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SPONTANEOUS THYROIDITIS IN THYMECTOMIZED AND IRRADIATED WISTAR RATS

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SUMMARY

Thyroiditis and autoantibodies to thyroglobulin were found to develop spontaneously in 60% of randomly bred Wistar rats subjected to thymectomy and whole body irradiation after weaning. Antinuclear antibodies were found in approximately 10% of these animals. Thyroiditis was seen in rats which were whole body irradiated without thymectomy but at a much lower incidence (22%). Control rats given irradiation to the thyroid region alone showed no such effects. The histology of the thyroid gland was similar to that seen in Hashimoto thyroiditis in man.

The majority of rats which developed severe thyroiditis were found to be lymphopenic and to have negligible mitogenic responses to phytohaemagglutinin as well as other changes which in view of the thymectomy and irradiation may be considered to be indicative of impaired T cell function.

It is concluded that these findings support the hypothesis that thymus-derived lymphocytes play an important part in the control of organ specific autoimmune disease.

INTRODUCTION

Recent studies on the pathogenesis of experimental thyroiditis have tended to re-establish the importance of the humoral component in the induction of organ specific autoimmune disease. Passive transfer of thyroiditis has been achieved in certain instances by the administration of antibody (Nakamura & Weigle, 1969; Vladutiu & Rose, 1971) and the spontaneous thyroiditis observed in the Obese strain of chickens is apparently also mediated by antibody, since bursectomy but not thymectomy prevents the disease (Wick *et al.*, 1970). Furthermore, studies in this laboratory indicate that, in man, target cells coated with certain thyroid autoantibodies or their complexes can be specifically lysed by normal lymphoid cells (Calder *et al.*, 1973). Thus the cytotoxic activity of mononuclear cells within autoimmune lesions may also be antibody dependent.

In consequence, the recent demonstration of the existence of physiological mechanisms

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which can control and inhibit antibody production (Gershon & Kondo, 1970; Baker *et al.*, 1970; Kerbel & Eidinger, 1971) may prove of major significance in the context of autoimmunity. Allison *et al.* (1971) have recently drawn attention to this possibility and have suggested that some autoimmune disease may be the consequence of the failure of such mechanisms to actively control autoantibody production.

Studies on the control of antibody formation indicate that this is mediated by thymus-derived lymphocytes (T cells) which in addition to their well established co-operative role in assisting antibody production by non-thymus processed B cells now also appear to have the ability to inhibit these under certain circumstances.

If T cells can operate in this manner to control autoantibody production then autoimmune phenomena might be expected to develop in animals in which a selective T cell deficiency has been induced. In support of this prediction an increase in the incidence of antinuclear antibodies has already been shown to occur following neonatal thymectomy in certain species (Thivolet *et al.*, 1967; Teague & Friou, 1969; Albini & Wick, 1973).

In the present study we report the occurrence of spontaneous thyroiditis in Wistar rats following the selective depletion of T cells by thymectomy and irradiation.

MATERIALS AND METHODS

Animals

Female Wistar rats from the randomly bred colony maintained within this unit were used throughout. In order to deplete them selectively of thymus-derived lymphocytes they were subjected to the procedure described by Harding *et al.* (1971). This involves repeated sub-lethal irradiation following thymectomy. In theory this procedure should eliminate mature lymphocytes without destroying the capacity of the bone marrow stem cells to regenerate lymphocytes which then cannot be thymus processed as a consequence of the thymectomy. All mature lymphocytes should thus be derived from the B cell compartment.

Thymectomy

This was carried out immediately after weaning at approximately 5 weeks of age by an adaptation of the method Miller (1960) described for mice. The operation was performed under sodium barbitone anaesthesia. At the termination of the experiment all rats were checked for thymic tissue remnants. One rat found to have a small quantity of residual thymic tissue was eliminated from this study.

Irradiation

Two weeks after weaning the rats were given five repeated doses of 200 rad at 14-day intervals. The rats were restrained in polystyrene boxes 75 cm from the source and irradiated at a rate of 66·4 rad/min using a Westinghouse unit run at 230 kv and 15 ma. Filtration was 0·5 mm copper and 1·0 mm aluminium. In order to assess the part played by irradiation both in relation to immunological competence and a direct effect on the thyroid, groups of rats were irradiated as follows: Group II, head and neck only (including the thyroid region). Group III, trunk and limbs (including the thymus). Group IV and V, whole body irradiated.

