Autoimmunity in Methylcholanthrene-Induced and Spontaneous Thyroiditis in Buffalo Strain Rats[1] (35945)

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Two forms of experimental thyroiditis can be produced in the Buffalo strain of inbred rat. It can be induced chemically by feeding the carcinogen, 3-methylcholanthrene (MC) (1), and immunologically by the injection of crude rat thyroid extract plus complete Freund's adjuvant and pertussis vaccine. Its occurrence has also been noted in untreated animals (2, 3). The NZB mice first described by Bielschowsky *et al.* (4) and the obese strain of chickens described by Cole *et al.* (5) have provided useful animal models of spontaneously occurring diseases associated with autosensitization. Their investigation has greatly advanced our understanding of autoimmune disease in man. Thyroiditis in the Buffalo strain of rat promises to be a unique experimental model relating autoimmunity, oncogenesis, and aging.

Following active immunization, Buffalo strain rats develop circulating antibodies to thyroid antigens demonstrable by tanned cell hemagglutination, indirect immunofluorescence, and precipitation. Histologically the thyroids of animals with methylcholanthrene-induced and spontaneous disease resemble the thyroids of animals with disease induced by immunization. This paper describes the occurrence of circulating autoantibodies in affected rats. An immunological basis of the carcinogen-induced and age-dependent forms of thyroiditis is thus inferred.

Materials and Methods. Animals. Buffalo strain and Lewis strain rats were obtained from Simonsen Laboratories, Gilroy, CA and Microbiological Associates, Bethesda, MD. Some Buffalo rats were bred in our own laboratories. Fischer rats were obtained from the National Institutes of Health, Bethesda, MD.

Antigens. Rat thyroid extract (RaTE) was made from freshly frozen Wistar rat thyroid (Pel Freeze Biologicals, Inc., Rogers, AR) according to a method described elsewhere (6). Fischer rat testis extract and Lewis rat liver extract were also prepared by this method. Adrenal extract was prepared in the manner of Werdelin and Witebsky (7).

Tanned cell hemagglutination (HA). Thyroid antibody titers were measured by a micromodification of the HA procedure (6). Two readings were made, the first at 1 hr and the second at 24 hr.

Double diffusion gel precipitation (PPT). The microplate modifications of Scheidegger (8) and of Wadsworth (9) were used.

Indirect immunofluorescence (IIF). Thyroid glands of Buffalo and Lewis strain rats were obtained at autopsy, quick frozen in liquid nitrogen, and sections 4 μ thick were cut after the tissue warmed to $-20°$ in the cryostat. The slides were stored at $-70°$ for up to 1 week before use.

Frozen sections were fixed in absolute methanol at -20 to $-30°$ for 10 min. After allowing the sections to air dry for 10 min, IIF was performed according to a standard procedure (10). Before use the antirat globulin rabbit conjugate was adsorbed with rat liver and kidney homogenate. All antisera were diluted at least 1:10.

Methylcholanthrene. The MC (Nutritional Biochemical Co., Cleveland, OH) was administered in laboratory chow (Purina) to 4-week-old Buffalo or Lewis strain rats by a previously described method (1). The dosage was 0.033 g/100 g of food.

Immunization. Rats were immunized ac-

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TABLE I. Thyroiditis in the Buffalo Rat.[a]

Age (weeks)	Methylcholanthrene induced[b] thyroiditis/total	%	Spontaneous thyroiditis/total	%
4–8	4/20	20	0/38	0
9–12	4/16	25	3/21	14
14–16	5/8	63	3/10	30
30			12/25[c]	48
43			6/21[d]	29
60–64			6/15[e]	40
85			0/6[f]	0

[a] All rats were females of Simonsen Laboratory stock, unless otherwise indicated.
[b] Treatment started at 4 weeks of age.
[c] Includes 23 retired breeders.
[d] All males.
[e] All rats obtained from Microbiological Associates.
[f] Two males.

cording to a method described in a previous work (6) except that the adjuvant used was Perrin's modification of Freund's complete adjuvant (Calbiochem, Los Angeles, CA).

Histology. The trachea and thyroid were removed *in toto* and fixed in phosphate buffered 10% formalin, pH 7.2. At least eight different levels per thyroid were cut and examined. Pathology was graded according to the method of Twarog and Rose (6).

