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Induction of Thyroiditis in Guinea-Pigs by Intravenous Injection of Rabbit Anti-Guinea-Pig Thyroglobulin Serum

I. LIGHT MICROSCOPIC STUDY

R. KÅRESEN AND T. GODAL

Department of Pathology, Norsk Hydro's Institute for Cancer Research, and Kaptein W. Wilhelmsen og Frues Bakteriologiske Institutt, Oslo University, Oslo, Norway

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Summary. An inflammatory reaction in the thyroid of guinea-pigs, induced by intravenous injection of rabbit anti-guinea-pig thyroglobulin serum, has been studied at time intervals ranging from ¼ hour to 20 days after injection. Specific staining methods for eosinophils have been used to demonstrate that the inflammatory infiltrate mainly consisted of eosinophil granulocytes, but that neutrophil granulocytes were also present at the earliest time intervals. PAS-staining revealed that the granulocytes contained rounded droplets of material with a PAS-reactivity comparable to that of the colloid. This was most clearly seen 12 and 24 hours after injection. The possibility that this material represents phagocytosed thyroglobulin perhaps in an antigen–antibody complex has been suggested. The number of mast cells was counted and degranulation, which was apparently most extensive 12 hours before maximum granulocyte infiltration, was observed. Possible mechanisms involved in eosinotaxis and uptake of antigen–antibody complexes in the granulocytes have been discussed.

INTRODUCTION

In previous reports (Godal and Kåresen, 1967a, b) we have described the development of thyroiditis in guinea-pigs after intraperitoneal injection of either rabbit anti-guinea-pig thyroglobulin serum or guinea-pig anti-guinea-pig thyroglobulin serum. After 24 hours, the first time interval studied, a massive infiltration of granulocytes, which were apparently eosinophils, was observed. In animals receiving rabbit anti-guinea-pig thyroglobulin serum, a subacute stage with mononuclear cells mainly of the histiocytic type, but also lymphocytes, was seen 5 days after injection. This was not observed in the animals receiving guinea-pig–anti-guinea-pig thyroglobulin serum. Ten and 20 days after injection the thyroiditis had apparently resolved, leaving nearly normal thyroids.

Sharp, Wortis and Dunmore (1967) have reported infiltration of granulocytes in the thyroid after intravenous injection of rabbit anti-guinea-pig thyroglobulin serum in guinea-pigs. They also included in their study the first 24 hours after injection, and found infiltration of granulocytes as early as after 1 hour. The infiltrates, in their case, consisted almost exclusively of eosinophils.

In the present investigation the antibody induced granulocyte infiltrates of the thyroid have been further explored, and also our results have been extended to include the first

24 hours. Since the neutrophil granulocyte of the guinea-pig has a pseudo-eosinophil character and may be difficult to distinguish from eosinophil granulocytes (Hirsch, 1965), different staining methods considered to be specific to eosinophils have been employed. Mast cells in the thyroid have also been studied as these cells may release their histamine containing granules when antigen–antibody reactions take place (Humphrey and Mota, 1959) and histamine has been suggested to be involved in eosinotaxis (Archer, 1963). The goal of this and the following studies with fluorescent antibody technique (Kåresen and Godal, 1969) and electron microscopy (Kåresen, 1969) is to throw light upon the mechanism behind the granulocyte invasion of the thyroid following intravenous injection of rabbit anti-guinea-pig thyroglobulin serum, and to look for any effect of the circulating antibody on the thyroid parenchymal cells.

EXPERIMENTAL METHODS

Animals

A total of fifty random-bred white female guinea-pigs, strain Pon-Sff-c, weighing 200–300 g were obtained from the National Institute of Public Health, Oslo. The animals were randomized and split in three groups as indicated in Table 1 by use of tables of random permutations (Moses and Oakford, 1963). Ten random-bred rabbits, weighing 2000–3000 g were used for production of antiserum.

TABLE 1

NUMBER OF GUINEA-PIGS KILLED AT DIFFERENT TIME INTERVALS AFTER INTRAVENOUS INJECTION OF RABBIT ANTI-GUINEA-PIG THYROGLOBULIN SERUM, RABBIT ANTI-FREUND'S ADJUVANT SERUM OR 0·9 PER CENT SALINE

Time (hours) after injection at killing	Material injected		
	Anti-thyroglobulin serum	Anti-Freund's adjuvant serum	0·9 per cent saline
1/4	2	2	1
1/2	2	2	1
1	2	2	1
3	2	2	1
6	2	2	1
12	2	2	1
24	2	2	1
120	2	2	1
240	2	2	1*
480	2	2	1

* The thyroid lobe for light microscopy was missing in this animal.