Group II and III rats, which received regional irradiation only, were first anaesthetized

and held on their backs in radially disposed troughs cut in a polystyrene block. Lead shielding, 0·4 cm thick, was then placed over the region of the rat to be protected. All rats were retained before killing for at least 60 days and in some cases 120 days after the last irradiation.

Preparation of rat thyroglobulin

Rat thyroids (stored at −20° before use) were homogenized in two volumes of phosphate buffered saline, pH 7·2, using a Silverson blender. The suspension was centrifuged at 30,000 *g* for 30 minutes at 4°C and the crude thyroglobulin was precipitated from the supernatant with ammonium sulphate (40% saturated). Further purification was achieved by gel filtration chromatography using Sephadex G-200. The exclusion peak was concentrated, dialysed at constant volume against distilled water and freeze dried.

Circulating antibodies to thyroglobulin

The tanned erythrocyte haemagglutination test was used for this purpose. Tanned chicken red cells were sensitized with 2 mg/ml of rat thyroglobulin (Herbert, 1967) and incubated overnight at room temperature with doubling dilutions of the sera from the various groups of animals. All sera were absorbed with 2 volumes packed chicken RBC prior to testing and diluted by the Takatsy microprocedure.

Phytohaemagglutinin transformation of lymphocytes

Cultures were set up in triplicate containing 1·0 ml Eagle's basal medium (Wellcome TC30), 0·1 ml heparinized whole blood and 0·1 ml PHA solution (equivalent to 2·5 μl stock Difco PHA-P). Control tubes, without PHA, were also set up for each sample. The culture tubes were loosely capped and incubated for 48 hr at 37°C in a humidified atmosphere of 5% CO_2 in air. One microcurie of ^{3}H-thymidine (Radiochemical Centre, Amersham) in 0·1 ml saline was then added to each culture and incubation continued for a further 24 hr.

The cultures were extracted by washing twice in PBS (pH 7·2), once in distilled water to lyse the RBC and then twice in 5% TCA and absolute methanol. The resulting precipitate was dissolved in 0·5 ml solubilizer (Soluene, Packard) and mixed with 5 ml scintillant (Instagel, Packard). Counts were recorded as cpm, quantity and background being constant. In order to compare samples, counts were converted to a transformation index (TI) defined as:

$$\text{TI} = \frac{\text{mean count of PHA-stimulated cultures}}{\text{mean count of unstimulated cultures}}.$$

Histology

Rat thyroids and other tissues were fixed in 4% formal saline and stained with haematoxylin and eosin. All slides were coded and examined blind. The degree of thyroiditis was scored as follows: − = normal thyroid structure; + = focal thyroiditis; + + = more severe and frequent foci of thyroiditis; + + + = diffuse thyroiditis with some loss of follicular architecture, + + + + = uniform severe thyroiditis with extensive loss of follicular architecture.

White cell counts

Total white cell counts were made in a haemocytometer and differential counts were

obtained from Leishman stained blood smears. Counts were expressed as cells per cubic mm blood.

Circulating antinuclear antibodies

Antinuclear antibodies were investigated by the indirect immunofluorescent technique with rat kidney sections as substrate. Test sera applied undiluted to the sections followed after washing by fluorescein-conjugated rabbit anti-rat immunoglobulin serum at 1 in 12 dilution (Wellcome).

RESULTS

Thyroiditis

The depletion of thymus-derived lymphocytes by thymectomy and irradiation was found to be associated with the appearance of spontaneous thyroiditis in 60% of the rats

TABLE 1. Summary of the influence of the various treatments on the incidence of spontaneous thyroiditis

Group	Treatment of group	No. of animals	Incidence of thyroiditis (%)
I	Untreated	50	0
II	Head and neck irradiated	10	0
III	Trunk and limbs irradiated	9	22
IV	Whole body irradiated	49	25
V	Whole body irradiated + thymectomy	42	60

χ^2 analysis of differences in incidence between groups: group I versus groups III, IV and V, $P<0{\cdot}001$; group V versus groups III and IV, $P<0{\cdot}001$; group III versus group IV, $P<0{\cdot}5$.

