

THE EFFECT OF THYMECTOMY ON THE COURSE OF
EXPERIMENTAL IMMUNE THYROIDITIS IN MICE

by

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ABSTRACT

The purpose of this project was to experimentally induce thyroiditis in the mouse and to examine any influence thymectomy might have on its development. Immunization was performed using homologous thyroid extract emulsified in Freund's adjuvant. The mice were thymectomized within 18 hours of birth using a suction technique. Results indicated that the incidence of thyroid lesions were reduced by 25 per cent with thymectomy. Serum antibody levels of an anti-thyroid nature were not reduced, however, as detected by a tanned cell haemagglutination technique. These results are in accordance with recent evidence that the thymus is important in the development of cellular immunity while immunoglobulin production is dependent on a separate immune system probably involving the bursa of Fabricius in chickens or its homologue in mammals.

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INTRODUCTION

Removal of the thymus in newborn rodents leads to a depression of immunologic responsiveness (Miller, 1961; Good et al, 1962). There is a measurable decrease in the circulating lymphocyte count as well as a reduced ability to reject foreign skin grafts. Serum antibody production is also reduced with respect to some of the antigens that have been studied (Fahey, et al, 1965; Humphrey, Parrot and East, 1964).

In the embryonic state the thymus is the first organ to show a lymphoid appearance characterized by a large population of lymphocytes. Whether or not these thymus lymphocytes supply all of the other lymphoid tissues is not yet determined, but certainly there is a migration of lymphocytes from the thymus (Fichtelius and Bryant, 1962). Considerable evidence also supports the humoral influence of thymus tissue on the development of immunologically competent cells (Osoba and Miller, 1964; Levey, Trainin and Law, 1963; Aisenberg and Wilkes, 1965).

The production of inflammatory lesions, consisting primarily of lymphocytes, by injection of the appropriate organ extract emulsified in a mixture of oil and bacterial proteins (Freund's adjuvant) is a well established phenomenon (See Whipple, 1965). One such condition is experimental thyroiditis, the development of which is considered to be a consequence of auto-immunity. Antibodies are synthesized to react against "self"

antigens and lesions containing large numbers of lymphocytes are observed in the thyroid (Terplan, Witebsky et al, 1960).

It would seem possible, therefore, that the thymus may exert some influence on the production of these auto-antibodies and lymphocytic lesions. The purpose of this investigation was to study the effects of thymectomy in newborn mice on the development of experimental immune thyroiditis. Recently, similar investigations have been carried out on guinea-pigs (Lepulescu, 1965) and chickens (Jankovic, 1965).

MATERIALS AND METHODS

A. Animals

The mice used were randomly bred from a Swiss albino colony maintained at the University of British Columbia for fifteen years. Litters were weaned at three weeks and fed mouse pellets and water *ad libitum*. The study included 17 thymectomized, 8 sham-operated and 17 control animals. Both sexes were selected at random.

B. Thymectomy

Thymectomy and sham operations were performed within eighteen hours of birth. The method followed was that of Osoba (1966). With experience the operative mortality was reduced to approximately ten per cent but the survival at the end of seven weeks approached fifty per cent. Thymectomized animals are more susceptible to infection and some of the fatalities may be associated with a wasting condition or runt disease (Parrot and East, 1962) although this doesn't usually occur until after seven weeks of age. Initially, maternal neglect and cannibalism severely reduced the survival rate. Several methods were attempted to alleviate this, including the utilization of foster mothers; the addition of Librium (Roche) tranquilizer to the drinking water; the use of mentholated vaporub (Vick's) on the incision and around the cage to interfere with

the olfactory sensitivity of the mother and the utilization of a plastic spray bandage (Aeroplast).

The highest survival rate, however, was obtained after injecting the mother with 0.15 cc. of a 2:1 dilution of saline and Nembutal. The anaesthetized mother was then left in the cage and the operated litter returned before the effect of the anaesthetic disappeared (approximately 1 hour). Also, it was much more effective to use experienced mothers. i.e. those having borne one or two previous litters.

Newborn animals were exposed to a foil covered ice block in an insulated chamber until body movements had ceased (4-5 min.). The animals' extremities were secured with elastic on an animal board. Reasonable sterile technique was observed by keeping instruments in alcohol and the operative area swabbed. A straight incision through the skin was made from the area of the sixth rib to the neck region anterior to the salivary tissue. Using a cotton swab the salivary tissue was then retracted anteriorly. Iris scissors were inserted under the clavicle and parallel incisions were made on either side of the sternum taking care to avoid major blood vessels since loss of a single drop of blood was usually fatal. The sternal cartilage was then completely removed exposing both lobes of the thymus. The thin fascia surrounding the gland was then removed using jewellers forceps. Gentle, controlled suction was applied with a mechanical vacuum pump fitted with a capillary tip. After unbinding the forelimbs to

close the chest cavity, two single stitches were made through the skin with 7-0 silk, 3/8 circle sutures. The animals were incubated under a lamp then returned to the mother.

