We gratefully acknowledge the fine technical assistance of Miss Joan C. Enterline. We also wish to thank Dr. Clifford J. Maloney, Dr. Suresh C. Rastogi and Mr. Sidney S. Spindel for their statistical analyses. We are particularly indebted to Dr. Paul D. Parkman for many helpful discussions.

1. Taylor-Robinson, C. H., McCarthy, K., Grylls, S. G., and O'Ryan, E. M., Lancet **1**, 1364 (1964).

2. Marcus, P. I. and Carver, D. H., Science **149**, 983 (1965).

3. Plotkin, S. A., Arch. Ges. Virusforsch. **16**, 423 (1965).

4. Vaheri, A., Sedwick, W. D., Plotkin, S. A., and Maes, R., Virology **27**, 239 (1965).

5. Liebhaber, H., Riordan, J. T., and Horstman, D. M., Proc. Soc. Exptl. Biol. Med. **125**, 636 (1967).

6. Mares, I. and Casny, J., Acta Virol. (Prague) **11**, 262 (1967).

7. Rawls, W. E., Desmyter, J., and Melnick, J. L., Proc. Soc. Exptl. Biol. Med. **124**, 167 (1967).

8. Rhim, J. S., Schell, K., and Huebner, R. J., Proc. Soc. Exptl. Biol. Med. **125**, 1271 (1967).

9. Rhim, J. S. and Schell, K., Proc. Soc. Exptl. Biol. Med. **125**, 602 (1967).

10. Rapp, F., Seligman, S. J., Jaross, L. B., and Gordon, I., Proc. Soc. Exptl. Biol. Med. **101**, 289 (1959).

11. Spendlove, R. S. and Lennette, E. H., J. Immunol. **89**, 106 (1962).

12. Rapp, F., Rasmussen, L. E., and Benyesh-Melnick, M., J. Immunol. **91**, 709 (1963).

13. Vogt, P. K. and Rubin, H., Virology **19**, 92 (1963).

14. Deibel, R. and Hotchin, J. E., Virology **8**, 367 (1959).

15. Wheelock, E. F. and Tamm, I., J. Exptl. Med. **113**, 301 (1961).

16. Thiel, J. E. and Smith, K. O., Proc. Soc. Exptl. Biol. Med. **125**, 892 (1967).

17. Parkman, P., Buescher, E., and Artenstein, M., Proc. Soc. Exptl. Biol. Med. **111**, 225 (1962).

18. Hopps, H., Bernheim, B., Nisalak, A., Tijo, J., and Smadel, J., J. Immunol. **91**, 416 (1963).

19. Parkman, P., Mundon, F., McCown, J., and Buescher, E., J. Immunol. **93**, 608 (1964).

20. McCarthy, K., Taylor-Robinson, C. H., and Pillinger, S. E., Lancet **2**, 593 (1963).

21. Stoker, M. and MacPherson, I., Nature **203**, 1355 (1964).

22. Smith, K. O. and Sundborg, S., in preparation.

23. Smith, K. O. and Hobbins, T. E., submitted for publication.

24. Smith, K. O., J. Bacteriol. **86**, 999 (1963).

25. Taylor, D. O. N., Klauber, M. R., Lennette, E. H., and Wiener, A., J. Natl. Cancer Inst. **40**, 147 (1968).

26. Dulbecco, R. and Vogt, M., J. Exptl. Med. **99**, 167 (1954).

Received June 17, 1968. P.S.E.B.M., 1968, Vol. 129.

Experimental Thyroiditis in Complement Intact and Deficient Mice Following Injections of Heterologous Thyroglobulins without Adjuvant* (33333)

R. M. NAKAMURA[1] AND W. O. WEIGLE[2]

Department of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, California 92037

Experimental thyroiditis has been induced in rabbits by injections of aqueous preparations of altered homologous (1) and certain heterologous thyroglobulins (2, 3). In either situation, the production of thyroiditis appears to be the result of a termination of a natural unresponsive state to autologous thyroglobulin (4, 5). Aqueous preparations of thyroglobulin are rapidly catabolized and do not persist as a sustained stimulus as in the case of injections of thyroglobulin incorporated in complete Freund's adjuvant; thus a secondary response to a subsequent injection

* Publication No. 287 from the Department of Experimental Pathology, Scripps Clinic and Research Foundation. The work was supported by United States Public Health Service Grant AI 07007-03 and Atomic Energy Commission Contract AT (04-3)-410.