In vitro *immunological methods*

The indirect hamagglutination procedure was used as previously described (Godal and Kåresen, 1967a). Complement fixation was carried out as described by Henriksen (1967).

The method of Ouchterlony was performed as described by Eriksen (1965). Constituents of the agar were 0·03 M barbital buffer at pH 8·2 with 0·003 per cent methyl orange and 1 per cent Difco Special Agar Noble.

Standard LKB equipment was employed for immunoelectrophoresis. The agar was the same as in gel precipitation, and was buffered with 0·05 M barbital buffer to pH 8·2.

Estimation of protein

Protein concentrations were determined by the biuret method or by optical density at 277 mμ as described by Kabat and Mayer (1961).

Antigens

Crude guinea-pig thyroglobulin prepared by $(NH_4)_2SO_4$ fractionation (Godal and Kåresen, 1967a), was purified further by gel filtration on Sephadex G-200 and gel electrophoresis. In gel filtration, thyroglobulin was identified in the main breakthrough peak. Fractions from this peak were precipitated with 50 per cent saturated $(NH_4)_2SO_4$. After dialysis against saline for 24 hours, the thyroglobulin (12 mg/ml) was subjected to gel electrophoresis under conditions similar to those used for immunoelectrophoresis. The thyroglobulin was removed from the gel by washing the gel in tubes containing a fine masked nylon net at the bottom. The final preparation of thyroglobulin was diluted to a concentration of 1 mg/ml and used for immunization of rabbits. Its purity was demonstrated by the single line obtained in immunoelectrophoresis on reaction with serum taken during the primary antibody response in rabbits. Their serum did not react with guinea-pig plasma in Ouchterlony plates or in immunoelectrophoresis. However, animals boosted by the same preparation also responded to an antigen of guinea-pig serum with the same electrophoretic mobility as thyroglobulin. The preparation of extracts of guinea-pig kidney, liver and spleen for gel precipitation studies has been described previously (Godal and Kåresen, 1967a).

Antisera

Five rabbits were immunized with guinea-pig thyroglobulin which was purified as described above, and mixed with equal volumes of Freund's complete adjuvant (Hyland laboratories), and five other rabbits with Freund's complete adjuvant mixed with equal volumes of 0·9 per cent saline. The technique of immunization and bleeding has been described previously (Godal and Kåresen, 1967a). Although the rabbits immunized with purified guinea-pig thyroglobulin primarily responded to thyroglobulin only, the primary response did not produce satisfactory levels of anti-guinea-pig thyroglobulin antibodies (Sharp *et al.*, 1967; Godal and Kåresen, 1967a). Consequently the rabbits were boosted. The contaminating antibody against guinea-pig serum was absorbed from the pooled rabbit antiserum with guinea-pig serum in three steps using guinea-pig serum/rabbit antiserum ratios of 1 : 100, 1 : 50 and 1 : 10. The antiserum then no longer reacted with guinea-pig serum in Ouchterlony plates. The pooled anti-thyroglobulin serum had a precipitating titre in gel of 1 : 100, and contained 703 μg antibody N/ml as determined by quantitative precipitation (Kabat and Mayer, 1961). Its haemagglutination titre was 1 : 100,000, and it fixed guinea-pig complement in antiserum dilutions up to 1 : 300. The control anti-Freund's complete adjuvant serum did not react with thyroglobulin and neither this serum nor the anti-thyroglobulin serum reacted with the kidney, liver and spleen extracts.

Injection of antiserum

Four millilitres of the pooled and absorbed anti-guinea-pig thyroglobulin serum was injected intravenously into the vena anonyma or lower part of vena jugularis of the guinea-pigs under light ether anaesthesia. Two control groups were injected with either 4 ml of rabbit anti-Freund's adjuvant serum or 4 ml 0·9 per cent saline.