Sham-operated animals were subjected to the same procedure. The sternum was removed but the thymus was left intact.

The animals sacrificed at seven weeks were examined both macroscopically and microscopically (medio-sternal tissue remnants were fixed, stained and sectioned) for residual thymus. These animals are described in the results.

Since the thymectomy technique involved surgical manipulation near the area of the thyroid the possibility of tissue injury was checked by histologic examination of a normal, thymectomized thyroid at seven weeks.

C. Induction of Thyroiditis

Mouse thyroids were collected and frozen. A crude extract was prepared by homogenizing the thyroid tissue in a Potter-type tissue homogenizer and a equal volume of isotonic mammalian saline was added. The extract was then centrifuged to separate the still solid tissue from the supernatant which was used as the antigen. Approximately one hundred mice were required to prepare 2 ml. of such an extract.

Thyroiditis was first produced using rats (Roitt, 1961) to test the technique since the initial attempts with mice were

unsuccessful.

A trial and error method of inducing histologic thyroid lesions was used for the mice since the procedure was not described elsewhere. This involved injections at intervals varying from one day to two weeks apart and sacrifices at similar intervals. Various concentrations of antigen were attempted. For the mice used, the most successful results were obtained by using a 0.05 cc. injection of thyroid antigen emulsified (by rapidly drawing and expelling through a B.D. 24 needle) in an equal volume of complete Freund's adjuvant (Difco). Injections were made near the base of the tail taking care to avoid major blood vessels. This allowed for a slow diffusion of antigen over a period of a few days. A similar booster was given at one week and sacrifice was made two weeks after the initial injection.

Autopsy was performed on Nebutal anaesthetized animals and the trachea with surrounding pretracheal muscle associated thyroid tissue were fixed in formal saline. The precaval veins were severed and the blood was collected using heparinized pipettes. Serum was stored at -10° C for haemagglutinations.

The thyroid tissue was dehydrated in alcohol, cleared in benzene and embedded in paraffin. Serial sectioning was done at 5 μ and the material was stained with Ehrlich's haematoxylin and counterstained with alcoholic eosin.

D. Haemagglutinations

D. Haemagglutinations

Tanned cell haemagglutinations were performed on the sera according to the method described by Fulthorpe, Roitt, et al (1961). Sheep red blood cells were washed and suspended in a phosphate buffer pH 7.2 to a final concentration of 1.0%. These cells were treated with tannic acid diluted 1:20,000. One half of them were coated with antigen while the remaining half was used for control tests.

Since mouse thyroglobulin is not available commercially, a partially purified extract was prepared by ammonium sulphate fractionation similar to the method of Kite, Argue, and Rose (1966) for monkey thyroids. From a pool of mouse thyroids a crude extract was prepared in the manner described above. This was centrifuged at 68,000 g in a Spinco Model L ultracentrifuge with a #50 rotor for 45 minutes to produce a clarified extract. Enough neutralized 4.0 M ammonium sulphate solution was added drop by drop at room temperature with constant stirring to bring the final concentration to 2.4 M. The small precipitate that formed was removed by centrifugation. The concentration was then adjusted to 2.0 M and the white precipitate that resulted was washed twice with 2.0M ammonium sulphate and then dissolved in 2 ml. of phosphate buffered saline. Overnight dialysis against the same phosphate buffered saline resulted in 3ml. of thyroglobulin solution. Protein determination by the Lowry method showed 2.84 mg./ml. This was brought to a 0.1% protein concentration in phosphate buffer and was

~~used as the antigen to coat~~ the tanned sheep red cells. By adding 0.2% formalin to the cells, they are usable for up to nine months.

Serum dilutions of 1:5 to 1:25 million were set up and 0.1 ml. of cells were added to 0.1 ml. of each serum dilution. Controls for each serum sample were done using uncoated sheep red cells. Haemagglutinations were attempted in depression slides but the shape of the concavity made readings impossible so haemagglutination tubes were used.

RESULTS

The nature of the investigation permitted a semi-quantitative analysis only. The two criteria selected to represent the experimental autoimmune condition were the incidence and severity of thyroid lesions and the detection of serum auto-antibodies. They are represented separately below.

A. Histologic Thyroiditis

(1) Scoring of Thyroiditis

Arbitrary criteria for identifying the severity of the thyroid lesions were established as described below.

