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The Biological Effects of Anti-Thyroid Antibodies

THYROID EOSINOPHILIA FOLLOWING PASSIVE TRANSFER OF ANTI-THYROGLOBULIN ANTIBODY

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Summary. Guinea-pigs that received a passive transfer of rabbit antiserum to guinea-pig thyroglobulin developed an eosinophilic infiltrate of the thyroid. The infiltrate varied in intensity in different guinea-pigs, developed over a 1–24-hour period, and resolved in 4–9 days. The antibody that was injected was localized to the interstitial areas of the thyroid gland. It is proposed that thyroglobulin is also present in the interstitial areas either normally or as a result of the antiserum injection and that the eosinophils appeared in response to the presence of thyroglobulin–anti-thyroglobulin complexes in these areas.

INTRODUCTION

Since the first report by Rose and Witebsky (1956), there has been considerable controversy about the role of circulating antibody in experimental thyroiditis. Several studies have shown no correlation between antibody titre as measured by agglutination and flocculation tests and the degree of thyroiditis seen histologically (Miescher, Gorstein, Benacerraf and Gell, 1961; McMaster, Lerner and Exum, 1961). Passive transfer and transplacental passage of thyroid antibodies have consistently failed to produce thyroid lesions (Roitt and Doniach, 1958; Irvine, 1960; Terplan, Witebsky, Rose, Paine and Egan, 1960; Sclare and Taylor, 1961; Rose, Kite and Doebbler, 1962; Roitt, Jones and Doniach, 1962).

On the other hand, Irvine (1960) and Pulvertaft, Doniach and Roitt (1961) have demonstrated specific cytotoxic effects of antiserum on thyroid cells in tissue culture. Roitt *et al.* (1962) found inflammatory reactions in the thyroid gland of rats after passive transfer of heterologous antiserum to rat thyroid if the recipients first received ^{131}I, X-irradiation or Freund's adjuvant. Koffler and Paronetto (1965) in fluorescent antibody studies with experimental thyroiditis have demonstrated the deposition *in vivo* of γ-globulin in the thyroid gland.

The present investigation was made to explore further the biological effects *in vitro* and *in vivo* of the organ specific antibodies occurring in response to immunization with thyroid antigen. In the course of these experiments, rabbit antiserum to guinea-pig thyroglobulin was transferred to guinea-pigs. This did not result in classical experimental thyroiditis; rather, eosinophilic infiltrates appeared in the thyroid glands of the recipients. These changes were confined to the thyroid gland and occurred specifically with antiserum to guinea-pig thyroglobulin. The course of development and resolution of this infiltrate is described and the possible underlying mechanisms are discussed.

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MATERIALS AND METHODS

Animals

The guinea-pigs used were 250–500 g random bred males obtained from several sources. New Zealand white rabbits were used for antiserum production.

Preparation of antigens

Guinea-pig thyroglobulin was prepared by the differential ultracentrifugation method of Edelhoch (1960). Further purification was achieved by passing this material through a Sephadex G-200 column at 5°, following the method described by Flodin and Killander (1962). Crude extracts of guinea-pig thyroid, stomach, liver and kidney were made by mincing the tissue, homogenizing in a glass–Teflon homogenizer for 2 minutes and then centrifuging at 1300 ***g*** for 10 minutes. The supernatant fluid was used as the crude extract.

Antiserum production

Rabbits and guinea-pigs were immunized with a series of injections of guinea-pig thyroglobulin or crude tissue extracts in complete Freund's adjuvant. *Mycobacterium tuberculosis* (H37Rv) was added to incomplete Freund's adjuvant (Difco) to a final concentration of 8 mg/ml. Three parts of antigen were emulsified in 1 part of adjuvant giving a final concentration of antigen of 3 mg/ml and a final concentration of mycobacterium of 2 mg/ml. Primary immunization consisted of 0·8 or 1·0 ml of the emulsion injected into numerous sites in the paw pads. Animals were boosted at 4-week intervals with 0·8 ml of the antigen–adjuvant emulsion containing 2 mg/ml of antigen protein. They were bled 1 week after the booster immunization. The sera of several animals were pooled and stored frozen.

Antibody titration

A modification of the Stavitsky (1954) method for tanned red cell agglutination was used. Sera were diluted in 1:150 normal rabbit serum using a separate pipette for each dilution. Three milligrams of guinea-pig thyroglobulin was used to coat 1 ml of a 33 per cent suspension of sheep red cells. The titre was expressed as the highest dilution of antiserum that gave at least a 2+ reaction.

Antiserum fractionation

The antiserum was separated into four fractions using DEAE-cellulose chromatography according to the methods reported by Peterson and Sober (1962). Elution was performed at 5° with buffers of increasing ionic strength at pH 7·8. The eluates were collected in a fraction collector and were assayed for protein concentration in a Beckman DB spectrophotometer at 280 mμ. Samples within the peaks were pooled and lyophilized. Lyophilized fractions were then dissolved in buffered saline for immunoelectrophoretic analysis and injection into animals.

Immunoelectrophoretic analysis

Immunoelectrophoretic analysis was carried out in veronal buffer at pH 8·2, 0·05 M using the micro-method described by Scheidegger (1955).

Antiserum absorption

Five-millilitre aliquots of antiserum were absorbed for 30 minutes at 37° followed by 36 hours at 5° with one of the following: 5 g of homogenized guinea-pig liver, stomach, thyroid or kidney; 2·5 ml of normal guinea-pig serum; or 0·75, 7·5 or 30 mg of guinea-pig thyroglobulin.