TABLE 2. The influence of the various treatments on the severity of spontaneous thyroiditis

Group*	No. of rats	Severity of thyroiditis†					Mean degree of thyroid‡ infiltration ± SE
		−	+	++	+++	++++	
I	50	50	0	0	0	0	0
II	10	10	0	0	0	0	0
III	9	7	2	0	0	0	0·22 ± 0·07
IV	49	37	7	2	3	0	0·4 ± 0·12
V	42	17	14	2	2	7	1·24 ± 0·23

Student's *t*-test analysis of differences in severity between groups: group I versus groups III, IV and V, $P<0{\cdot}001$; group III versus group IV, $P<0{\cdot}15$; group III versus group V, $P<0{\cdot}50$; group IV versus group V, $P<0{\cdot}001$.

* Treatment of groups as detailed in Table 1.

† Gradation of thyroid lesions as described under Materials and Methods.

‡ Lesions were scored as follows: − = 0; + = 1; ++ = 2; +++ = 3; ++++ = 4.

so treated (Table 1, group V). The severity of lymphoid infiltration and destruction of normal thyroid architecture varied greatly between individuals (Table 2, Group V). In the most severe cases the thyroid gland was goitrous, with a severe degree of infiltration and extensive loss of follicular structure (Fig. 1a). The infiltrate was composed of the cellular elements which are characteristically found in autoimmune lesions: lymphocytes, monocytes and plasma cells. Plasma cells appeared to be more prominent in some animals (Fig. 1b) than was usually seen in actively induced experimental thyroiditis in the same strain of rats. Thyroiditis was also seen in rats which were given whole body irradiation (group IV) although this was both less severe and less frequent than in the group which was thymectomized as well as irradiated (Tables 1 and 2). The group given irradiation to the trunk and limbs only (group III) and in which the head and neck were shielded in order to avoid irradiation of the thyroid gland but permitted irradiation of the thymus, was found to have a similar incidence and severity of thyroiditis as the whole body irradiated group (Tables 1 and 2). This indicated that direct irradiation damage to the thyroid was not necessary for the development of thyroiditis. This inference was further substantiated by the absence of thyroiditis in those rats which had only their head and neck region irradiated and in which the chief lymphoid aggregations in the body were shielded (Table 1, group II and Fig. 2). Similarly, thyroiditis was absent in all untreated rats (Table 1, group I).

Circulating antibody to thyroglobulin

A summary of the thyroglobulin autoantibody studies is presented in Table 3. It will be noted that the distribution within groups closely parallelled the incidence of thyroiditis. Group V had the highest incidence and the highest titres. This was followed by groups IV and III. No antibodies were detectable by the haemagglutination procedure in Groups I and II.

TABLE 3. Summary of the influence of the various treatments on the incidence and titre of autoantibody to thyroglobulin

Group*	No. of animals	Incidence of thyroglobulin antibodies (%)	Haemagglutination titre $\log_2 \pm$ SE
I	50	0	0
II	10	0	0
III	9	22	$0{\cdot}56 \pm 0{\cdot}52$
IV	52	23	$0{\cdot}79 \pm 0{\cdot}21$
V	41	68	$2{\cdot}65 \pm 0{\cdot}36$

χ^2 Analysis of differences in incidence between groups: group I versus groups III, IV, V, $P < 0{\cdot}001$; group V versus groups III, IV, $P < 0{\cdot}001$; group III versus group IV, $P < 0{\cdot}5$.

Student's *t*-test analysis of differences in mean titre: group I versus groups III, IV, V, $P < 0{\cdot}001$; group V versus groups III, IV, $P < 0{\cdot}02$; group III versus group IV, $P < 0{\cdot}1$.

* Treatment of groups as detailed in Table 1.

Although there was a correlation between thyroiditis and autoantibody incidence when groups were compared, it was not found possible to correlate the severity of thyroiditis with antibody titre to thyroglobulin in the individual animal (Fig. 3).

