlation between circulating antibodies and incidence or degree of thyroiditis is at best vague. In addition, it has been impossible to transfer the disease passively with massive doses of high titer antithyroglobulin serum.

The experiments described here were designed to study the problem of whether incidence of experimental immune thyroiditis in the guinea pigs bears a closer relationship to formation of circulating antibodies or to delayed type of hypersensitivity against thyroglobulin. Use was made of the fact that when minimal amounts of the immunizing antigen are heavily conjugated with a haptene (picryl chloride), serum antibody production is decreased without interfering with the ability of the antigen to induce delayed type of hypersensitivity.

Materials and methods. Thyroglobulin was prepared according to the method of Derrien(5) as modified by Roitt. Thyroid glands of 80 guinea pigs were frozen immediately after removal. The tissue was cut into very thin slices while maintained in a frozen state. One part of thyroid tissue was placed in 3 parts by weight of physiological saline and kept overnight at 4°C. The saline extract was subsequently filtered through surgical gauze and centrifuged at 8000 g for 10 minutes at room temperature. One hundred ml of the clear supernatant was brought to 42% ammonium sulfate saturation by the dropwise addition of 72.5 ml of saturated ammonium sulfate solution at room temperature. The 42% saturated ammonium sulfate extract was stirred for 30 minutes and the precipitate was then centrifuged at 6000 g for 10 minutes at room temperature. The supernatant was discarded and the precipitate redissolved in 50 ml of a 45% saturated ammonium sulfate solution. Subsequently, the ammonium sulfate concentration was slowly decreased by dropwise addition of 10.75 ml of distilled water to a final ammonium sulfate saturation of 37%. The suspension was continuously stirred for 2 hours, then centrifuged at 6000 g for 10 minutes. The thyroglobulin left in the supernatant was precipitated by dropwise addition of 4.1 ml of a solution of saturated ammonium sulfate (final saturation = 42%). The precipitated thyroglobulin was finally centrifuged at 6000 g at room temperature for 10 mintues and the supernatant subsequently discarded. The precipitated thyroglobulin was dissolved in saline and dialyzed against saline. Nitrogen content was determined by the micro Kjeldahl method (6.8 mg N/ml).

Preparation of picrylated thyroglobulin. 0.1 g of crystallized picryl chloride was dissolved in acetone and mixed with 1.9 g of cellulose (Hyflo-Super-cel, Johns Manville, COMPOC Calif.). The acetone was evaporated and a concentration of 5% picryl chloride adsorbed on cellulose remained.

* This work was supported by a grant from Nat. Inst. Health, U.S.P.H.S.

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Twenty-five ml of a 4 mg/ml solution of guinea pig thyroglobulin was brought to pH 8.5 by dropwise addition of a solution of 10% sodium carbonate. This solution was mixed with 300 mg of the picryl chloride cellulose powder (equivalent to 15 mg of picryl chloride). This procedure results in slow picrylation of thyroglobulin. With increasing picrylation of the protein, the pH of the solution falls. The pH was continuously adjusted to 8.5 by dropwise addition of 10% sodium carbonate. Picrylation was continued for 6 hours with constant stirring of the solution. At the end of this period, the solution was centrifuged and the supernatant withdrawn. The powder was suspended in an equal amount of distilled water and recentrifuged. The supernatant was mixed with the previous one and dialyzed against distilled water for 2 days(6).

Immunization of guinea pigs. Two groups of guinea pigs were immunized with picrylated thyroglobulin, emulsified in an equal amount of complete Freund's adjuvant. One group of guinea pigs were immunized with a total of 500 μg of thyroglobulin, the other with 50 μg administered in each case in 2 series of injections separated by a 7-day interval. Initial injections were in the 2 front foot pads, the second injections in the rear foot pads. The material was administered in a 0.1 ml volume in each foot pad. Seven to 10 days after the second injection, the animals were skin tested by intradermal injection of 50 μg thyroglobulin in 0.1 ml of normal saline. The skin reaction was read and graded at the end of 24 hours as described elsewhere(6). The presence of circulating antithyroglobulin antibody was determined by the passive hemagglutination technic(7).§ The animals were sacrificed after the skin tests were read and blood was removed for antibody determination. Complete autopsies were performed and sections of thyroid gland, liver, spleen, adrenal, lung and kidney were taken. Hematoxylin and eosin stained sections were made of both lobes of the thyroid gland and sections of the other organs were examined in most animals.