Histological techniques

At the time intervals after injection shown in Table 1, the animals were killed by ether. One thyroid lobe was removed and cut with a razor blade through the long axis into two halves. The halves were fixed in either ethanol–formalin = 9 : 1 for haematoxylin and eosin staining, Dominici staining (Litt, 1963) and peroxidase reactions (Rytömaa, 1960), or in Karnovsky's fixative (Karnovsky, 1965) for staining of mast cells with toluidine blue (Padawer, 1959) and the periodic acid–Schiff (PAS)-reaction (Culling, 1963). The Dominici staining and peroxidase reaction after Undriz as modified by Rytömaa are considered to stain eosinophil granulocytes specifically (Litt, 1963; Rytömaa, 1960). The other thyroid lobe and the regional lymph node of the thyroid were fixed for fluorescent antibody and electron-microscopic studies to be reported (Kåresen and Godal, 1969; Kåresen, 1969). Pieces of liver and kidney were fixed in ethanol–formalin and stained with haematoxylin and eosin. The material for light microscopy was embedded in paraffin, and was cut in approximately 6 μ thick sections by the same person and with the same equipment.

Counting of cells in the thyroid

All counting was done 'blind', i.e. with the slides randomly mixed and coded by another person than the one counting. Only cells where the nuclei were included in the section

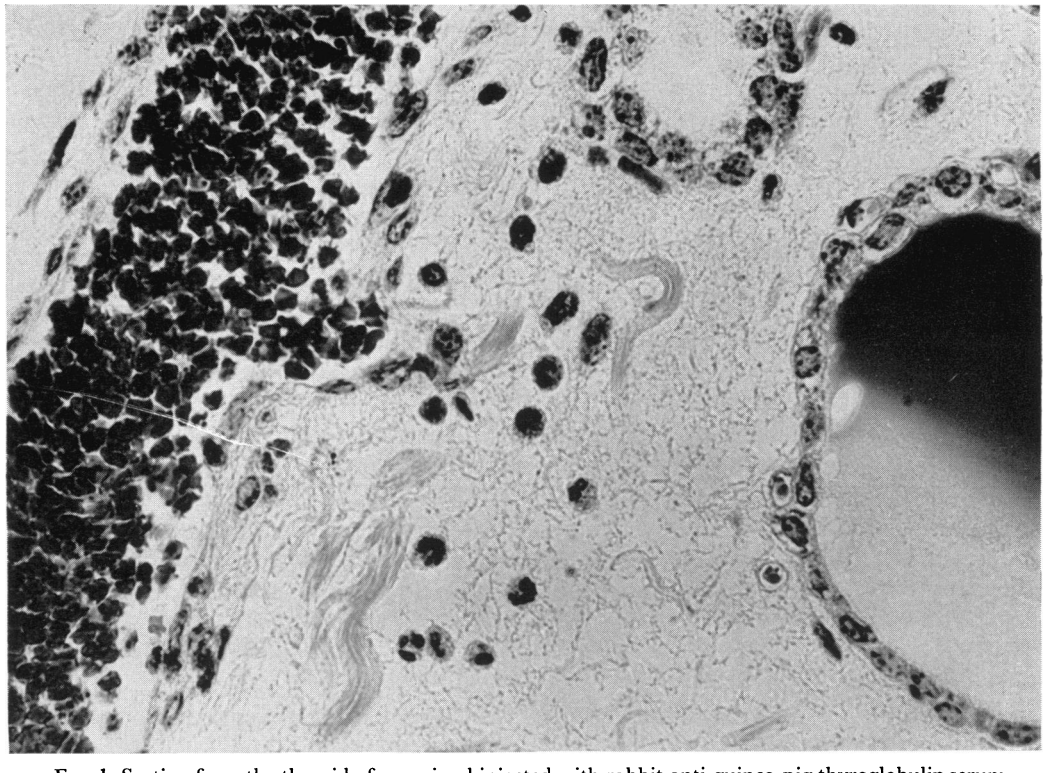

FIG. 1. Section from the thyroid of an animal injected with rabbit anti-guinea-pig thyroglobulin serum and killed $\frac{1}{4}$ hour after injection. Note granulocytes in the interstitium. To the left a blood vessel containing mainly red blood cells. To the right a follicle. Dominici staining, × 670.

were counted. When necessary the focus was adjusted to ensure that all cells were included. It was assumed that there was no systematic variation in thickness of the sections between control groups and experimental animals.

Mast cells were counted in two different sections from the thyroid of each animal. Counting was done with a Zeiss Standard RA 34 microscope, with a 40× objective and an 8× ocular fitted with a quadratic frame, omitting the peripheral part of the vision field. In each section five areas were counted, all on the long axis of the section; two at each end, one at the mid-point and the other two halfway between the end and the mid point. The areas to be counted were selected at low magnification and with the preparation slightly out of focus, thus making it impossible to see the cells that were to be counted. Only cells with evident metachromatically stained granules were counted.