[1] Recipient of a fellowship from the Arthritis Foundation.

[2] Recipient of United States Public Health Service Research Career Award 5-K6-GM-6936.

of an aqueous preparation of native homologous thyroglobulin can be studied. In a study designed to investigate the latter phenomenon, it was noted that the autoimmune thyroiditis, once induced in rabbits, can later be enhanced and perpetuated for limited periods of time by subsequent injections of aqueous native thyroglobulin (6). On the other hand, similar injections of native thyroglobulin in normal rabbits have not resulted in any detectable immune response (4). Furthermore, it has been demonstrated that when rabbits were injected with a mixture of heterologous (cross-reacting) thyroglobulins without adjuvant, the thyroid lesions induced were very similar to lesions induced by injections of homologous thyroglobulin in complete Freund's adjuvant (3).

In contrast to rabbits, an extensive study of experimental thyroiditis in mice has not been reported. Mice are of particular interest for studies in thyroiditis because of the availability of inbred complement intact and deficient strains (7). The inherited complement deficiency of certain inbred strains of mice was shown to be due to an isolated lack of the murine analogue of the fifth component of human complement (C'5) (8). Present experiments were designed to study the immune response and production of thyroiditis in several strains of mice following injections of an aqueous mixture of heterologous thyroglobulins.

Materials and Methods. Mice. The experiments were performed with 20–30 g adult mice of the following strains: Swiss Webster, A/JAX, B10-D2/SN new line, B10-D2/SN old line, DBA/1J and DBA/2J. Except for the Swiss Webster, the strains were inbred and obtained from Jackson Laboratories, Bar Harbor, Maine. Mice of the A/JAX, B10-D2/SN old line and DBA/2J were complement deficient (7). All experiments utilized male mice since it has been shown that normal female mice contain a significantly lower amount of the late components of complement and specifically the analogue of human components C'5, C'6, and C'7 (8).

Isolation and purification of thyroglobulin. Bovine, equine, human, and mouse thyroglobulins were isolated and purified as previously described (1) using a modification of the ultracentrifugation method described by Edelhoch (9). Fresh bovine and horse thyroids were obtained from local slaughter houses. Human thyroid tissue was obtained from adults at autopsy shortly following death. Mouse thyroids were excised from many different strains of mice and stored at −20° until a sufficient number of glands was accumulated for isolation of the thyroglobulin. Purified thyroglobulin preparations were stored at 4° in 0.15 *M* NaCl with the addition of 1:10,000 parts of merthiolate.

Nitrogen determination. Protein nitrogen determinations were performed by a modification of the micro-Kjeldahl technique using the Technicon AutoAnalyzer (10).

Injection and bleeding of animals. Various groups of mice were injected with a mixture of equal parts of aqueous preparations of human, equine, and bovine thyroglobulins. Injections of a similar mixture of heterologous (cross-reacting) thyroglobulins were very effective in the production of thyroiditis in rabbits (3). The mice were injected intraperitoneally with 0.5 mg of a mixture of the heterologous thyroglobulins daily for 4 days and 1.0 mg was injected on the fifth day. A second and third series of injections were given with a rest period of 2 weeks between each course of injections. The first four injections of the second and third series of injections were given subcutaneously and the fifth 1.0-mg injections was given intraperitoneally. All animals were bled from the axillary artery under ether anesthesia 7 days after the last injection.

Antibody analyses. Hemagglutinating antibodies were measured by the Boyden technique (11) with the use of the Takatsy spiral loop dilution procedure (12). A 2.5% suspension of tannic acid-treated sheep erythrocytes was sensitized with 0.5 mg of mouse thyroglobulin/ml. Test sera were first heated at 56° for 30 min and then absorbed twice with an equal volume of packed sheep erythrocytes. In order to minimize error due to carry-over with the spiral loop dilution procedure, high titered sera were first quantitatively diluted in 0.15 *M* NaCl containing

TABLE I. Production of Thyroiditis and Autoantibody in Complement Intact and Deficient Mice.