Results. Table I shows the incidence of spontaneous and MC-induced thyroiditis in the Buffalo rat. No spontaneous disease was observed before the rats were 12 weeks of age. Thereafter the incidence increased. The apparent decrease in the 43-week-old age group may be due to the fact that all of the animals were males. In the Buffalo strain, MC induced thyroiditis in an age group in which spontaneous disease has not been seen. The incidence in older ages was also greater in groups treated with this drug. Lewis rats did not develop thyroiditis after similar feeding of MC for 12 weeks. It has been reported (3, 11) that there is a lesser incidence of disease in Buffalo males than females, and that Fischer, ACI, Marshall, and Osborne Mendel strain rats do not develop disease after treatment with MC.

Actively immunized Buffalo strain rats have antibodies demonstrable by HA. In contrast to actively immunized rats, animals with spontaneous and MC-induced disease infrequently had antibody by this test. However, they did regularly have antibodies detectable by indirect immunofluorescence. Of these rats (Table II), 13 of 16 with thyroid lesions rated $\geq 3+$ had antibodies to thyroid antigen demonstrable by IIF. Only 3 of this group had antibodies that could be detected by HA. This finding is surprising because of the usual greater sensitivity of the tanned cell hemagglutination method. It suggests that these antibodies differ somewhat from those induced by active immunization. Nevertheless, the PPT technique showed that serum from an MC-treated rat produced a line of identity with serum from an immunized rat and serum from a rat with spontaneous disease in its reaction with thyroid extract (Fig.

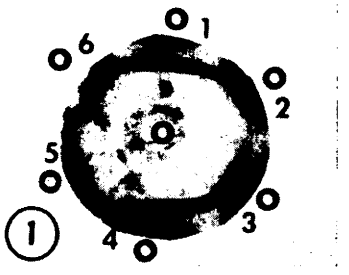

FIG. 1. Gel precipitation: (center well) RaTE 1:20; (wells 1 and 4) serum from an animal with immunologically induced thyroiditis; (wells 6 and 3) serum from an animal with MC-induced disease; (wells 2 and 5) serum from an animal with spontaneous disease.

TABLE II. Relationship Between Severity of Thyroiditis and Circulating Antibody.

	Index of Pathology											
	1+			2+			3+			4+		
	Positive			Positive			Positive			Positive		
	Total	HA	IIF	Total	HA	IIF	Total	HA	IIF	Total	HA	IIF
Methylcholanthrene induced	3	0	1	0	0	0	0	0	0	8	3	8
Spontaneous	5	0	0	7	0	0	4	0	1	4	0	4

1). This result does not exclude the possibility that other antibodies are present in MC-treated or aged rats, reactive with different thyroid antigens. These sera did not react in HA, IIF, or PPT with rat liver, adrenal, or testis.

When serum from two MC-treated rats, which contained precipitating antibodies, were allowed to diffuse against RaTE separated by electrophoresis in gel, a precipitation arc was visible in the α-globulin region, the position normally associated with thyroglobulin. In Fig. 2, the position of this line can be equated to that obtained with serum from an actively immunized Buffalo rat.

In IIF (Fig. 3) the colloid of thyroid reacted specifically. Parathyroid tissue visible in the same section was not stained. This localization again suggests a major antigen is thyroglobulin.

The thyroid used in these tests came from Buffalo strain rats. Since rats of this strain are syngeneic, the antibodies may be regarded as autoantibodies. The antibodies also reacted in an identical fashion with the thyroid of Lewis rats.

Three of 10 Buffalo strain rats had naturally occurring antibodies (HA titers 8, 8, 4), whereas none of 6 Lewis rats had such naturally occurring antibodies to thyroglobulin detectable by HA. A single intravenous injection of 0.5 ml of aqueous rat thyroid extract into the 6 Lewis rats did not produce antibody detectable by HA after 14 days. One of 10 Buffalo strain rats treated in this manner exhibited a marked increase of a preexisting titer. Before injection the animal had a hemagglutination titer of 4. On day 5 after injection, it had a titer of 128. On day 14, it had a titer of 1024.