Eosinophil and neutrophil granulocytes in the thyroid were counted in one section from each animal stained by the Dominici method. This method was preferred because the neutrophil granules were also faintly stained. The neutrophil was thus more easily identified than in peroxidase stained sections where the cytoplasm of neutrophils remained unstained. However, preliminary assays, using either method, gave similar counting results of eosinophils. Counting was done with a 100× oil objective and the same ocular as for mast cells. Granulocytes which had penetrated the follicles were omitted, as they

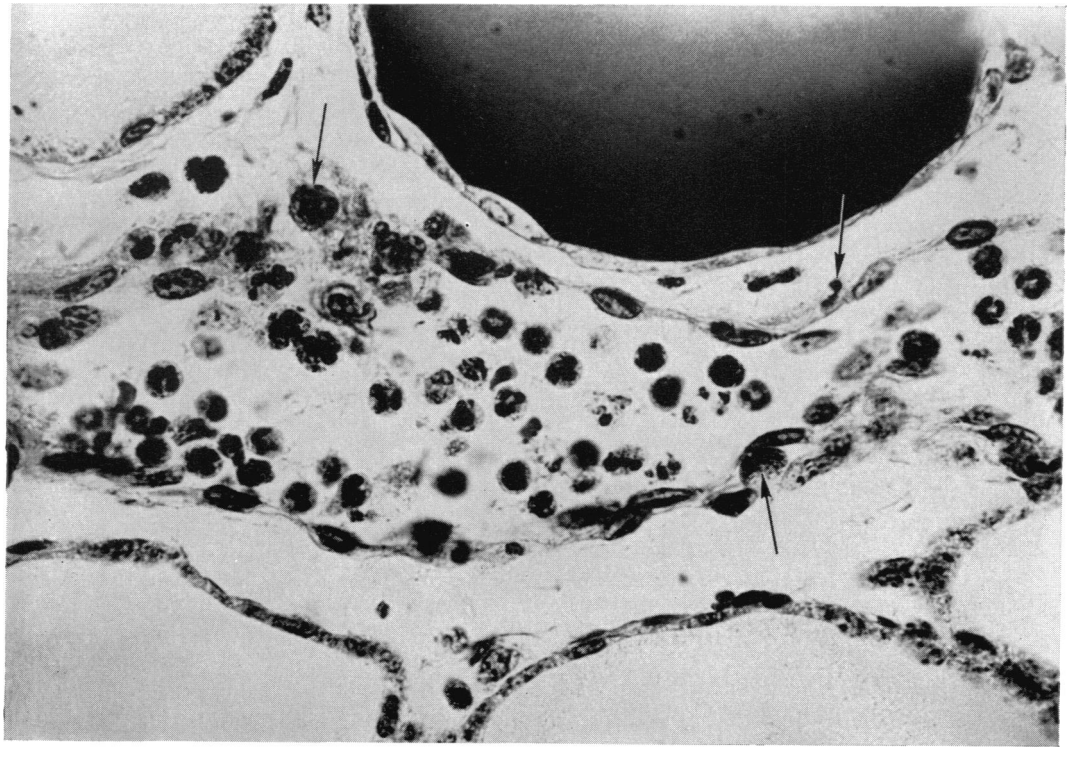

FIG. 2. As Fig. 1 but the animal was killed ½ hour after injection. A blood vessel can be seen filled with leucocytes, mainly granulocytes, some of which are penetrating the vessel wall (arrows). Dominici staining, × 670.

were often too degraded to make an identification possible at the latest time intervals. Counting was started at one end of the section, and the granulocytes in twenty adjacent frames were counted.

RESULTS

MORPHOLOGICAL OBSERVATIONS ON THE THYROID

A quarter of an hour after injection, signs of inflammation were found in the animals receiving rabbit anti-guinea-pig thyroglobulin serum. Granulocytes, both eosinophils and neutrophils, were lining the vessel walls and were also found in the interstitium of the thyroid (Fig. 1). After ½ hour this was more evident (Fig. 2). After the first 3 hours the granulocytes were present in about equal numbers, perhaps with a slight preponderance