Figure 1 represents the appearance of normal mouse thyroid tissue.

i. Grade I-mild thyroiditis

Characteristically there was a reduction in follicle size with leucocytes adhering to capillary walls and some perivenular lymphocyte accumulation. (See Figure 2) This condition is similar to that described for lesions produced by Freund's adjuvant alone (Pearson, Waksman and Sharp, 1961).

ii. Grade II-definite thyroiditis

In this instance there is substantial colloid loss. Epithelial follicle cells are often sloughed off (Figure 3) and are seen in the lumen together with large numbers of foreign cells. Mononuclear cells are the most prevalent and macrophages can be identified. Polymorphonuclear leucocytes are present and often the

follicle is filled with a great deal of cellular debris. (See Figure 4) In this condition up to one-half of the gland (either or both lobes) was occupied with inflammatory foci.

iii. Grade III-severe thyroiditis

The normal architecture is almost completely lost and the follicles are widely separated by fibrous lymphocytic stroma. Intact follicles contain little colloid and invaded follicles often remain as cellular islands, (Figure 5). More than one-half of the gland was infiltrated and the invasive cells occupied the surrounding connective and muscle tissue, (Figure 6, 7 and 8).

(2) Comparison of Immunized Control, Thymectomized and Sham-Operated Animals

A comparison of the induced thyroid lesions is represented in Table 1. The control animals, Group I, were litters selected at random and immunized on the fifth and seventh week. Fourteen of the seventeen animals (82 %) showed recognizable thyroid changes as described. The mean severity of the lesions was 2.2 .

Thymectomized animals were divided into three classes depending on the residual thymus tissue recognizable upon autopsy. Considering the twenty-one animals in Group II that underwent neonatal thymectomy, immunization and subsequently survived to seven weeks of age, fifteen (Group III) showed only a microscopic remnant or complete thymectomy. Group IV comprised seven animals that had no trace of residual thymus. The percentage of animals exhibiting thyroiditis was reduced by fifteen to twenty-five per cent

PLATE I

Figure 1. Normal mouse thyroid follicles X1,000.

Figure 2. Grade I thyroiditis with perivenular leucocyte accumulation and microfollicular architecture. X200.

Figure 3. Definite thyroiditis. Epithelial cells of the follicle are being sloughed off into the lumen. X1800.

Figure 4. Cellular infiltrate of a follicle showing mononuclear cells, macrophages, and cellular debris. X1700.