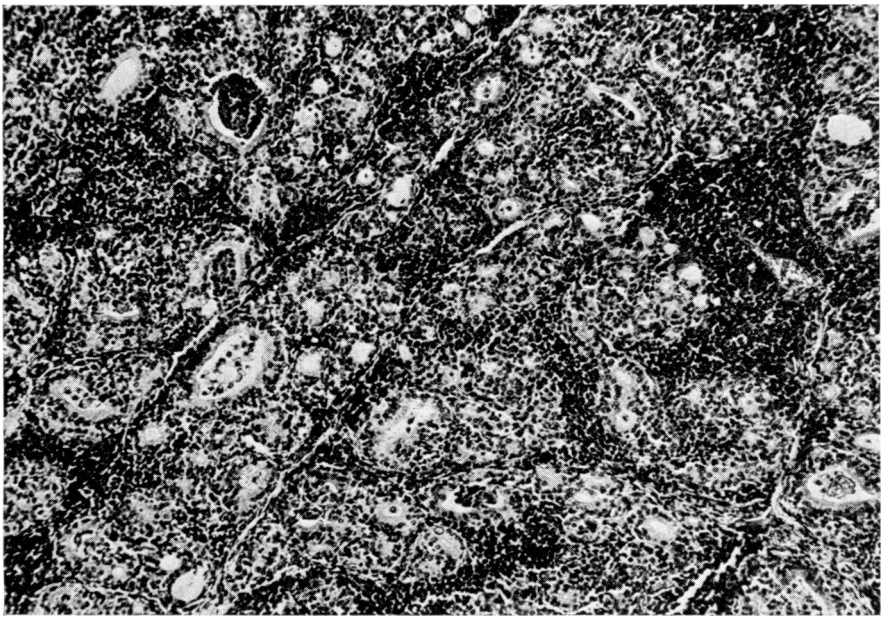

FIG. 1a. Thyroid of a rat after thymectomy and irradiation (5 × 200 r). Note the severe inflammatory reaction (grade + + + +) with involvement and destruction of follicles (H & E, × 80).

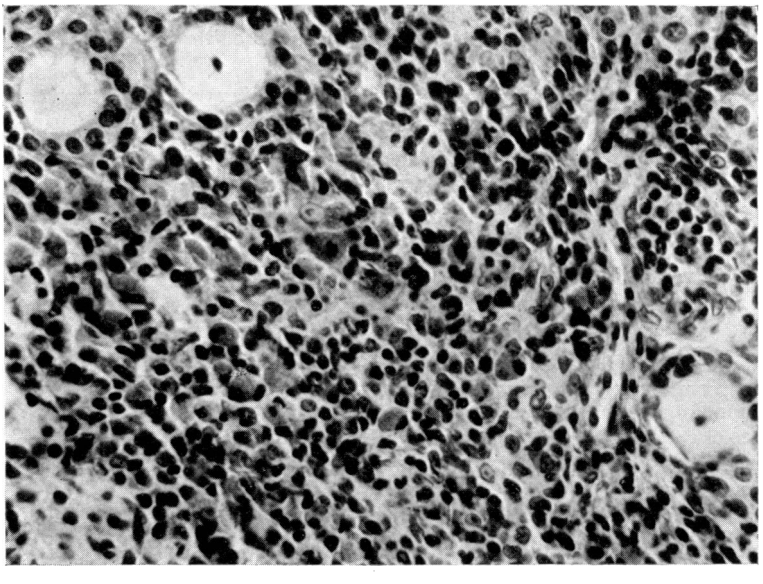

FIG. 1b. Thyroid of a rat after thymectomy and irradiation (5 × 200 r). There is a mononuclear infiltration including many plasma cells (H & E, × 400).