Discussion. Apparently Buffalo strain rats are easily sensitized to thyroid antigens. The evidence that the MC-induced and spontaneous diseases are immunologic is as follows: The thyroid is the only organ which is infiltrated. Hypophysis, skeletal muscle, parathyroid, liver, adrenal, kidney, spleen, and salivary gland are normal. Antibodies specific for thyroid antigen are frequently present in high titer as demonstrated by 3 different serologic tests. Pathologically the

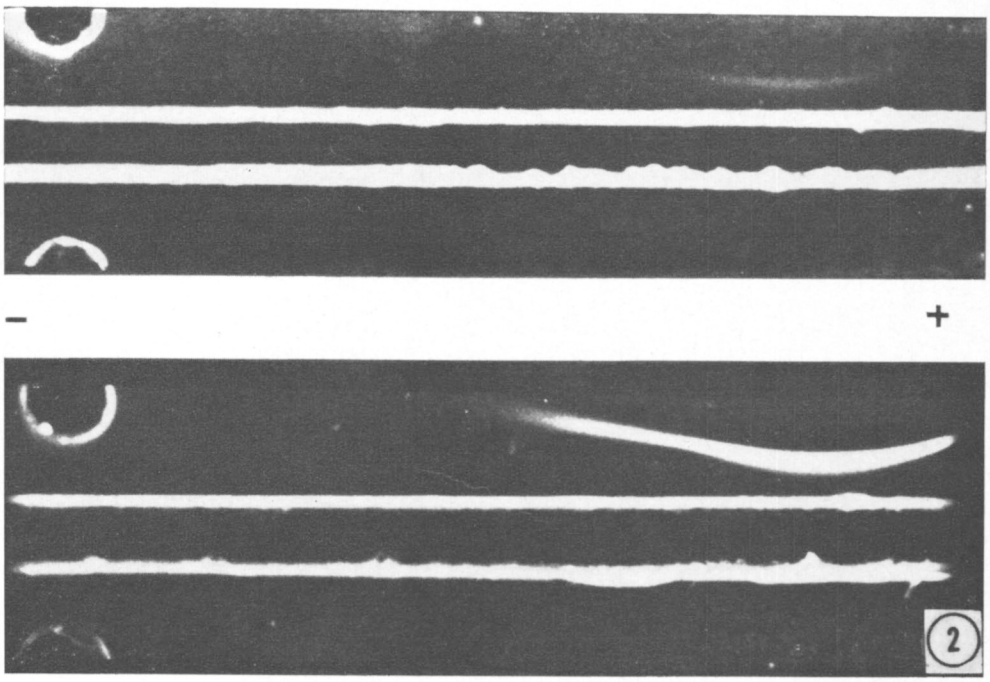

FIG. 2. Immunoelectrophroesis: (upper trough) serum from an animal with MC-induced disease; (lower trough) serum from an animal with immunologically induced disease. The well above each trough was filled with undiluted RaTE. The well below each trough was filled with normal rat serum, with which there was no reaction.

MC-induced and spontaneous diseases are the same as the immunologically induced disease (Fig. 4). The major antigen migrates in the α-globulin region and is present in rat colloid. It is resistant to boiling for 2 min. This evidence suggests that thyroglobulin, a

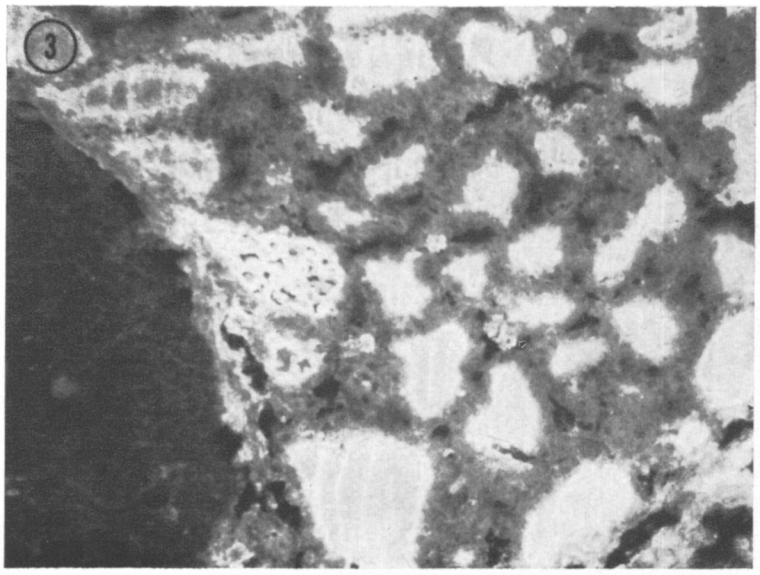

FIG. 3. Indirect immunofluorescence: serum from an animal with methylcholanthrene-induced disease reacted with a normal rat thyroid.