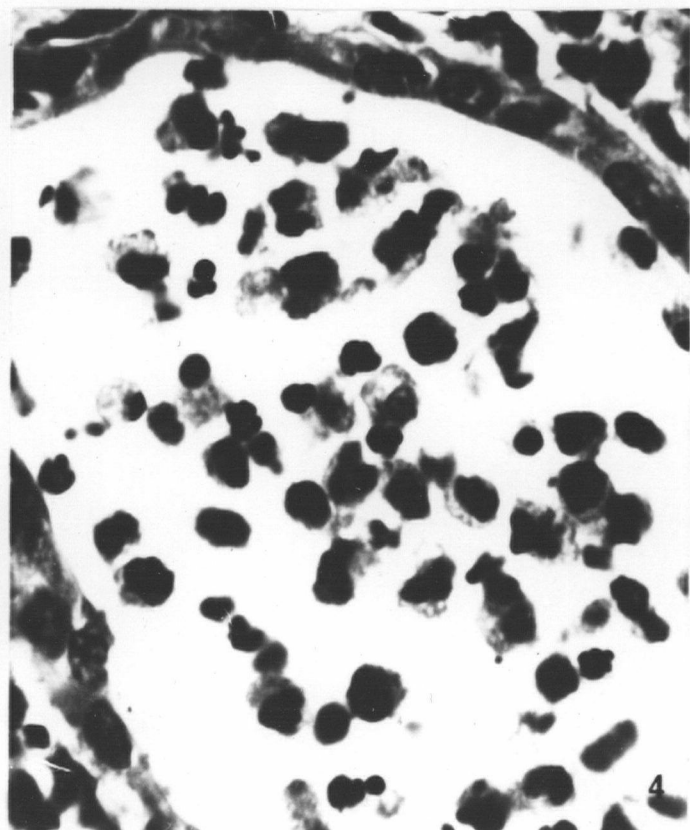
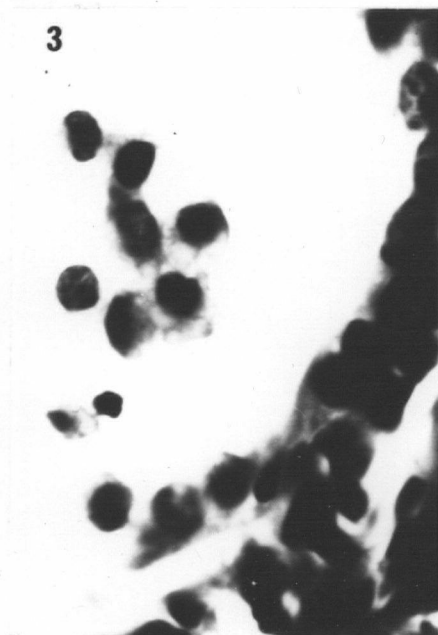
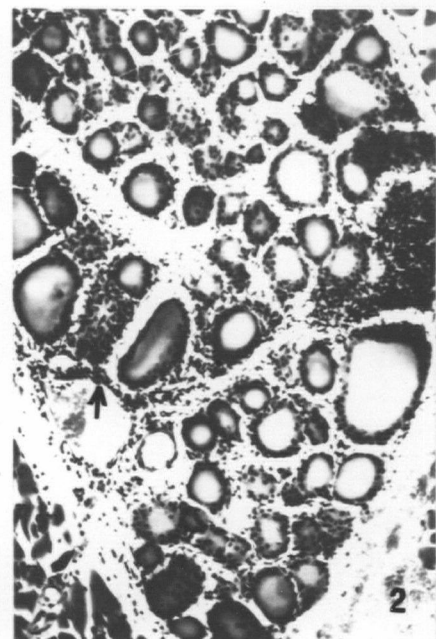
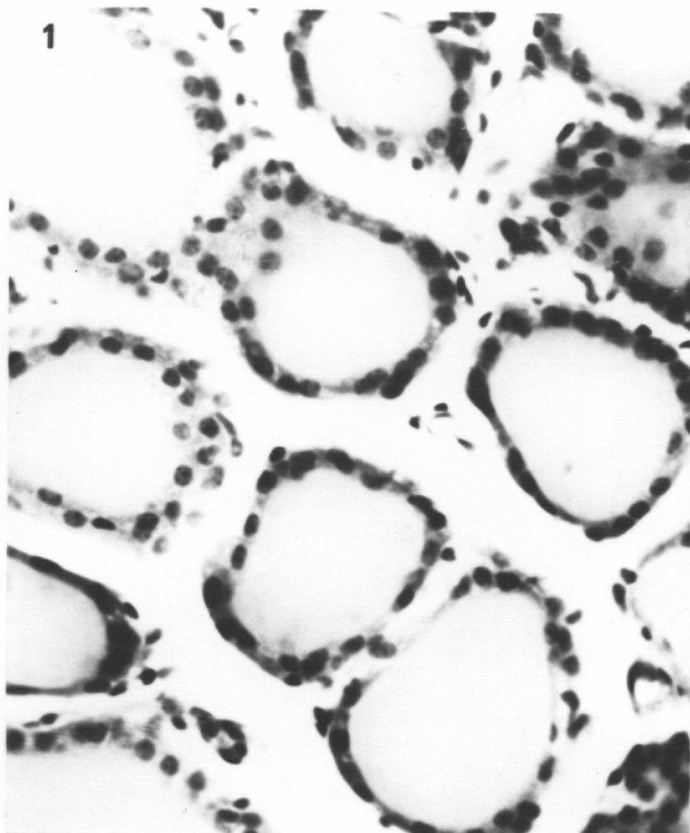


PLATE II

- Figure 5. Definite thyroiditis. Normal tissue structure is lost and remaining follicles appear as cellular islands of infiltrated cells. X200.
- Figure 6. Definite thyroiditis with follicles in various stages of infiltration by lymphocytes. X200.
- Figure 7. Mononuclear cells have invaded muscle and connective tissue surrounding the thyroid. Grade III thyroiditis. X160.
- Figure 8. Grade III thyroiditis. Low power of one thyroid lobe showing mononuclear cells in surrounding muscle and connective tissue. X40.