Strain	Incidence of thyroid lesions[a]	Ab titer[b] (mean ± SE)
Complement deficient		
A/JAX	22/27	13.8 ± 0.18
B10-D2/SN old line	0/18	7.8 ± 0.54
BSA/2J	0/14	12.0 ± 0.43
Complement intact		
Swiss Webster	11/25	12.4 ± 0.28
B10-D2/SN new line	0/16	7.6 ± 0.62
DBA/1J	0/14	12.0 ± 0.47

[a] Number of animals demonstrating definite thyroid lesions/total number of animals in each group.

[b] Antibody titer is determined as the $\log_2$ of the reciprocal of the highest serum dilution showing agglutination of sheep erythrocytes sensitized with homologous mouse thyroglobulin. Results are expressed as the mean titer ± the standard error of the mean.

1:100 normal mouse sera previously absorbed with washed packed sheep red cells.

Histological examination. Immediately after sacrificing the animals, both lobes of the thyroid were removed and fixed in Bouin's solution. Four to five sections through the largest part of the thyroid gland lobes were stained with hematoxylin and eosin. When different degrees of inflammation were observed in different lobes of the glands, the stronger reaction was taken as the result. The degree of thyroiditis was arbitrarily graded from 1+ to 3+ according to the extent of infiltration of the cross section of the thyroid gland: 1+ = 2–4 small infiltration areas; 2+ = infiltration of 25–50%; 3+ = infiltration of 50–75% of the thyroid tissue.

Results. The average mean hemagglutinating titers and incidence of thyroid lesions in the various groups of mice are given in Table I. The sera of the complement intact Swiss Webster mice and complement deficient A/JAX mice showed the highest mean hemagglutinating titer ($\log_2$) to mouse thyroglobulin of 12.4 and 13.8, respectively. In addition, both the Swiss Webster and A/JAX strains were found to be susceptible to thyroid lesions. Twenty-two of a total of 27 mice in the A/JAX group showed inflammatory lesions of the thyroid glands: 12 mice had 1+ lesions, 9 had 2+ lesions, and only 1 mouse demonstrated a 3+ thyroiditis. Eleven of 25 Swiss Webster mice showed evidence of thyroiditis, 7 animals showed a 1+ lesion and 4 animals showed a 2+ thyroiditis. No definite correlation between antibody titer and severity of thyroid lesions was observed in either group. A representative 2+ thyroid lesion observed in the mice is shown in Fig. 1. Primarily mononuclear cells were seen in the lesions. There were many lymphocytes with scattered plasma cells, histiocytes and a few polymorphonuclear leukocytes (Fig. 2). A focal disruption of the thyroid follicular architecture with inflammatory cells within the lumen of the follicle was observed.

The complement intact and deficient B10-D2/SN new and old line, DBA/1J and DBA/2J strains demonstrated a definite immune response to injections of the heterologous thyroglobulins with production of hemagglutinating antibody to homologous mouse thyroglobulin. The mean antibody titers within each of the respective B10-D2/SN and DBA groups were similar and not significantly different. Both DBA/1J and DBA/2J groups of mice showed a similar mean antibody titer of 12.0 which was significantly higher than the mean titers of 7.6 and 7.8 found in B10-D2/SN new and old line, respectively. None of the B10-D2/SN and DBA strains showed any definite thyroid lesion.

Discussion. The above data demonstrate thyroiditis can be induced in certain complement intact and deficient strains of mice by multiple injections of a mixture of heterologous (cross-reacting) thyroglobulins without adjuvant. The complement deficient A/JAX mice demonstrated the highest incidence of thyroiditis, and the highest mean hemagglutinating antibody titer to mouse thyroglobulin was observed in their sera. The complement deficient mice lack C'5 activity (8), while having normal C'1, C'4, C'2, and C'3 activities. Present data do not substantiate a necessary role for components which are activated after C'3 in the production of thyroiditis. In a study of nephrotoxic serum nephritis in mice, it also was observed that