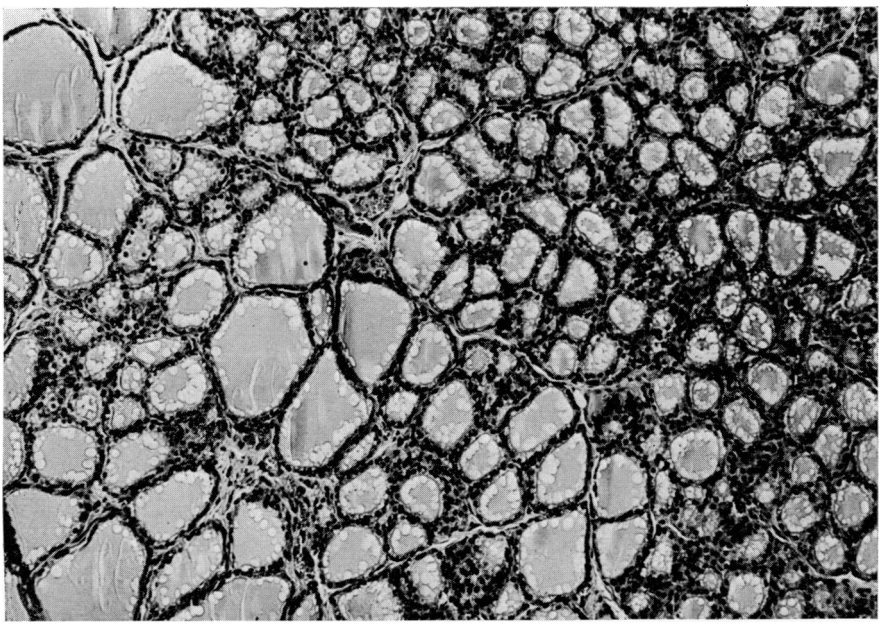

FIG. 2. Thyroid of a rat after head and neck irradiation (including thyroid region) only. There is no inflammatory reaction and follicular architecture is normal (H & E, × 80).

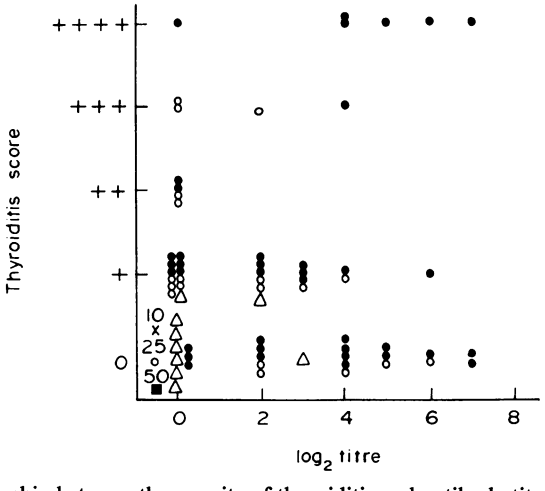

FIG. 3. The relationship between the severity of thyroiditis and antibody titre to thyroglobulin in individual rats. Grading of thyroiditis as detailed in Materials and Methods. ●, Thymectomized and irradiated rats; ○, whole body irradiated rats; △, trunk irradiated rats; ×, head and neck irradiated; ■, untreated.

Circulating antibody to nucleoprotein

The sera of four out of forty rats of the thymectomized and irradiated group were found to contain antibodies to nuclear material by immunofluorescence. In contrast, no positive sera were found in any of the other four groups.

Other possible autoimmune phenomena

Evidence of other pathological effects of possible autoimmune origin was also sought in all rats showing thyroid changes. Histological sections of adrenal, gastric mucosa, liver and kidney were examined for cellular infiltration. All were found to be normal.

Similarly, attempts to demonstrate anti-erythrocyte antibodies in the sera by the direct Coombs' method were also negative.

The effect on general aspects of immunological competence

In order to assess the degree of immunological deficiency induced by the various treatments, and to attempt to relate this to the severity of lesions, a number of simple parameters were investigated (Table 4). Thymectomized and irradiated rats (group V) had a significantly

TABLE 4. Summary of the effect of the various treatments on general aspects of immunological competence

Group*	No. of animals	Mean weight (g) ± SEM		Mean white cell count (10^3/mm^3 ± SEM)		Mean PHA TI
		Carcase	Spleen	Total	Lymphocytes	
I	14	204 ± 56·7	0·72 ± 0·21	8·9 ± 2·6	7·7 ± 2·4	40·5 ± 14·2
II	10	208 ± 69·7	0·80 ± 0·28	7·4 ± 2·5	5·7 ± 2·0	19·8 ± 9·9
III	9	210 ± 74·7	0·86 ± 0·31	6·4 ± 2·6	5·1 ± 2·1	12·7 ± 5·4
IV	33	195 ± 35·7	0·69 ± 0·13	6·6 ± 1·4	4·7 ± 1·0	5·6 ± 1·4
V	25	183 ± 39·2	0·94 ± 0·22	5·4 ± 1·4	3·3 ± 1·0	1·7 ± 0·4

Student's *t*-test analysis of differences between treatments. Carcass weight: group I versus V, $P < 0{\cdot}01$. Spleen weight: group I versus V, $P < 0{\cdot}05$. TWC count: group I versus $P < 0{\cdot}001$; group I versus II, $P < 0{\cdot}05$. Lymphocyte count: group I versus V, $P < 0{\cdot}001$; group I versus IV, $P < 0{\cdot}01$; group I versus II, $P < 0{\cdot}02$; group IV versus III, $P < 0{\cdot}05$. PHA TI: group I versus IV and V, $P < 0{\cdot}001$; group I versus III, $P < 0{\cdot}02$.