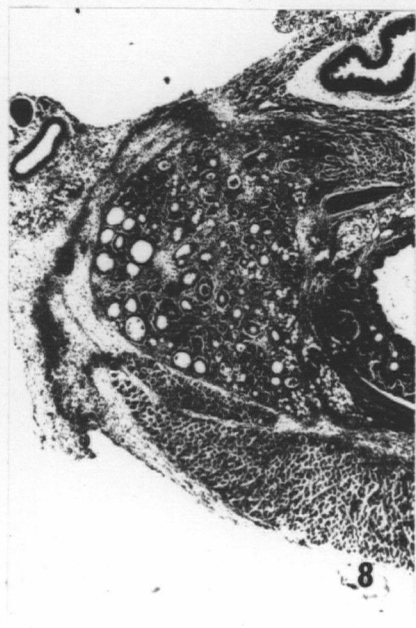
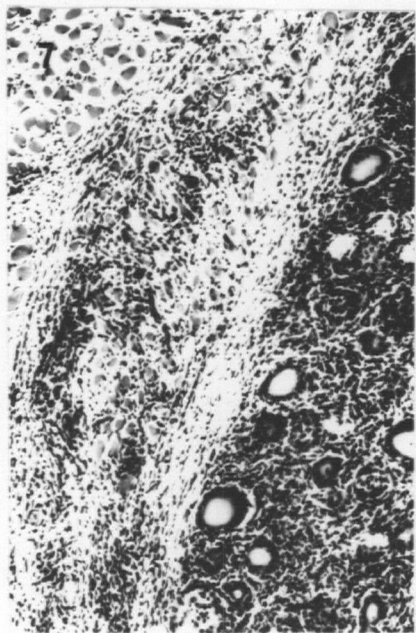
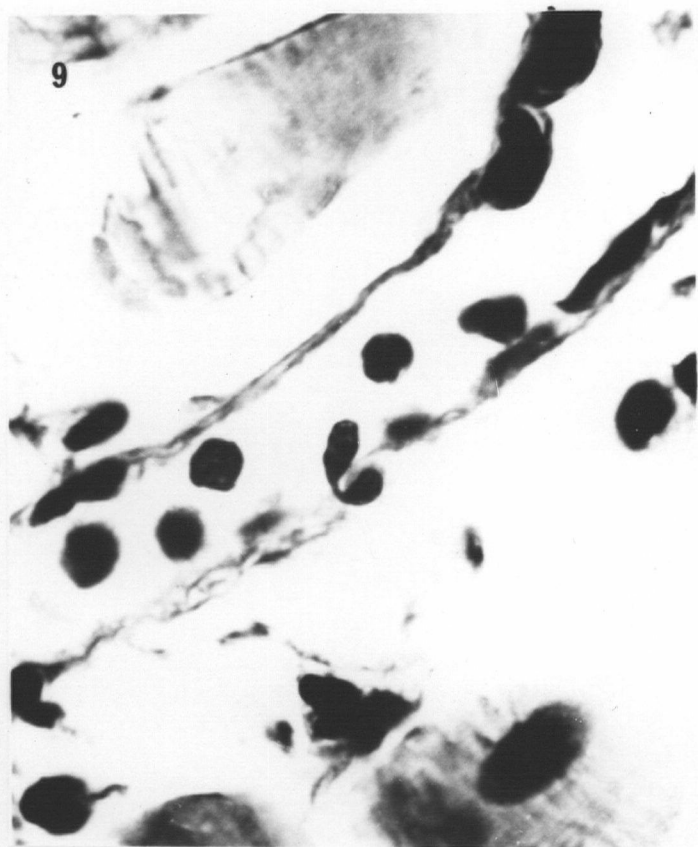


PLATE III

Figure 9. Distorted shape of a lymphocyte penetrating the endothelium of a venule in perithyroid muscle. X2,000.



in the thymectomized animals. Moreover, the mean severity of the thyroiditis was reduced to 1.7 and 1.3 in the thymectomized groups.

Group V represents the eight sham-operated animals all exhibiting severe thyroiditis.

Histologic examination of the thyroid of a non-immunized, thymectomized animal revealed no trace of abnormal thyroid tissue which may have resulted from thymectomy per se, or from damage during the operative procedures.

B. Thyroid Auto-Antibodies

The results of the tanned cell haemagglutinations for thyroid auto-antibodies are summarized in Table 2. Titre values were taken as the highest value that showed positive agglutination. Using control, uncoated red blood cells all the sera showed a negative response. Serum was not collected from all the animals referred to in the histologic study. Some of the titres were greater than $1: 25 \times 10^6$ and were not taken to the endpoint. This test is primarily of diagnostic value for auto-antibodies and is used extensively in studies of theoretical problems associated with auto-immunity. In a study involving mice it was particularly valuable since only a few drops of serum are required. Moreover, the test is 1,000 times more sensitive than the precipitation reaction. Direct agglutination titres do not correlate perfectly with antibody levels as determined by quantitative techniques. Fulthorpe, Roitt, Doniach and Couchman (1961) have compared anti-thyroid antibody

levels determined by radioactive coprecipitation (Roitt and Doniach, 1959) and quantitative precipitation methods (Heidelberger and Kendall, 1935) with direct haemagglutinations. High haemagglutination titres do correlate with high serum antibody concentrations.

There is, therefore, no significant difference between the anti-thyroid antibody titres in the control and thymectomized groups. All animals showed a high titre diagnostic of auto-antibodies. Four animals that showed no trace of histologic thyroiditis had anti-thyroid antibody titres higher than 1:500,000. Also, five animals with no residual thymus tissue had auto-antibody titres of 1:250,000 or higher.