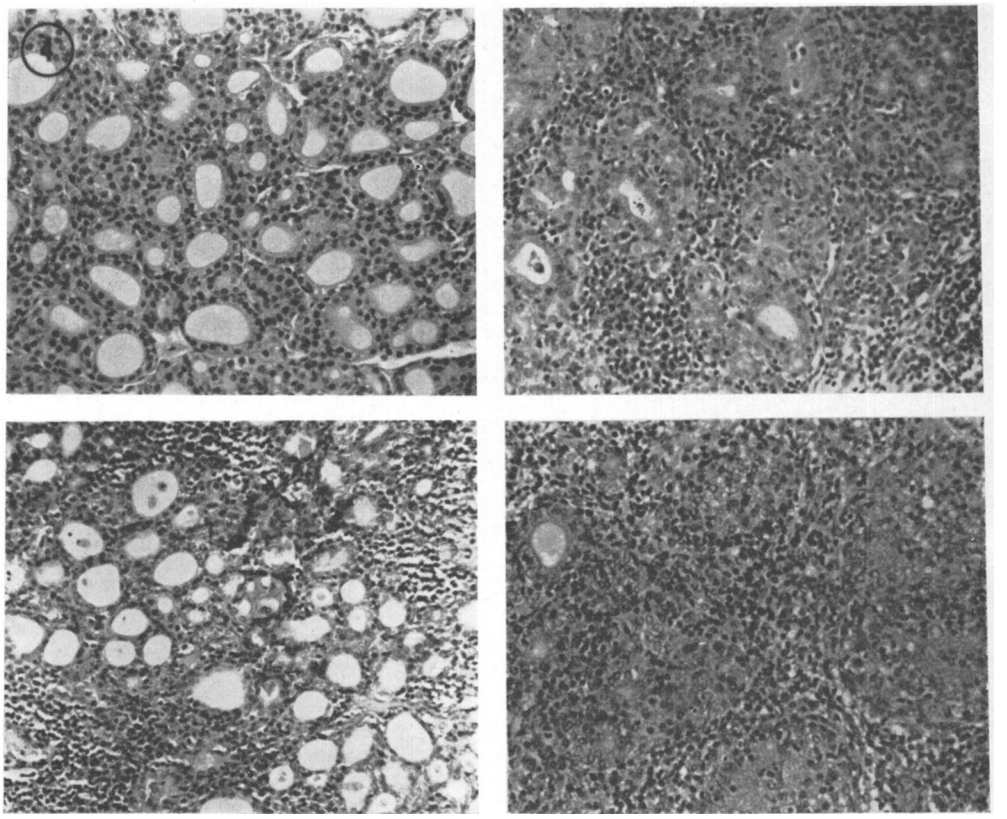

FIG. 4. (upper left) Normal Buffalo rat thyroid, H & E (× 150); (upper right) MC-induced thyroiditis; (lower left) spontaneous thyroiditis; and (lower right) immunologically induced thyroiditis.

well-recognized and characterized autoantigen, is an immunologic stimulus. Other thyroid antigens may also play a role. Positive IIF is well correlated with the presence of disease. Antibody without disease has not yet been detected. Aqueous RaTE, normally nonantigenic in rats, is capable of boosting antibody titers in animals with spontaneous disease, indicating preexisting or natural autosensitization.

The Buffalo strain thus possesses heightened susceptibility to autosensitization by thyroid antigen. This heightened susceptibility appears to be organ specific, since Buffalo rats are remarkably resistant to Heymann's nephritis and experimental allergic encephalomyelitis (12). This tendency to develop autoimmune thyroiditis seems to be potentiated by feeding MC, a known carcinogenic agent. MC has a direct effect on the thyroid (13). It may also induce disease indirectly via the immune system due to its immunosuppressive actions (14, 15). It is known that immunosuppressants can enhance certain immune responses (16).

Summary. Spontaneous, 3-methylcholanthrene-induced, and immunologically induced thyroiditis have been investigated in the Buffalo strain of inbred rat. Circulating autoantibodies to thyroid antigen are present in each form of the disease. Antibodies to other organs, or mononuclear cell infiltrations in them were not found. The major stimulus appears to be thyroglobulin. The evidence indicates that these 3 different forms of disease have an autoimmune basis.

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