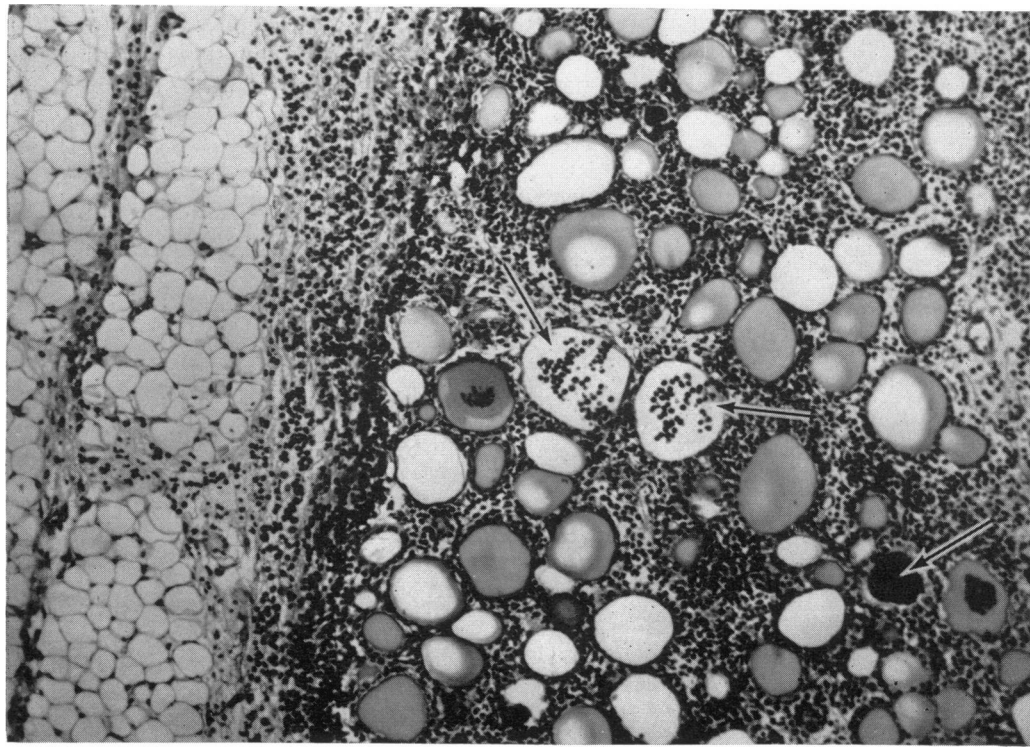

FIG. 3. As Fig. 1 but the animal was killed 24 hours after injection. There is heavy infiltration of granulocytes in the interstitium, and some follicles have also been penetrated by granulocytes(arrows). Note the especially heavy infiltration in the pericapsular region. Dominici staining, ×275.

of neutrophils, but the accumulation of inflammatory cells was rather slow. Thereafter their number rapidly increased reaching a maximum after 24 hours (Fig. 3). By now the eosinophil granulocyte was clearly the dominating cell as evidenced both by the Dominici and peroxidase staining (Fig. 6). The infiltration seemed to be especially intense in the pericapsular region (Fig. 3). Five days after the injection of the anti-thyroglobulin serum there

was still a rather severe thyroiditis. By now, however, nearly all inflammatory cells were eosinophil granulocytes and this was also the case after 10 days.

Infiltration of granulocytes and a few mononuclear cells into follicular lumina could be observed after 12 hours, but this phenomenon was more pronounced after 24 hours (Figs. 3 and 4). In these follicular infiltrates, the eosinophil granulocyte was even more