Histological examination

The specimens were fixed in 10 per cent formalin or in Bouin–Hollande fluid for 24 hours, embedded in paraffin, sectioned at 4–6 μ and stained by haematoxylin and eosin or by a modified Dominici stain as described by Litt (1963). With the Dominici stain the eosinophils had bright orange granules, neutrophils had pale lilac granules, the cytoplasm of plasma cells was intensely blue, and the thyroid follicles and cell nuclei took various shades of blue and violet. Evaluation of sections was made independently by at least two observers.

Fluorescent antibody studies

Tissue sections were prepared according to the technique described by Allansmith, Goihman-Yahr and Buell (1964) which included snap-freezing in isopentane cooled with liquid nitrogen, lyophilization for 7 days at −40°, embedding in degassed paraffin and cutting sections (4 μ) on an ordinary microtome. These sections were placed in xylene, ethanol and pH 7·4 buffered saline, and staining was carried out for 20 minutes at room temperature using fluorescent goat anti-rabbit γ-globulin or fluorescent normal goat serum. All antisera were characterized for specificity by immunoelectrophoresis before use. In some cases pre-treatment was carried out with non-fluorescent normal goat serum, goat anti-rabbit γ-globulin or rabbit anti-guinea-pig thyroglobulin. Sections were washed in buffered saline for 15 minutes after each staining procedure and were mounted in 50 per cent glycerol buffer. For fluorescent microscopy a Zeiss fluorescence microscope fitted with a high pressure mercury vapour lamp (Osram HBO-200) was used. An exciter filter Schott UG-2 and barrier filter Schott GG4 (O/1) were used. With these filters the autofluorescent areas appeared blue so that there was a clear distinction from the bright green of the specific γ-globulin fluorescence. In sections stained with fluoresceinated normal goat serum the colloid, follicular cells and interstitial areas were blue. Eosinophils showed a very bright yellow-green fluorescence and red blood cells appeared yellow.

RESULTS

THYROID CHANGES AT VARIOUS TIMES AFTER INTRAVENOUS INJECTION OF RABBIT ANTISERUM TO GUINEA-PIG THYROGLOBULIN

Table 1 summarizes the course of events following a single intravenous injection of 2 ml of rabbit antiserum to guinea-pig thyroglobulin. As early as 1 hour after injection, small numbers of eosinophils were lining vessel walls around and within the gland. This we have termed a slight change. It progressed until the time of maximum eosinophilic infiltration at 24 hours after injection. This was a consistent finding; 49/53 glands examined at 24 hours were positive and most of these showed diffuse infiltration with large numbers of eosinophils. At 4–8 days after injection some thyroids appeared normal and infiltrates, when they occurred, were minimal. By 2–4 weeks after injection the thyroid appeared normal. We have noted similar eosinophilic infiltrates after a single intraperitoneal

injection of 2 ml of antiserum to thyroglobulin (8/9). Repeated intraperitoneal injections of antiserum resulted in minimal thyroid eosinophilia and no other abnormality when recipients were examined at 3–4 weeks (6/11).

THE HISTOLOGICAL APPEARANCE OF THE EOSINOPHILIC INFILTRATES

Eosinophils are seen with some frequency in the stomach, small intestine, spleen, lymph nodes and lungs of normal guinea-pigs, but only an occasional eosinophil is seen in the thyroid of normal animals. The normal guinea-pig thyroid (Fig. 1) has a regular arrangement of closely approximated colloid follicles separated only by narrow interstitial areas containing small blood vessels. In contrast (Fig. 2) 24 hours after the intravenous injection of 2 ml of rabbit antiserum to guinea-pig thyroglobulin the follicles were separated by dense cellular infiltration in the interstitial areas. These infiltrating cells were almost

TABLE 1

THYROID EOSINOPHILIA AFTER INTRAVENOUS INJECTION OF RABBIT ANTISERUM TO GUINEA-PIG THYROGLOBULIN

Time of death	No. positive/No. injected	Extent of infiltrate
1 hour	4/4	Slight
6 hours	5/6	Slight to moderate
12 hours	2/3	Slight to moderate
24 hours	49/53	Moderate to marked
48–72 hours	8/9	Slight to moderate
4–5 days	7/10	Slight to moderate
8 days	3/9	Slight
2–4 weeks	0/5	None

exclusively eosinophils (Fig. 6). The degree of eosinophilia seen in different animals varied from small numbers of perivascular cells without any real disruption of gland architecture in some cases to such marked infiltrates in other cases that the normal follicular arrangement was altered. We have never noted any of the glandular disorganization with mononuclear cell infiltrates that is typical of classical experimental thyroiditis (Figs. 3 and 5). Thus the eosinophilic lesion differs from that seen in experimental thyroiditis in that the infiltrate consists almost exclusively of eosinophils and regresses without destructive changes in the gland. These changes are confined to the thyroid. Examination of liver, kidney, lymph nodes, adrenal, pancreas, spleen, lung and stomach shows no more eosinophils than can be seen in normal guinea-pigs, and no other abnormalities have been noted. Normal guinea-pigs have fairly large numbers of eosinophils in their lymph nodes. For this reason counts were performed using Dominici stained tissues according to the method of Litt (1963) which revealed no increase of eosinophils in the nodes of injected animals. Furthermore, eosinophilic infiltrates have not occurred in the thyroid or other organs following injection of normal rabbit serum or antiserum to guinea-pig serum, liver, kidney or stomach.