§ The authors wish to acknowledge thanks to Dr. Noel R. Rose, Univ. of Buffalo School of Medicine, for checking the titers of many sera in his laboratory.

Other groups of guinea pigs were immunized in a parallel series of experiments with tissue homogenates of the adrenal, liver and kidney emulsified in Freund's adjuvant as described above. Untreated animals maintained under the same conditions with identical housing and diet served as an additional control group.

Examination and evaluation of thyroid gland. Both lobes of the thyroid gland were examined in each animal and compared with control animal groups. The lesions were graded according to the following criteria: (+) Focal collections of inflammatory cells with some swelling of scattered follicular epithelial cells. Occasional desquamation of epithelial cells and macrophages within the follicles. No gross alteration of colloid. (++) Confluent areas of inflammation consisting principally of lymphocytes and plasma cells, but without extensive derangement of the normal follicular architecture. Moderate to marked colloid depletion associated with a varying degree of alteration was noted. (+++) Gross disruption of the normal follicular pattern with generalized involvement of both lobes. Areas of granulomatous inflammation in which no recognizable normal structures could be identified.

Results. At the end of the experimental period the animals immunized with thyroglobulin were in good condition and appeared to have gained weight at approximately the same rate as the control groups.

In the group of animals immunized with a course of 50 μg of picrylated thyroglobulin 30 of 43 or 69% developed delayed type of hypersensitivity to thyroglobulin. Of these 8 had strong reactions while 22 had weaker though distinctly positive reactions. Two of the 43 or 4.7% had circulating antithyroglobulin antibody. Twenty-five of the 43 animals developed thyroiditis. In the animals immunized with 500 μg picrylated thyroglobulin, incidence of delayed type of sensitivity was 74% (47 of 67 animals). Ten of these displayed a strong skin reaction to thyroglobulin while 37 had somewhat weaker reactions. Twenty-one of 67 animals or 31% had demonstrable circulating antibody, 38 of

TABLE I. Incidence of Thyroiditis and Immune Response to Thyroglobulin in Animals Immunized with Picrylated Thyroglobulin.

Picrylated thyroglob., μg	No. of animals	% of animals: Circulating antibody	Delayed hypersensitivity	Thyroiditis +	++	+++	Total
50	43	4.7	69.7	44	9.3	4.7	58
500	67	31.3	74	35.8	16.5	4.6	56.8

the 67 developed thyroiditis. These data are summarized in Table I. In addition, incidence of circulating antibody was approximately the same in the animals with thyroiditis (9 of 31) and those without any lesion (9 of 29). The percentage of animals with titers above 512 was slightly higher in the former group (Table II). Of a total of 61 animals with thyroiditis in both groups, 50 or 82%, had a positive delayed skin test. Sixteen of 61 animals or 26% had strongly positive reactions in this group. In a group of 45 animals without thyroiditis 62% or 28 of 45 had positive skin reactions. Only one of these animals had a strongly positive skin test (Table III).

No lesions were found in any other organ of the animals immunized with picrylthyroglobulin, with the exception of the liver and lung where small infarcts were occasionally noted. In one instance, a small foreign body granuloma in the lung was seen. This was interperted as a reaction to one of the constituents of Freund's adjuvant. Fig. 1 shows the histologic pattern of the thyroid gland of a typical control animal. Inflammatory cells were never observed. The control animals included groups immunized with Freund's adjuvant alone, and others immunized with organ extracts of liver, adrenal and kidney in Freund's adjuvant. Figures 2 and 3 demonstrate the typical lesion graded as ++ in a guinea pig which received a total immunizing dose of 500 μg picrylthyroglobulin. There is marked atrophy of the parenchyma with loss of colloid in addition to a generalized infiltration by mononuclear cells, principally plasma cells, lymphocytes and a few macrophages.