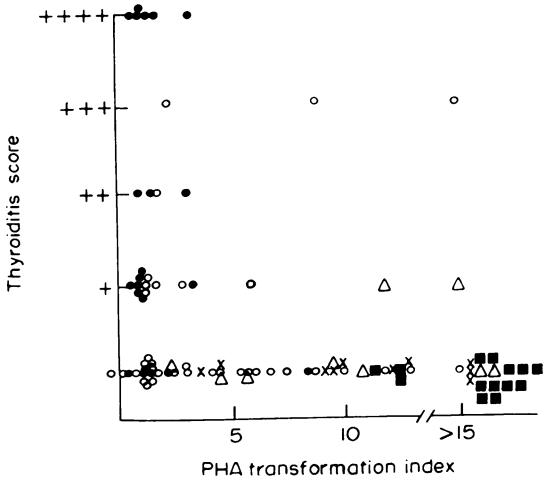

FIG. 4. The relationship between the severity of thyroiditis and the mitogenic response to PHA in individual rats. Grading of thyroiditis as detailed in Materials and Methods. ●, Thymectomized and irradiated rats; ○, whole body irradiated rats; △, trunk and limb irradiated rats; ×, head and neck irradiated rats; ■, untreated rats.

lower mean PHA transformation index when compared to any other group. The whole body irradiated group (group IV) also had a significantly lower mean TI than groups I, II and III. Only the head and neck irradiated group (group II) did not differ from the mean TI of the normal rats (group I). Thymectomized and irradiated rats were also found to be very susceptible to spontaneous infection and to have significantly lower body weights and increased spleen size than those in other groups. The total white cell and lymphocyte counts were also reduced as compared to the normal group. With the exception of the total white cell count of group II (head and neck irradiated only) the total WBC and the lymphocyte counts of the others were significantly different from the untreated group. Thus none of the treated groups could be regarded as having made a complete recovery after irradiation, although the degree of recovery differed greatly with the different forms of treatment. The relationship between the mitogenic response and the incidence and severity of thyroiditis in individual rats is examined in Fig. 4. It will be seen that although depressed transformation indices generally accompanied thyroiditis, thyroiditis did not necessarily accompany depressed transformation indices.

DISCUSSION

Meuwissen *et al.* (1969) showed that the mitotic response to phytohaemagglutinin in neonatally thymectomized rats is significantly lower than in normal rats. Doenhoff (1971) has shown a similar effect in adult thymectomized, lethally irradiated and bone marrow reconstituted mice. Using the same protocol that we have used in the present study, Harding *et al.* (1971) showed that 2 weeks after the completion of treatment, thymectomized and sublethally irradiated young rats had their mitotic response to PHA reduced by a factor of fifty-six as compared to normal rats and concluded that this procedure was effective in depleting rats of thymus-processed lymphocytes. Our findings are in agreement with these workers and further show that this effect may persist for a prolonged time after treatment (at least 60 days).

The present study has shown that severe thyroiditis may develop in rats subjected to this treatment and since no evidence of thyroid damage was seen in the group which received only local irradiation to the thyroid region, pathological change cannot be attributed directly to irradiation *per se*. Furthermore, evidence of mild thyroiditic change was seen in some of the rats which were irradiated over the trunk and limbs but in which the thyroid was shielded. As the majority of rats with severe thyroiditis were lymphopenic and had depressed mitogenic responses to PHA it would appear that this lesion is primarily related to the depletion of thymus-processed lymphocytes.

Judging by the character of the cellular infiltrate and also by the observation that autoantibody to thyroglobulin could frequently be demonstrated in the treated groups, this condition appears to be of autoimmune origin. However, we have no evidence at the moment to rule out the possibility that the procedures employed may have caused the activation of a latent virus.