THYROID CHANGES AFTER INJECTION OF ANTISERUM TO OTHER ANTIGENS

Rabbit antiserum was made against a more highly purified guinea-pig thyroglobulin achieved by passing it through a Sephadex G-200 column. Unlike the thyroglobulin prepared by ultracentrifugation, the G-200 material failed to react with rabbit antiserum

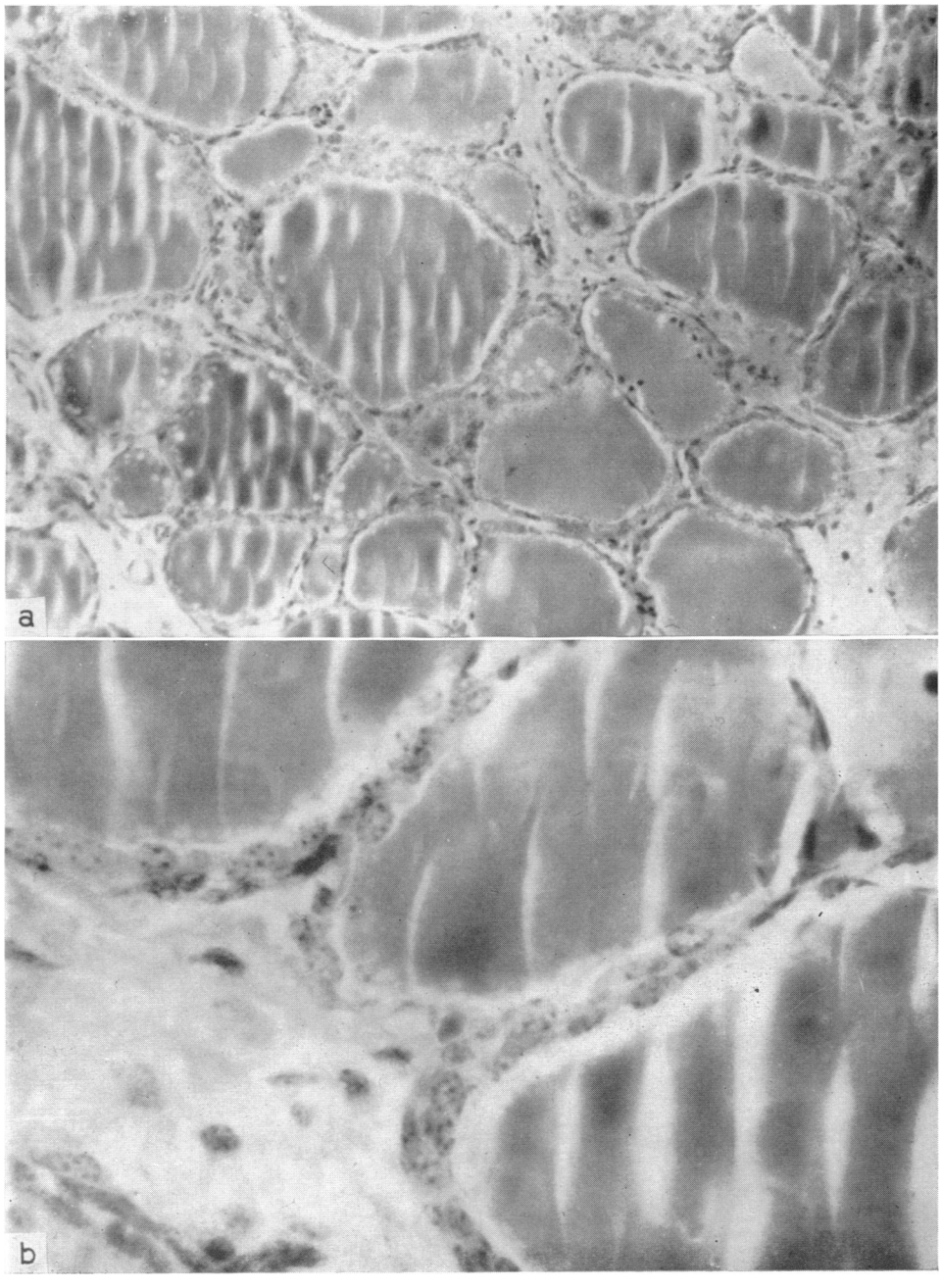


FIG. 1. (a) Normal guinea-pig thyroid showing the normal variation in acinar structure. In the interstitial areas that separate the follicles are small blood vessels, some of which contain erythrocytes and occasional leucocytes. Dominici stain, × 75.

(b) Higher power view of the normal thyroid showing the closely approximated colloid follicles and the sparse cellularity of the interstitial area (lower left corner). Dominici stain, × 300.

(*Facing p.* 42)