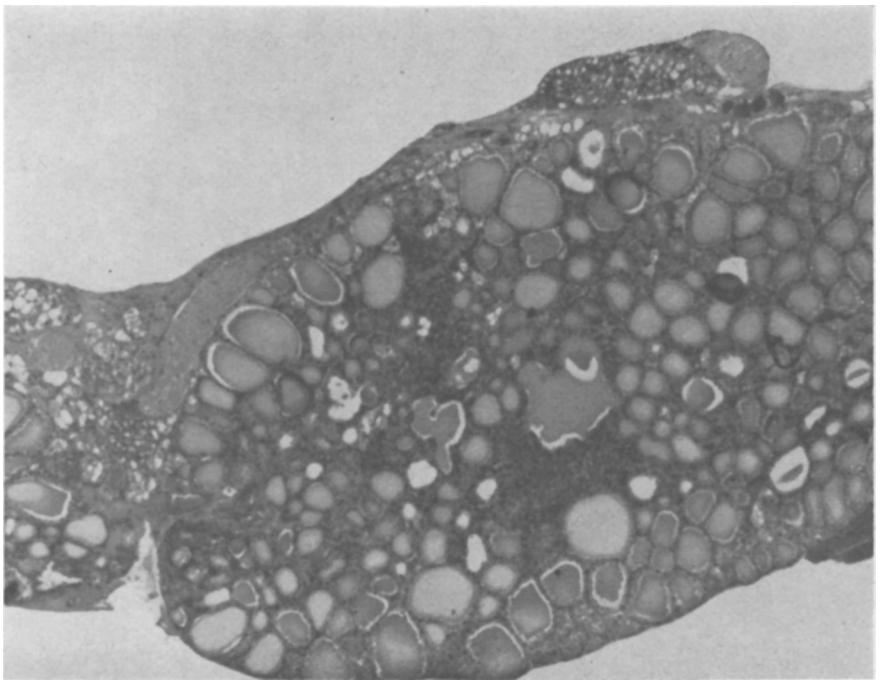

FIG. 1. Thyroid lesion in A/JAX mouse following injections of an aqueous mixture of heterologous thyroglobulins. H and E stain, magnification 36×.

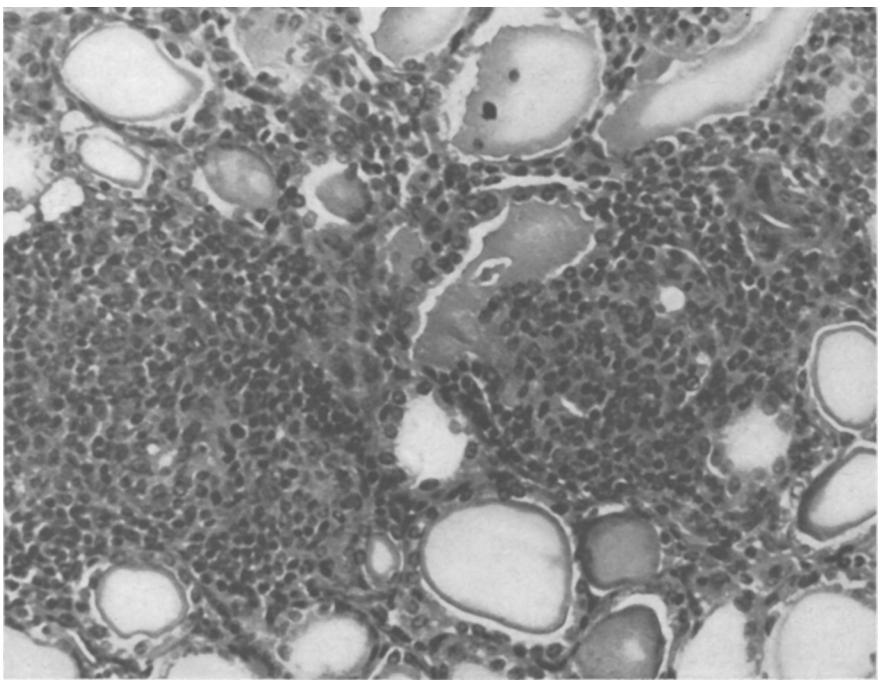

FIG. 2. Microphotograph of thyroid lesion observed in mice. Primarily mononuclear inflammatory cells with focal disruption of the follicles were seen. H and E stain, magnification 125×.

all complement intact and deficient mice, regardless of strain variation, developed a membranous glomerulonephritis during the autologous phase; and the severity of nephritis did not appear to be related to the presence or absence of a complete complement system (13). However, in a subsequent study, small differences in the severity of nephrotoxic serum nephritis were noted when complement containing progeny from the hybridization of B10-D2 old line females with B10-D2 new line males were compared to the complement deficient B10-D2 old line strain (14). A similar role of the late-acting components of complement in the minor intensification of thyroiditis cannot be ruled out by the present experiments.