An increase in the severity of thyroiditis which occurs spontaneously in obese strain chickens has also recently been observed following neonatal thymectomy (Welch *et al.*, 1973) and it seems highly probable that a similar mechanism of pathogenesis may be involved to that observed in the present study. Both situations implicate B lymphocytes rather than those derived from the thymus as the major lymphoid cell type infiltrating the

thyroid and, presumably, the chief effector of thyroid damage. The precise manner by which B cells produce thyroid destruction in this situation is uncertain but from this study it would seem unlikely that circulating antibody of the haemagglutinating type alone has any significance in this respect. However, local antibody production within the thyroid may be important as plasma cell types were prominent in histological sections of the present series. Furthermore, Clinton & Weigle (1972) have suggested that there is a correlation between the appearance of anti-thyroglobulin plaque-forming cells in the thyroid and the development of thyroid lesions in experimental thyroiditis in rabbits. It is possible that antibody produced locally within the thyroid subsequently confers specific cytotoxicity on normal B lymphocytes and/or other cells of the monocytic series. These may then destroy thyroid cells in a similar manner to the *in vitro* system recently described (Calder *et al.*, 1973) wherein thyroglobulin coated chicken erythrocytes are lysed by normal human lymphocytes in the presence of Hashimoto serum.

Assuming an autoallergic origin for the thyroiditis observed, this study provides evidence to support the hypothesis that under normal circumstances, the thymus plays an important part in the prevention of autoimmune reactivity. Our observations may be most readily explained by assuming that B cells autoreactive with thyroid antigens are prohibited from expression by the presence of thymus-derived lymphocytes. The removal of T lymphocytes by thymectomy and irradiation, and to a lesser extent by irradiation alone, may then lead to clonal expansion and subsequent thyroiditis. This inhibition may be produced by an active controlling T cell mechanism. Alternatively, Welch *et al.* (1973) have suggested that the elimination of thymus-derived lymphocytes merely provides extra biological 'space' for B lymphocytes. Of these two possibilities, the concept of an active controlling mechanism is more attractive and is consistent with studies on the haemolytic anaemia of NZB mice where Playfair (1971) has reported that the transfer of thymus cells from 2-week-old NZB mice routinely to other mice from the age of 1 month significantly delayed the onset of positive direct Coombs' tests. Similarly, Teague & Friou (1969) have shown that the transfer of thymus cells from young strain A mice to old syngeneic animals which frequently develop antinuclear antibodies results in decreased levels or their disappearance. The manner by which such an active controlling system may function (i.e. whether control operates centrally by inhibiting the development of auto-reactive clones, or peripherally by preventing the infiltration of the thyroid) remains to be elucidated.

If an active T cell control process is involved it is necessary to explain why many rats with apparently defective T cell function failed to develop thyroiditis. One possibility is that these particular rats did not possess B cells which were potentially auto-reactive with thyroid antigens. In this connection it may be significant that the incidence of spontaneous thyroiditis in the thymectomized and irradiated group is much the same as can be obtained by active induction of thyroiditis using thyroid extract in Freund's complete adjuvant in normal rats of the same strain (Penhale, unpublished data). This explanation would also account for the comparative rarity of other coincidental autoimmune phenomena in the thymectomized and irradiated series.

The occurrence of antinuclear antibodies in some thymectomized irradiated rats parallels similar findings in other species following neonatal thymectomy (Thivolet *et al.*, 1967; Teague & Friou, 1969; Albini & Wick, 1973). Albini and Wick also noted the association between the occurrence of spontaneous thyroiditis and antinuclear antibodies in the Obese strain of chicken. The reason for this association is unknown.

The present findings also indicate that T cell co-operation does not seem to be essential for autoantibody induction as the majority of the rats in the thymectomized and irradiated group were found to have thyroglobulin antibodies. These findings also suggest that the model of autoimmunity proposed by Weigle (1971), in which loss of specific tolerance of auto-reactive T cells is thought to lead to stimulation of B cells, is unlikely to apply to the present model.

Finally it would appear that this model represents a closer analogy to the clinical situation in several features, such as spontaneity and duration, than the actively induced experimental disease and may therefore prove of value in the elucidation of the basic pathogenesis of autoimmune thyroid disease in man.

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