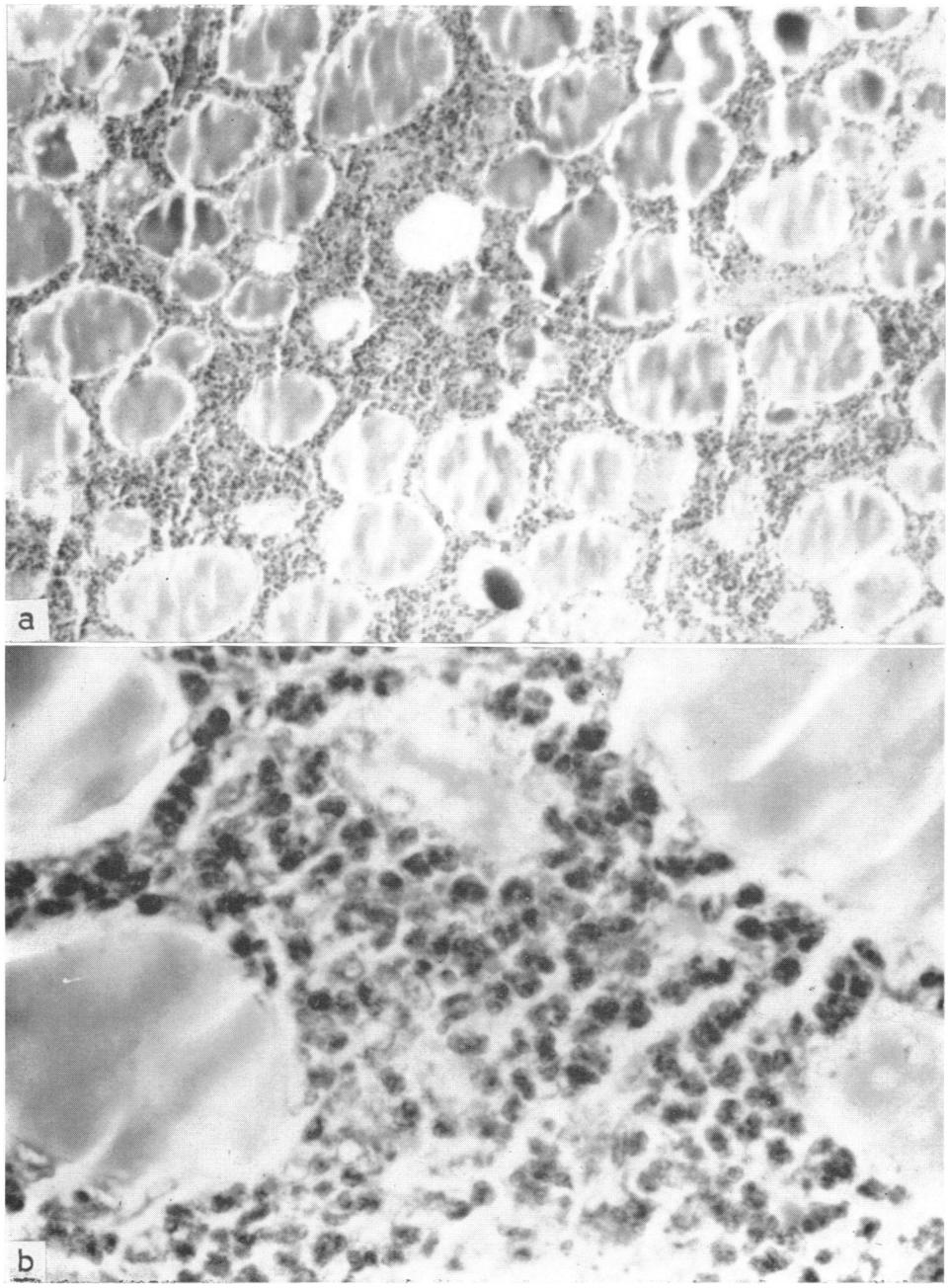


FIG. 2. (a) Guinea-pig thyroid 24 hours after intravenous injection of 2 ml of rabbit antiserum to guinea-pig thyroglobulin. The normal appearing follicles are separated by interstitial areas that are enlarged due to dense infiltration by eosinophils. Dominici stain, × 75.

(b) Higher power view demonstrating the change that is referred to as a 'marked infiltrate' in Table 1. The dark-staining cells are all eosinophils. Dominici stain, × 300.