The severity of the immune response and incidence of thyroid lesions in mice varied from strain to strain. The Swiss Webster and A/JAX strains had the highest mean hemagglutinating antibody titer to mouse thyroglobulin in their sera in addition to being susceptible to thyroid lesions. However, there was no correlation between antibody level and severity of thyroid lesions in the mice within these strains. Similar lack of correlation of antibody level and severity of thyroid lesions has been observed in other species (2, 15). All of the complement intact and deficient strains of B10-D2/SN new line, DBA/1J, B10-D2/SN old line and DBA/2J produced autoantibody to thyroglobulin; however thyroid lesions were not observed in any of these strains. Such genetic differences in susceptibility to immune injury have been reported in experimental thyroiditis of guinea pigs (16) and nephrotoxic serum nephritis in mice (13).

Summary. Experimental thyroiditis and thyroglobulin autoantibodies have been produced in both complement intact and deficient mice by injections of a mixture of heterologous (cross-reacting) thyroglobulins without adjuvant. The severity of the immune response and incidence of thyroid lesions in mice varied from strain to strain. The Swiss Webster and A/JAX strains had the highest mean hemagglutinating antibody titer to homologous thyroglobulin in addition to being susceptible to thyroid lesions, whereas the complement intact and deficient strains of B10-D2/SN new line, DBA/1J, B10-D2/SN old line and DBA/2J showed autoantibody production without any definite thyroid lesions. Present data do not substantiate a necessary role for components of complement which are activated after the third component of complement in the production of either thyroiditis or autoantibodies in mice.

The authors gratefully acknowledge the excellent technical assistance of Mrs. Judith Bolls.

1. Weigle, W. O., J. Exptl. Med. **121**, 289 (1965).
2. Rose, N. R., Kite, J. H., Jr., and Doebbler, T. K., *in* "Mechanisms of Cell and Tissue Damage Produced by Immune Reactions," (P. Grabar and P. Miescher, eds.), Benno Schwabe, Basel (1962).
3. Weigle, W. O. and Nakamura, R. M., J. Immunol. **99**, 223 (1967).
4. Nakamura, R. M. and Weigle, W. O., J. Immunol. **98**, 653 (1967).
5. Nakamura, R. M. and Weigle, W. O., J. Immunol. **99**, 357 (1967).
6. Weigle, W. O., J. Exptl. Med. **122**, 1049 (1965).
7. Cinader, B., Dubiski, S., and Wardlaw, A. C., J. Exptl. Med. **120**, 897 (1964).
8. Nilsson, U. R. and Müller-Eberhard, H. J., J. Exptl. Med. **125**, 1 (1967).
9. Edelhoch, H., J. Biol. Chem. **235**, 1326 (1960).
10. Ferrari, A., Ann. N. Y. Acad. Sci. **87**, 792 (1960).
11. Boyden, S. C., J. Exptl. Med. **93**, 107 (1951).
12. Takatsy, G., Acta Microbiol. Acad. Sci. Hung. **3**, 191 (1955).
13. Unanue, E. R., Mardinay, M. R., Jr., and Dixon, F. J., J. Immunol. **98**, 609 (1967).
14. Lindberg, L. H. and Rosenberg, L. T., J. Immunol. **100**, 34 (1968).
15. Miescher, P., Gortstein, F., Benacerraf, B., and Gell, P. G. M., Proc. Soc. Exptl. Biol. Med. **107**, 12 (1961).
16. McMaster, P. R. B., Lerner, E. M., II, and Mueller, P. S., Science **147**, 3654 (1965).

Received June 18, 1968. P.S.E.B.M., 1968, Vol. 129.