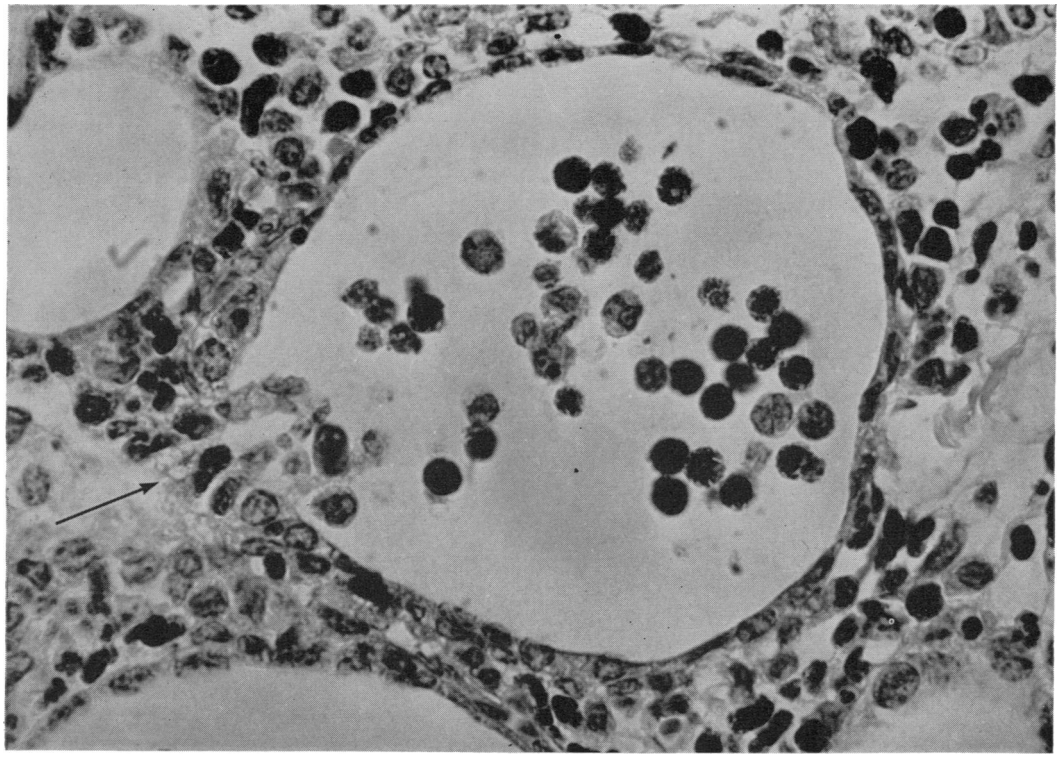

FIG. 4. As Fig. 1 but the animal was killed 24 hours after injection. Infiltration of granulocytes and a few mononuclear cells into the follicular lumen can be seen. Area where the continuity of the follicle is broken by invading cells is marked by an arrow. Dominici staining, × 670.

dominant than in the interstitium, and in some cases seemed to be the only inflammatory cell. After 5 days, but most evident after 10 days, signs of necrosis, pyknotic nuclei and fragmentation of the cytoplasm were seen in these leucocytes. Also the peroxidase reaction had diminished, but it was still faintly evident after 10 days.

Although some apparently released eosinophil granules were seen among the inflammatory cells, no sign of necrosis or of a diminishing peroxidase reaction was evident in the eosinophils in the interstitium.

In agreement with earlier results (Sharp *et al.*, 1967; Godal and Kåresen, 1967a), the inflammatory reaction was found to regress slowly. After 20 days the thyroids appeared nearly normal except for a few eosinophils in the interstitium and remnants of necrotic cells in some of the follicular lumina (Fig. 5).

No injury to the epithelial cells detectable by light microscopy was found at any time interval, except for the areas where the continuity of the follicle was broken by penetrating

leucocytes (Fig. 4). Neither was there seen thrombosis or injury to the vessels. In the control groups leucocytes were rarely observed and when seen mainly free in the lumina of larger vessels.

Mast cells, stained metachromatically with toluidine blue, were rather numerous in the thyroid of control animals. All the cells were located to the loose connective tissue of the interstitium and mainly in the neighbourhood of the blood vessels. No difference in the distribution or staining character of the mast cells were noted in animals injected with anti-thyroglobulin serum during the first 6 hours after injection. Twelve and 24 hours, and to a lesser degree 5 days after injection, some of the mast cells appeared to be degranulated, having less granules and often only a slight metachromasy when compared to the mast cells of controls.