HISTOLOGIC THYROIDITIS

COMPARING CONTROL, SHAM-OPERATED AND THYMECTOMIZED ANIMALS

TREATMENT	NUMBER OF ANIMALS	SEVERITY OF LESIONS				MEAN SEVERITY	PER CENT THYROIDITIS
		-	+	++	+++		
GROUP I IMMUNIZED CONTROL	17	3	1	2	11	2.2	82
GROUP II TOTAL THYMECTOMIZED	21	7	2	3	9	1.7	67
GROUP III ^A THYMECTOMIZED (<10% RESIDUAL THYMUS)	15	6	2	1	6	1.7	60
GROUP IV THYMECTOMIZED (NO RESIDUAL THYMUS)	7	3	1	1	2	1.3	57
GROUP V SHAM-OPERATED	8	0	0	0	8	3.0	100

^A INCLUDES ANIMALS WITH NO RESIDUAL THYMUS AS REPRESENTED IN GROUP IV

TABLE I

HAEMAGGLUTINATIONS

SERUM DILUTION

NUMBER
OF
ANIMALS

	25 ⁻²	50 ⁻²	25 ⁻³	50 ⁻³	25 ⁻⁴	50 ⁻⁴	25 ⁻⁵	50 ⁻⁵	25 ⁻⁶	UNCOATED CONTROL CELLS
IMMUNIZED CONTROL	1				2	1		2 ^Δ	5 ^Δ	—
TOTAL THYMECTOMIZED			1		3	1	4 ^Δ		3 ^Δ	—
SHAM - OPERATED					2					—

^Δ HIGHEST TITRE RECORDED

TABLE II

DISCUSSION

The importance of the thymus in the development of the immune system has been the subject of many investigations and recently more than one symposium, (See: The Thymus in Immunobiology, 1964, Good and Gabrielson, -editors; The Thymus, 1964 - Defendi, editor; and The Thymus: Experimental and Clinical Studies, 1966 - Wolstenholme and Porter, editors.). The thymus appears to be the centre of lymphoid-cell proliferation and differentiation (Miller, 1964). The exact nature of the influence of the thymus is not entirely clear, but there is substantial evidence for a humoral factor or factors. Such studies have been done on mice, (Osoba and Miller, 1964 and Levey, Trainin and Law, 1963) on rabbits (Trench, Watson et al, 1966) and on hamsters (Wong et al, 1965).

It is also established that the immune system is not always a normal, beneficial homeostatic mechanism. A state where there is an abnormal immunological reaction against self-antigens may be defined as auto-immunity (Dameshek, 1966). It would seem possible then, that the functioning of the thymus may influence autoimmunity.

Autoimmunity may be associated with antigens that are normally in isolated regions which become accessible to the immune system only after injury, possibly after the immune system has differentiated , and are therefore recognized as foreign. Such may

be the case with thyroglobulin (Rose et al, 1965), seminiferous spermatozoa (Feltkamp et al, 1965), lens of the eye (Halbert, Manski and Ehrlich, 1965) and elements of the central nervous system (Waksman, 1965).

The second broad possibility for autoimmune development may involve an aberrant recognition system by abnormal immunocytes. Dameshek, (1963) uses this term to describe a complex of cells proliferating in response to an antigen. There is considerable evidence to show that a normal immune response follows the following sequence of events. The macrophages phagocytose foreign material which contain antigen and digest it (Roberts and Haurowitz, 1962). Material or information derived from this may be picked up by small lymphocytes which have been shown to gather about the macrophages (Sharp and Burwell, 1960) and in response to the antigen these lymphocytes are transformed into large, primitive looking blast cells or immunoblasts (Tanaka et al, 1963) which possess large numbers of ribosomes. The subsequent proliferation of the blast cells is then considered to lead to the formation of plasma cells (involved in serum antibody production) and lymphocytes, (involved in cell-mediated immunity) or both, although experimental evidence is still uncertain. Following the immune reaction, memory cells are thought to remain , capable of rapidly reacting to the same antigen if it is encountered again. According to Gowans and Mc Gregor, (1965) these are almost surely the lymphocytes.

Autoimmunity as a consequence of some abnormality in this immunocyte complex has been hypothesized by Burnet (1959) in his " forbidden clone " concept. He suggests that a somatic mutation could lead to the proliferation of a clone of cells that lose their immunologic tolerance to self-antigens. Such a situation could be initiated in the thymus. This speculation resulted from the appearance of germinal centres within the thymus associated with certain known autoimmune disorders. In contrast to other lymphoid organs the thymus usually lacks germinal follicles. However, haemolytic anaemia which develops spontaneously in NZB/BL mice (Bielchowsky, Helyer and Howie, 1959) and myasthenia gravis (Castleman and Norris, 1949) have been associated with thymic germinal centres. This particular anaemia can be transmitted from mouse to mouse by injecting spleen cells into the animals (Holmes, Gorrie and Burnet, 1961) which might be the "forbidden clones" of Burnet's hypothesis.

More recently, the effects of thymectomy have been studied on various animals with autoimmune disorders. In adult mice even, thymectomy near the time of immunization reduced the severity of allergic aspermatogenesis (Vojtiskova and Pokorna, 1964).