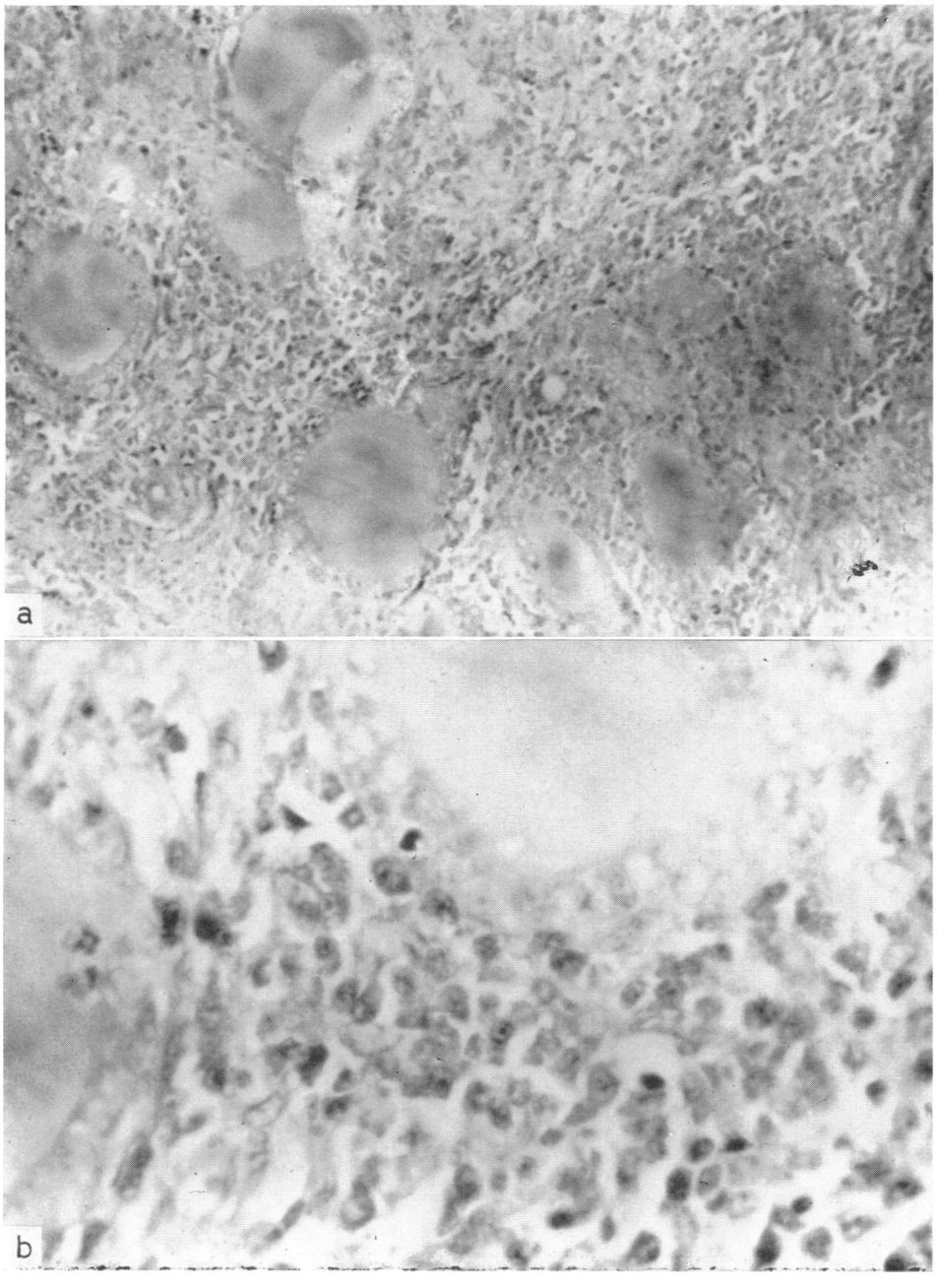


FIG. 3. (a) Guinea-pig thyroid showing experimental thyroiditis 3 weeks after footpad injection with guinea-pig thyroglobulin in complete Freund's adjuvant. There is marked disruption of the normal architecture with only a few follicles remaining. Dominici stain, × 75.

(b) Higher power view of experimental thyroiditis showing the mononuclear character of the cellular infiltrate which is in marked contrast to that following the passive transfer of antiserum. Dominici stain, × 300.

to whole guinea-pig serum by agar gel diffusion. Intravenous injection of guinea-pigs with 2 ml of this antiserum resulted in no changes at 30 minutes (0/5), slight changes at 1 hour (2/3) and almost uniformly provoked moderate to marked eosinophilic changes at 24 hours (13/14). Intraperitoneal injection of 2 ml of rabbit antiserum to whole crude thyroid extract also produced eosinophilic infiltrates of varying intensity at 24 hours (4/4).

SPECIFICITY STUDIES OF SERA POSSESSING EOSINOPHILIA PROVOKING ACTIVITY

In the experiment summarized in Table 2 a potent rabbit antiserum to guinea-pig thyroglobulin was subjected to various types of absorption and following this was tested for anti-thyroglobulin haemagglutination titre and eosinophilia provoking activity. The unabsorbed sera and the sera absorbed with saline, guinea-pig liver, kidney or serum had

TABLE 2

THYROID EOSINOPHILIA 24 HOURS AFTER INJECTION OF ABSORBED RABBIT ANTISERUM TO GUINEA-PIG THYROGLOBULIN

Addition of 5 ml of rabbit antiserum to guinea-pig thyroglobulin	Haemagglutination titre after absorption	No. positive/No. injected
None	1:2,000,000	1/1
2·5 ml saline	1:2,000,000	4/4
Guinea-pig thyroglobulin		
2·5 ml 12 mg/ml	1:1,000	0/4
2·5 ml 3 mg/ml	1:20,000	0/4
2·5 ml 0·3 mg/ml	1:200,000	4/4
Crude guinea-pig thyroid		
2·5 ml 0·8 g/ml	0	0/3
2·5 ml normal guinea-pig serum or guinea-pig liver, kidney or stomach	1:2,000,000	12/12
2·5 ml 2 g/ml		

a titre of 1:2,000,000 and induced eosinophilia in every case. Absorption with increasing amounts of guinea-pig thyroglobulin led to progressive lowering of the haemagglutination titre. When the titre fell to 1:20,000 or less there was no eosinophilia provoking activity. Absorption with crude guinea-pig thyroid resulted in complete loss of haemagglutination titre and eosinophilia provoking activity. Thus, specific absorption of the serum with guinea-pig thyroglobulin but not with other tissues removed the ability to agglutinate sensitized red cells and to provoke eosinophilic lesions. Dilution of the antiserum to a titre of less than 1:100,000 also eliminated significant eosinophilia provoking activity although traces of eosinophils have occasionally been found using antisera with a titre as low as 1:20,000.

FRACTIONATION OF ANTISERUM TO LOCALIZE EOSINOPHILIA PROVOKING ACTIVITY

Fig. 4 shows the results of fractionation of rabbit antiserum to guinea-pig thyroglobulin on a DEAE-cellulose column. As the molarity of the phosphate buffer passing through the column was increased in a stepwise manner from 0·007 to 0·15 M while maintaining a constant pH of 7·8, four protein peaks were obtained. When tested by immunoelectrophoretic analysis, Fraction I consisted only of γ-globulin. Fractions II and III also contained γ-globulin in progressively lesser amounts along with other serum components. No

γ-globulin was demonstrable in Fraction IV, which consisted of α-globulin and albumin. When 50 mg of each fraction was dissolved in physiological saline and injected intravenously into guinea-pigs it was found that Fraction I produced striking eosinophilia, Fraction II induced a few eosinophils, but Fractions III and IV were inactive. Injection of as little as 5 mg of Fraction I has resulted in eosinophilic changes.