In the group of 35 animals immunized with liver, adrenal, and kidney extracts in Freund's adjuvant, no lesion which could be classified as + was noted in the thyroid gland.

TABLE II. Circulating Antibody in Guinea Pigs Immunized with a Total Dose of 500 μg.

	No. of animals	Circulating antibody titer: 16-512	512	Total
		%	%	%
Thyroiditis	31	12.9 (4)*	16.1 (5)	29 (9)
No thyroiditis	29	24.1 (7)	6.9 (2)	31 (9)

* No. of animals in parentheses.

TABLE III. Comparison of Severity of Thyroiditis with Intensity of Delayed Skin Reaction to Thyroglobulin.

	Degree of thyroiditis	% of animals with positive delayed skin test to thyroglobulin		
61 animals with thyroiditis	+	52.4	39.4	weak*
			13	strong†
	++	21.4	11.6	weak
			9.8	strong
	+++	8.2	5.0	weak
			3.2	strong
	Total	82	56	weak
			26	strong
45 animals without thyroiditis		62	58	weak
			4	strong

* Weak reaction characterized by moderate induration and erythema.

† Strong reaction with marked induration usually associated with hemorrhage.

Discussion. The results of this series of experiments clearly indicate that there exists a close relationship between incidence of delayed type of hypersensitivity to thyroglobulin and incidence of immune thyroiditis in this experimental model. No such correlation could be found with respect to circulating antibodies. These conclusions are based in part on the special exprimental conditions, utilizing the fact that in the course of immunization with minimal doses of antigen there is a phase in the immune response where little or no circulating antibody is demonstrable.

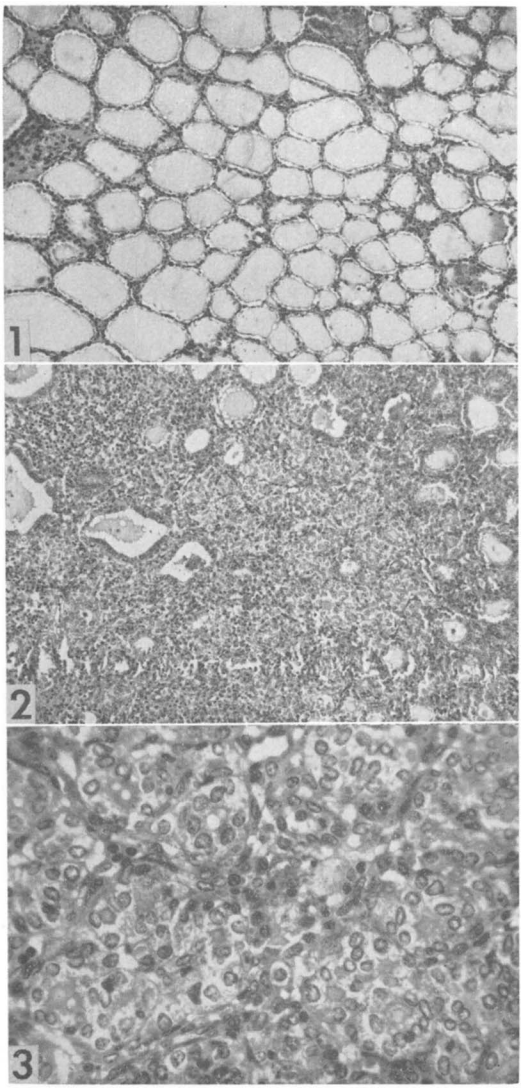

FIG. 1. Thyroid gland, guinea pig. Control animal treated with Freund's adjuvant. Hematoxylin and eosin. × 60.

FIG. 2. Thyroid gland guinea pig. Immunized with a course of 500 μg pierylated thyroglobulin in Freund's adjuvant over 17 days. Alteration of normal architecture as a result of collapse of follicles and apparent loss of colloid. Graded as (++). Hematoxylin and eosin × 60.

FIG. 3. Thyroid gland, guinea pig. Same general field as Fig. 2 in area of follicle collapse. Hematoxylin and eosin × 600.

Were thyroiditis mediated by circulating antibody little or no evidence of a thyroid gland lesion should occur under these conditions. This is in contrast to the actual findings in these experiments.