In auto-immune strains of mice (Howie and Helyer, 1966) neonatal thymectomy causes the precocious development and an increase in acuity of the disorder. Moreover, normal thymectomized mice grafted with autoimmune thymus develop serological and histological evidence of auto-immune disease.

In birds, the bursa of Fabricius seems to be a primary lymphoid organ that shares some of the attributes of the mammalian thymus (Glick et al, 1956; Mueller, et al, 1962). Neonatal thymectomy in chickens as well as in mammals considerably retards the rejection of skin homografts (Warner, 1962) despite seemingly normal antibody production. However, surgical or hormonal bursectomy suppresses the antibody forming capacity of chickens. Bursal homografts in such animals restores the impaired antibody response (Jankovic et al, 1963).

Jankovic and workers (1963) have also shown that neonatal thymectomy in chickens does significantly reduce experimental allergic encephalomyelitis. This disease can be produced equally well in bursectomized and normal chickens by injection of spinal cord in complete Freund's adjuvant. Another experimental autoimmune condition studied in chickens is induced thyroiditis (Jankovic et al, 1965) produced by injection of homologous thyroid tissue in complete Freund's adjuvant. Again, the severity of the lesions was reduced by thymectomy performed at hatching while bursectomy had no effect. Auto antibody determination however, was not studied.

This evidence strongly suggests that the aggressive agent in the development of predominantly mononuclear cell lesions associated with both induced allergic encephalomyelitis as well as thyroiditis is the lymphocyte and that the autoantibody in the serum is of less obvious significance.

In the present study the results demonstrate a definite reduction in the severity of thyroiditis lesions following neonatal thymectomy in mice. This is most probably a direct consequence of the lymphocytopenia resulting from thymectomy. This type of mononuclear cell invasion may be occurring in conjunction with a reaction of the delayed hypersensitive type (Roitt, 1967). Dermal reactions to homologous mouse thyroid extract were attempted, but a quantitative determination of the reaction both macroscopically and histologically was rather unsatisfactory for any comparison to be made. Spiegelberg and Miescher, (1963) used the immunosuppressive drug, 6-mercaptopurine. They were able to reduce the delayed response to thyroglobulin and also reduce the severity of thyroiditis without significantly affecting the antibody titre. In Jankovic's studies (1965) with thyroiditis and allergic encephalomyelitis, the delayed hypersensitivity response to thyroglobulin and spinal cord antigens was also reduced with thymectomy. Roitt and Doniach (1967) claim that delayed hypersensitivity reactions probably operate in the pathogenesis of many of the experimentally induced auto-immune conditions in animals. However, the evidence is not nearly so convincing for the human auto-immune diseases. A synergism between cell-bound antibodies associated with the lymphocytes and serum antibodies is a possibility that has yet to be determined.

The early changes in a delayed hypersensitivity reaction following intradermal injection of an antigen consist of perivenular cuffing of lymphocytes derived from the blood. Gowans, (1962) shows

that emigration of lymphocytes from blood into lymph nodes takes place from the venules and in pathological conditions the migration is again from the venules. This is in contrast to the essentially polymorphonuclear leucocyte response seen in the Arthus reaction, which depends upon the precipitation of antigen-antibody complexes within the vessel wall. Arthus or immediate reactions occur soon after injection of antigen. They can be passively transferred by serum. Delayed reactions, however, reach a maximum at about 24 hours. The inflamed area is filled with mononuclear cells and the condition cannot be transferred via the serum. The photomicrographs described on page 11 also demonstrate a similar perivenular lymphocyte penetration. The cell shape of the lymphocyte is extremely pliable as the micrograph (Figure 9) indicates. This is also evident in cinematographic studies of emperipolesis in lymphocyte-thyroid culture (Acton, 1964).

Jankovic (1965) describes, with photomicrographs, direct participation of thymus lymphocytes in the invasion of the thyroid. Approximately 65% of normal chickens show small thymic lobes either in the adipose tissue of the thyroid area or directly attached to the thyroid gland, separated only by a thin layer of connective tissue. In cases of thyroiditis the thymus-thyroid barrier has disappeared and apparent massive migration of mononuclear cells directly from the thymus cortex to the thyroid can be seen. There was however, no evidence of germinal centres or inflammation within the thymus itself.

The multiple antigenicity of the thyroid extract prepared for immunization may have affected the areas of tissue that showed cellular infiltrates. Such a crude extract contains a very large number of antigenically distinct proteins. Thus, parathyroid proteins may be present as well as various muscle and connective tissue proteins. This could account for the mononuclear cell lesions in the muscle tissue described as Grade III thyroiditis. Jankovic (1965) shows the concomital occurrence of parathyroiditis with the development of thyroiditis. Tolnai (1966) was able to demonstrate mononuclear cell invasion near the injection site at least, of homologous skeletal muscle extract emulsified in Freund's adjuvant. It should be emphasized however, that the antigen used for the haemagglutinations was a more highly purified thyroglobulin product and that cross-reactions with antibodies other than thyroglobulin auto-antibodies should therefore have been reduced.