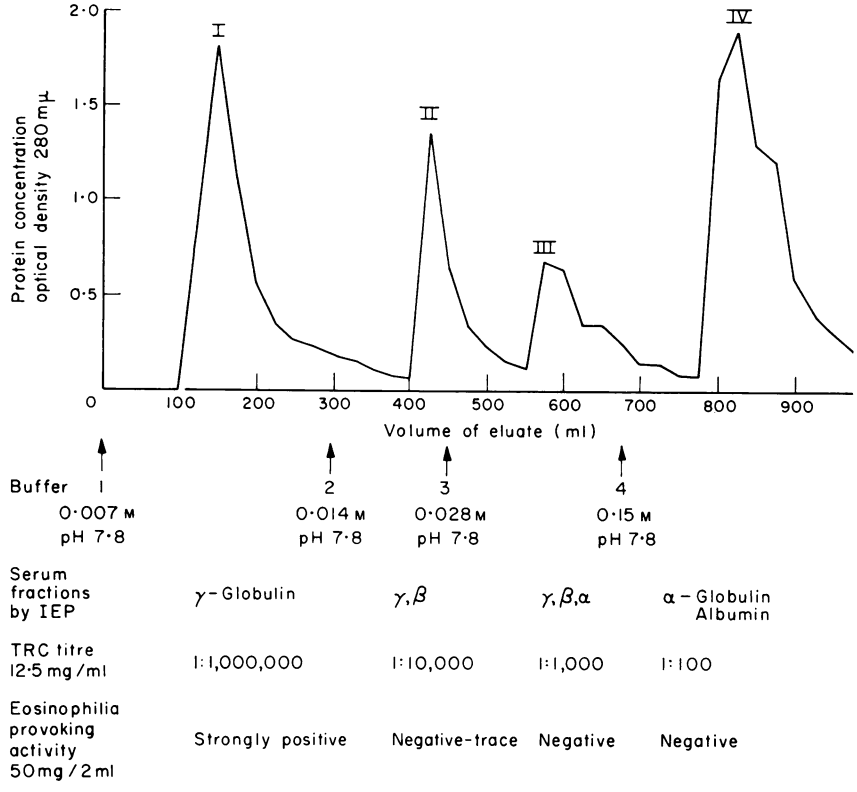
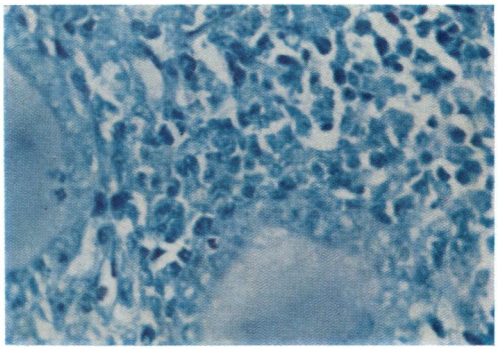


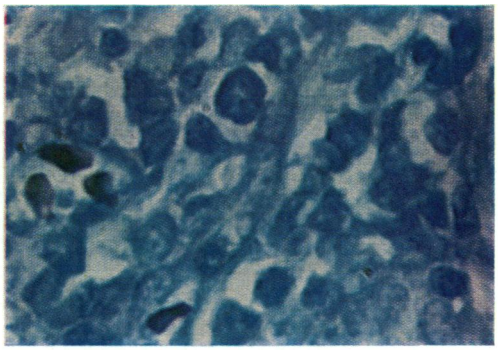
FIG. 4. DEAE-cellulose fractionation of rabbit anti-thyroglobulin serum. The arrows mark the points at which buffers 1–4 are added. They have a pH 7·8 and increase in molarity from 0·007 to 0·15 M. Below each of the peaks I–IV are listed the serum fractions found by immunoelectrophoresis (IEP), the tanned red cell (TRC) titre (red cells coated with guinea-pig thyroglobulin) of a 12·5 mg/ml concentration of the fraction, and the eosinophil provoking activity noted 24 hours after intravenous injection of guinea-pigs with 50 mg of the lyophilized fraction dissolved in 0·15 M Nacl.

LOCALIZATION OF INJECTED γ-GLOBULIN BY THE FLUORESCENT ANTIBODY TECHNIQUE

Guinea-pigs received an intravenous injection of 2 ml of rabbit antiserum to guinea-pig thyroglobulin and were killed at 1, 6 and 24 hours. The thyroid sections when treated with xylene, ethanol, buffer and fluorescent goat anti-rabbit γ-globulin showed wavy strands of specific green fluorescence in the interstitial areas (Fig. 7a). There was no fluorescence in the follicles or follicular cells. The eosinophils which were present in the interstitial areas (as confirmed by Dominici staining of adjacent sections) showed a very bright green fluorescence. When a section of the same thyroid was treated with non-fluoresceinated goat anti-rabbit γ-globulin, washed with buffer and then stained with fluorescein conjugated goat anti-rabbit γ-globulin there was no fluorescence (Fig. 7b).