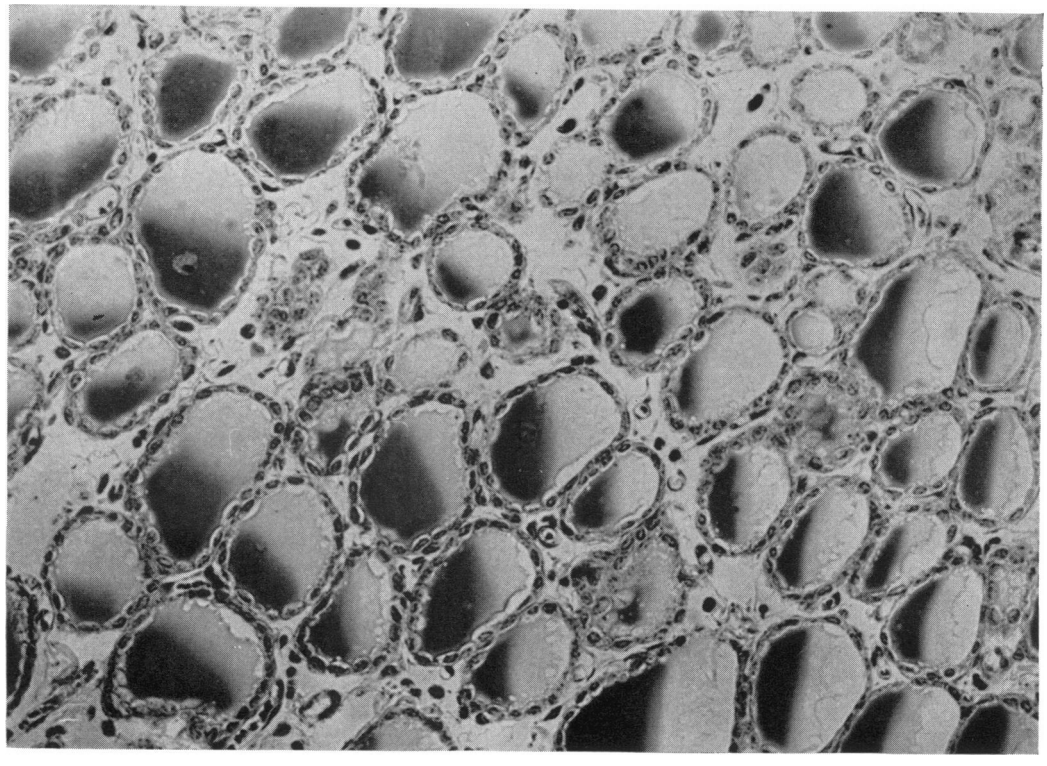

FIG. 5. As Fig. 1 but the animal was killed 20 days after injection. The appearance of the gland is very close to that seen in a normal animal. Only a few eosinophil granulocytes are still left. Dominici staining, × 275.

In sections stained according to the PAS method the usual strong positive reaction of the colloid was noted in the follicles of all animals. No PAS-positive material could be found in the epithelial cells. In the interstitium and in the pericapsular region, large thin walled vessels filled with a slightly PAS-positive amorphous material, but without blood cells, were seen. These vessels were assumed to be lymph vessels. The intensity of their staining reaction was far less than that of the colloid, and was similar to that of the plasma of blood vessels in the neighbourhood (Fig. 7).

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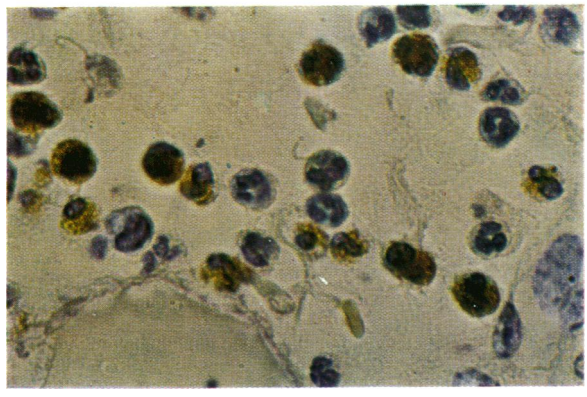

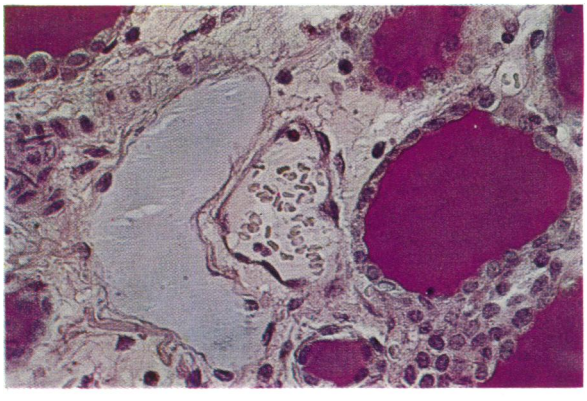

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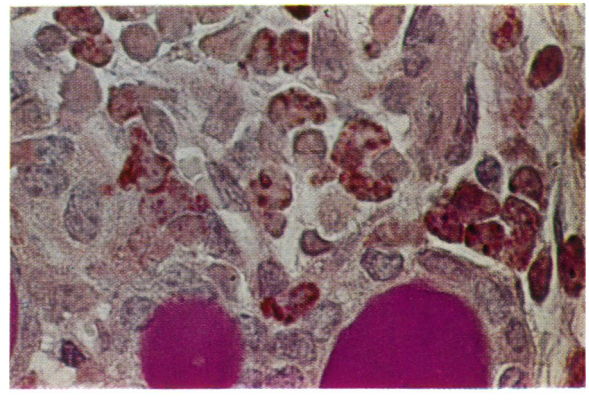

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FIG. 6. Section from the thyroid of an animal injected with rabbit anti-guinea-pig thyroglobulin serum and killed 24 hours after injection. Eosinophil granulocytes are stained yellow-brown, while neutrophils remain unstained. Peroxydase-reaction, × 865.