Further information regarding the aggressive participation of the lymphocyte has been accumulated from passive transfer experiments. Allergic encephalomyelitis has been induced passively by the inoculation of lymph node cells taken from animals immunized with brain tissue in Freund's adjuvant under conditions where the transferred lymphoid cells were able to survive (Aström and Waksman, 1963). Parallel experiments using large volumes of serum from immunized animals have failed to produce lesions. As yet, passive transfer of lymphoid cells has not successfully induced experimental thyroiditis.

The results outlined in this text indicate that a high titre of thyroid auto-antibody does not necessarily imply the co-existence of lesions in the gland itself. Rose et al (1965) using rabbits injected with alum-precipitated thyroid extract demonstrated that they possessed high titres of circulating antibody for long periods of time, but develop no thyroid lesions nor significant delayed hypersensitivity. Mc Master, Lerner, and Exum (1961) attempted to correlate the severity of lesions with the delayed hypersensitivity reaction and antibody levels. For example, in guinea pigs immunized with thyroglobulin and incomplete Freund's adjuvant low levels of thyroglobulin antibody could be detected without inflammatory changes in the gland or in dermal reactions. Again, it should be noted that one or more features of autoimmunity may be acting synergistically or they may be separated in time only. A detailed history of the disease in mice was not made after five weeks of development.

The use of Freund's adjuvant is common in the experimental production of autoimmunity. By emulsifying small concentrations of the antigen in complete Freund's adjuvant which contains killed *Mycobacterium* severe pathological manifestations and circulating auto-antibodies are consistently produced. However, in a few cases, lesions have been induced without the participation of adjuvant. Levine and Wenk, (1965) have shown that a number of strains of rats will succumb to experimental encephalomyelitis when injected with homologous nervous tissue only, but the dose required was 200 - 400 times larger than with adjuvant. Thyroiditis has been induced in r

rabbits by using heterologous thyroglobulin (Terplan, Witebsky, Rose, Paine and Egan, 1960). The mechanism by which the adjuvant enhances the immunologic reaction is not clear, but possibly the mycobacterium somehow acts to initiate the differentiation of the immunocyte complex allowing for a more widespread reaction to the homologous antigen.

The findings represented in this study are in accordance with a similar study performed on young guinea-pigs by Lupulescu et al (1965) insomuch as the reduced nature of histologic thyroid lesions with thymectomy is concerned. They, however, were unable to detect any circulating antibodies by the complement fixation method in thymectomized animals. Also in the immunized animals circulating antithyroid antibodies were not detected regularly and the titre varied. This is in sharp contrast to the present finding and it may be a consequence of the detection methods used. Rose et al (1965) were able to detect antibodies to thyroglobulin using the tanned cell haemagglutination method in monkeys, but were able to produce little or no complement fixation with soluble thyroid proteins.

The phylogeny of the immune system has been extensively reviewed by Papermaster et al (1962). The dissociation of cellular immunity which is dependent on the thymus from immunoglobulin development on the bursa in birds has also been established. This has promoted attempts to describe a mammalian equivalent to the bursa. According to Good and workers (1966) the " Primordial tissue for the mammalian immuno globulin producing system is presumed to be another gut-associated

glandular-lymphoid tissue, lympho-epithelial in type, relatively large in the embryo and neonate, and involuting at about the time of sexual maturity. " Candidates so far include pharyngeal and palatine tonsils and recently Cooper, Paterson and Good (1966) obtained evidence that the rabbit appendix and Peyer's patches function like a bursa. With these considerations plus preliminary results on DNA synthesis rates in the gut epithelium of guinea-pigs which show exceedingly high rates, comparable to the thymus and bursa, Fichtelius (1967), postulates that the entire gut-epithelium of mammals may function in immunoglobulin development as does the avian bursa of Fabricius.

This investigation seems to support the previously reported evidence (Fichtelius, 1967) suggesting that the immunoglobulin synthesizing system may be somewhat divorced from the system concerned with cellular immunity. Thymectomy definitely reduced the cellular manifestations of induced thyroiditis while the immunoglobulin levels were seemingly unaffected. It would appear, also, that the existence of thyroglobulin auto-antibodies is not in itself diagnostic of thyroiditis. This does not exclude the possibility that the auto-antibodies preview the development of thyroid lesions. Some of the unanswered aspects of autoimmunity might be further studied in animals with completely dissociated thymus and bursa dependent immune systems. Future studies on lower vertebrates may prove to be worthwhile.

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