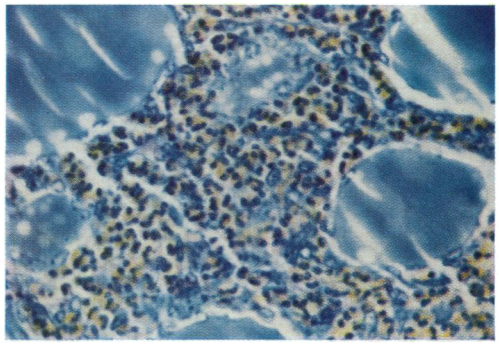
5(a)

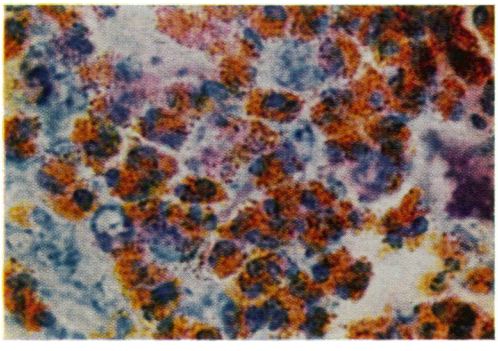




5(b)

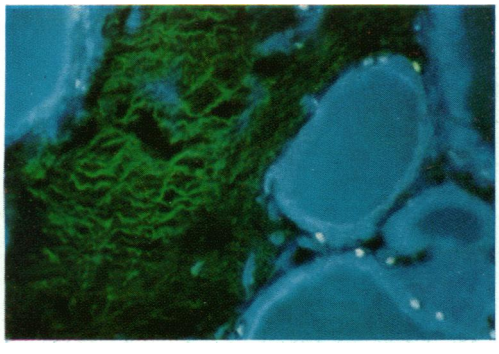
6(a)

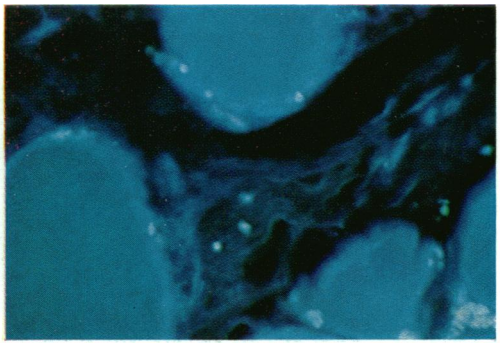




6(b)

7(a)





7(b)

FIG. 5. (a) Experimental thyroiditis (the same field as Fig. 3b) demonstrating more clearly the mononuclear character of the infiltrate. Dominici stain, × 100.

(b) Higher power view of experimental thyroiditis showing mononuclear cells and several erythrocytes with absence of eosinophils. Dominici stain, × 250.

FIG. 6. (a) Thyroid eosinophilia (the same field as Fig. 2b) demonstrating the interstitial eosinophilic infiltration. Dominici stain, × 100.

(b) Higher power view showing the orange-red granules that clearly identify the infiltrating cells as eosinophils. Dominici stain, × 250.

FIG. 7. (a) Guinea-pig thyroid 1 hour after intravenous injection of 2 ml rabbit antiserum to guinea-pig thyroglobulin. Green fluorescent material is present in the interstitial area after treatment of the section with fluoresceinated goat anti-rabbit γ-globulin. × 60.

(b) Same thyroid as in (a) but with pre-treatment with non-fluoresceinated goat anti-rabbit γ-globulin before staining with fluorescein conjugated goat anti-rabbit γ-globulin. No fluorescence is present. × 60.

(*Facing. p.* 44)

Thus an effective blocking had occurred by pre-treatment with the non-fluoresceinated serum. Eosinophils still showed a bright fluorescence which is probably due to the strong tendency for these cells to stain non-specifically with the addition of protein–fluorescein conjugates *in vitro*. Pre-treatment with normal goat serum instead of goat anti-rabbit γ-globulin did not block the interstitial fluorescence. Staining of a thyroid section from the same animal with fluoresceinated goat anti-rabbit γ-globulin absorbed with rabbit γ-globulin resulted in no fluorescence. Absorption with guinea-pig γ-globulin did not remove the fluorescent activity of the goat anti-rabbit γ-globulin. Also, the fluoresceinated goat anti-rabbit γ-globulin reacted with rabbit γ-globulin and not with guinea-pig γ-globulin by agar gel diffusion. When a guinea-pig was injected with 2 ml of rabbit antiserum to guinea-pig kidney instead of the anti-thyroglobulin serum, staining of the thyroid with fluorescein conjugated anti-rabbit γ-globulin revealed no fluorescence. The kidney section of such an animal showed faint fluorescence in the glomeruli and blood vessels. The thyroids of animals injected with rabbit antiserum to guinea-pig thyroglobulin and killed at 6 and 24 hours showed progressively less interstitial fluorescence and increasing numbers of eosinophils when stained with fluoresceinated goat anti-rabbit γ-globulin. Identical staining of the sections of lymph node, kidney and liver of these animals revealed no specific fluorescence. Eosinophils in the lymph node again showed bright fluorescence. Sections of the thyroid glands were treated directly with rabbit antiserum to guinea-pig thyroglobulin followed by buffer and then fluoresceinated anti-rabbit γ-globulin and this revealed typical thyroglobulin fluorescence to be present in the follicles. Thus the absence of rabbit γ-globulin in the follicles of glands with eosinophilic infiltrates is not due simply to an absence of antigen.

To avoid the complicating factor of heterologous serum reactions we have begun investigation of the results of passive transfer of guinea-pig antiserum. Thus far only one of four animals injected with guinea-pig antiserum to guinea-pig thyroglobulin has had small numbers of eosinophils in the thyroid. Since an haemagglutination titre of about 1:100,000 is necessary for activity in the case of the rabbit antiserum this may be a limiting factor as guinea-pig antisera rarely approach such a titre.