The lack of a positive correlation between circulating antithyroglobulin antibody and thyroiditis is also apparent from the following data. Whereas only 5% of guinea pigs immunized with 50 μg of picrylated thyroglobulin had detectable circulating antibodies at a low range of titers, 31% of guinea pigs treated with the 10-fold larger immunizing dose exhibited demonstrable circulating antibodies. If thyroiditis had been a direct consequence of antibody formation, the incidence should be much higher in the latter group. However, in both groups the proportion of animals with thyroiditis was the same. Furthermore, among the animals immunized with 500 μg of antigen the percentage of animals with circulating antibodies was almost identical in the groups with and without thyroiditis.

In contrast to the data with respect to circulating antibody, a good positive correlation was noted between delayed type of sensitivity to thyroglobulin and experimental thyroiditis. In both groups of animals, incidence of delayed hypersensitivity as well as incidence of thyroiditis was the same (Table I). The proportion of more severe thyroiditis seemed to be slightly higher in the group immunized with the higher dose, but the difference was relatively small. Table II shows the relationship between thyroiditis and delayed hypersensitivity from another aspect. The proportion of weak skin reactions is particularly high in animals with mild thyroiditis. If one focuses on intensity of the skin reaction as a point of reference, it is interesting to note, that out of 57 animals with a weak skin test, 33 (58%) exhibited thyroiditis, whereas in the group of 19 animals with a strong skin test (marked induration with hemorrhage), 17 (89%) of the animals exhibited thyroiditis.

The percentage of positive skin tests was also quite high in the 45 animals without thyroiditis (62%), although weak reactions were predominant in this group. Does this finding contradict the thesis that delayed hypersensitivity to thyroglobulin is the pathogenetic pathway of experimenal thyroiditis? If this relationship is correct, then the first stage

would be the development of delayed hypersensitivity to thyroglobulin and thyroiditis would appear only subsequently. The finding of a relatively high incidence of delayed type of hypersensitivity in animals without thyroiditis is, therefore, consistent with the hypothesis. This raises the question whether a critical level of sensitivity or some additional factor is necessary to elicit the immunologically mediated tissue damage.

Summary. 1. Among each of 2 groups of guinea pigs immunized with 50 μg and 500 μg of picrylated thyroglobulin in Freund's adjuvant respectively, 57.2% (63 of 110 animals) developed thyroiditis 17 days following the initial dose of antigen. Delayed skin reactivity to intradermal thyroglobulin was observed in 72.7% of the animals. There was no significant difference in incidence of thyroiditis and delayed skin reactivity in the 2 groups of animals. However, circulating antithyroglobulin antibody as determined by passive hemagglutination, was found in 4.6% (2 of 43 animals) in the low antigen group and 30% (18 of 60 animals) in the high antigen group.

2. The incidence of thyroiditis and delayed skin thyroglobulin reaction were positively correlated, while no such correlation between thyroiditis and circulating antithyroglobulin antibody was apparent.

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Received March 3, 1961. P.S.E.B.M., 1961, v107.

Radiosensitivity of Swine from Irradiated Parentage. (26520)

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Various parameters have been studied in the progeny of irradiated humans(1,2), dogs (3), and rodents(4.5), but we are not aware of any studies on the radiation sensitivity of progeny from irradiated large mammals. Some change in radio-sensitivity might be expected for life shortening effects have been reported in offspring of irradiated mice(6) and rats (7). The present report concerns the radiation response of offspring from parent swine exposed to the mixed gamma-neutron flux from a nuclear detonation.

Material and methods. Offspring from 3 parent categories of predominantly Landrace swine were studied. The *Progeny of Irradiated Parents* group was composed of 89 pigs sired by a boar surviving exposure to 268 rads (55% LD 50/30). The sows had received doses from 268 rads to 515 rads (mean dose 351 rads, 72% LD 50/30). Conditions of exposure for the parent swine have been described(8,9). *Progeny of an Irradiated Male* consisted of 44 offspring resulting from mating an irradiated boar (268 rads) with sows which had been placed as controls during field studies on the effects of a nuclear detonation (8,9). Dosimetric data indicated that these sows received less than 1 rad during the field