In order to compare the pathological changes in the thyroid after passive transfer of antiserum to those following active immunization, guinea-pigs were immunized with guinea-pig thyroglobulin in complete Freund's adjuvant. Early changes of thyroiditis were present at 1 week and by 3–4 weeks after immunization marked thyroid infiltrates were present in most animals. Mononuclear cells predominated and eosinophils were rare, but in occasional animals comprised 5–10 per cent of the infiltrating cells. Animals killed at various times from 30 minutes to 6 days after active immunization showed no thyroid eosinophilia prior to the development of thyroiditis.

DISCUSSION

It has been shown that rabbit antiserum to guinea-pig thyroglobulin when passively transferred to guinea-pigs gives rise to an eosinophilic infiltrate of the thyroid. Since there have been a number of previous attempts to produce thyroid changes by the passive transfer of anti-thyroid sera it is somewhat surprising that eosinophilic infiltrates have not been reported earlier. There are several possible explanations. A very high haemagglutinating titre of antibody is necessary and this requirement may not have been met in earlier investigations. Since the thyroid eosinophilia is transient the eosinophil response may be

missed if the gland is not examined at the appropriate time. Also, there may be species differences in an experimental eosinophilia response (Litt, 1963).

The fluorescent labelling studies have shown that the rabbit antibody to guinea-pig thyroglobulin which is injected is localized specifically in the interstitial areas of the thyroid where the eosinophils are also seen. The interstitial pattern of immunoglobulin localization is similar to that shown by Koffler and Paronetto (1965) in guinea-pigs with experimental thyroiditis. They were also able to demonstrate guinea-pig γ-globulin in the follicles. We have not been able to show that the injected rabbit antibody is present in the follicles, even though appropriate fluorescent testing reveals that guinea-pig thyroglobulin is present there. Koffler and Paronetto (1965) had to deal with the possibility that they might be demonstrating only a component from an inflammatory exudate rather than a specific deposition of immunoglobulin. They pointed out that a non-immunological avidity of tissue for γ-globulin was not excluded. In our studies the fact that it is rabbit antibody directed against guinea-pig thyroid antigen and not against other guinea-pig tissues that localizes in the thyroid, and the failure of guinea-pig γ-globulin to absorb out the fluorescent activity of the goat anti-rabbit γ-globulin make it likely that it is there because of the presence of antigen and hence represents a real immunological event.

The fact that anti-thyroglobulin antibody probably in conjunction with thyroglobulin is found in the interstitial areas of the guinea-pig thyroid gland is of particular interest in view of the suggestion that thyroglobulin is a 'hidden antigen' confined to the follicles. There are previous studies that suggest that thyroglobulin may not be inaccessible. Hjort and Pedersen (1962) reported that there is circulating thyroglobulin in a high percentage of normal infants. More recently Assem, Trotter and Belyavin (1965) have demonstrated free thyroglobulin as well as anti-thyroglobulin antibodies in normal adult sera. White (1959) and Koffler and Paronetto (1965) have shown that antibody to thyroglobulin passes into the follicles in thyroiditis. Studies by Daniel, Pratt, Roitt and Torrigiani (1966a, b) indicate that thyroglobulin is present in the lymph leaving the thyroid gland of the normal monkey and rat. Hence the thyroglobulin antigen may be accessible for combination with antibody in the interstitial areas.

It seems clear that the injected anti-thyroglobulin antibody plays a role in eliciting the eosinophilic response in the thyroid. However, we do not know the precise mechanism by which eosinophils appear. There are a number of recent reports relating eosinophils to antigen–antibody complexes. Litt (1964a) has demonstrated the uptake of specific antigen–antibody complexes by eosinophils. Antigen or antibody alone or antigen plus non-specific antibody were not taken up. Sabesin (1963) has also shown phagocytosis of antigen–antibody complexes by eosinophils. *In vivo* peritoneal exudate studies by Litt (1961) required the presence of antigen and antibody for eosinophilia to occur. Litt (1964b) and Cohen, Kantor and Gatto (1961) have reported that injection of antigen–antibody complexes in the footpads of animals evokes an eosinophilic response in the draining lymph nodes. Archer and Hirsch (1963) have shown *in vitro* that horse eosinophils were attracted to and readily engulfed antigen–antibody complexes. Thus it would seem that a reasonable explanation of the eosinophilic reaction is that eosinophils are migrating to the sites of antigen–antibody combination.

Another explanation for the eosinophilic infiltrates is that the bound antibody is acting as a sessile antigen and that we are witnessing a very early immune response to foreign protein (Speirs, 1958). However, Litt (1963) has shown that eosinophils do not migrate to the site of antigen alone so that this explanation would require that antibody against

the rabbit serum was being formed within the 1st hour after injection. If this were true one would expect to see an increase in eosinophils in the regional lymph nodes (Litt, 1964c), and this is not the case.

What is the significance of the thyroid eosinophilia and why does typical experimental thyroiditis not result? Soluble immune complexes have been shown to play an important role in the initiation of a number of pathological states such as anaphylaxis, serum sickness, experimental glomerulonephritis and the Arthus reaction (Weigle, 1961). Litt (1964a) has suggested that since eosinophilic leucocytes can wall off these noxious agents by phagocytosing them, they may be involved in a defensive mechanism. The infiltration followed by the gradual disappearance of the eosinophils from the thyroid, leaving the gland without any pathological change that we can detect, might be indicative of such a protective function.

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