FIG. 7. As Fig. 6 but the animal was killed ½ hour after injection. Follicles with their contents of strongly PAS-positive colloid can be seen. In the central part of the picture a vessel with red blood corpuscles can be found. Close to this vessel a rather large lymph vessel appears. Both of them demonstrate a similar slight PAS-reactivity of their amorphous contents. PAS-reaction, × 350.

FIG. 8. As Fig. 6 but the animal was killed 12 hours after injection. Granulocytes with rounded droplets of strongly PAS-positive material in their cytoplasm can be seen. The intensity of the staining reaction is similar to that of the colloid of the follicles in the lower part of the picture. PAS-reaction, × 865.

(*Facing p.* 854)

In animals receiving anti-thyroglobulin serum, amorphous material was noted among the fibres and cells of the interstitium. This material had a PAS-reactivity similar to that of the contents of lymph and blood vessels. In contrast to this the leucocytes in the interstitium of these animals contained rounded droplets of strongly PAS-positive material which were thought to be intracellular vesicles. The staining intensity of these vesicles was similar to that of the colloid (Fig. 8). They could be detected 1 hour after injection, but were most evident after 12 and 24 hours. Because of the character of the staining method, clear distinction between the cell types present was impossible. However, granulocytes with both bilobular and multilobular nuclei contained strongly PAS-positive vesicles, thus implying that both neutrophils and eosinophils were involved. Five, 10 and 20 days after injection the leucocytes still present in the interstitium contained no PAS-positive vesicles. Also leucocytes in the follicular lumina revealed strongly PAS-positive vesicles, most evident 12 and 24 hours after injection of anti-thyroglobulin serum, but still seen after 5 and 10 days. Twenty days after injection this phenomenon no longer could be observed.

A few follicles in most of the sections from the control animals contained a small number of mononuclear cells, and some of these had PAS-positive material in the cytoplasm. This so called colloidophagy is a well-known phenomenon in different species including the guinea-pig. (Hellwig, 1951). However, cells with PAS-positive vesicles intrafollicularly in the experimental animals far outnumbered those in the controls. Further the Dominici and peroxidase methods indicated that nearly all cells in this location were eosinophil granulocytes, a cell type never seen in the follicular lumina of the controls.

The liver and the kidney from all animals appeared normal except the livers from two of the controls. They had macroscopically multiple small tumours, which microscopically turned out to be small abscesses with a surrounding granulomatous reaction.

COUNTING OF CELLS IN THE THYROID

Mast cells, eosinophil and neutrophil granulocytes were counted. The results are shown in Table 2 and Fig. 9.

As seen there was a reduction in the number of mast cells in the experimental animals. The lowest number was found 12 hours after the injection of anti-thyroglobulin serum.

TABLE 2

NUMBER PER mm^2 OF MAST CELLS, EOSINOPHIL AND NEUTROPHIL GRANULOCYTES IN THE THYROID OF GUINEA-PIGS TREATED BY THE INTRAVENOUS INJECTION OF ANTI-THYROGLOBULIN SERUM, ANTI-FREUND'S ADJUVANT SERUM OR 0·9 PER CENT SALINE

Time (hours)	Anti-thyroglobulin serum*			Anti-Freund's adjuvant serum and saline†		
	Mast cells	Eosinophils	Neutrophils	Mast cells	Eosinophils	Neutrophils
1/4	77	20	28	64	0	3
1/2	65	5	25	71	0	2
1	44	18	125	71	0	2
3	48	30	88	58	0	0
6	41	173	58	75	2	2
12	15	605	283	49	0	0
24	40	1465	603	69	3	5
120	37	570	13	96	0	3
240	63	173	5	75	0	0
480	67	18	5	61	7	3

* The number at each time-interval represent the mean of the counts in two animals.

† The number at each time-interval represent the mean of the counts in three animals, two injected with anti-Freund's adjuvant serum and one with 0·9 per cent saline.

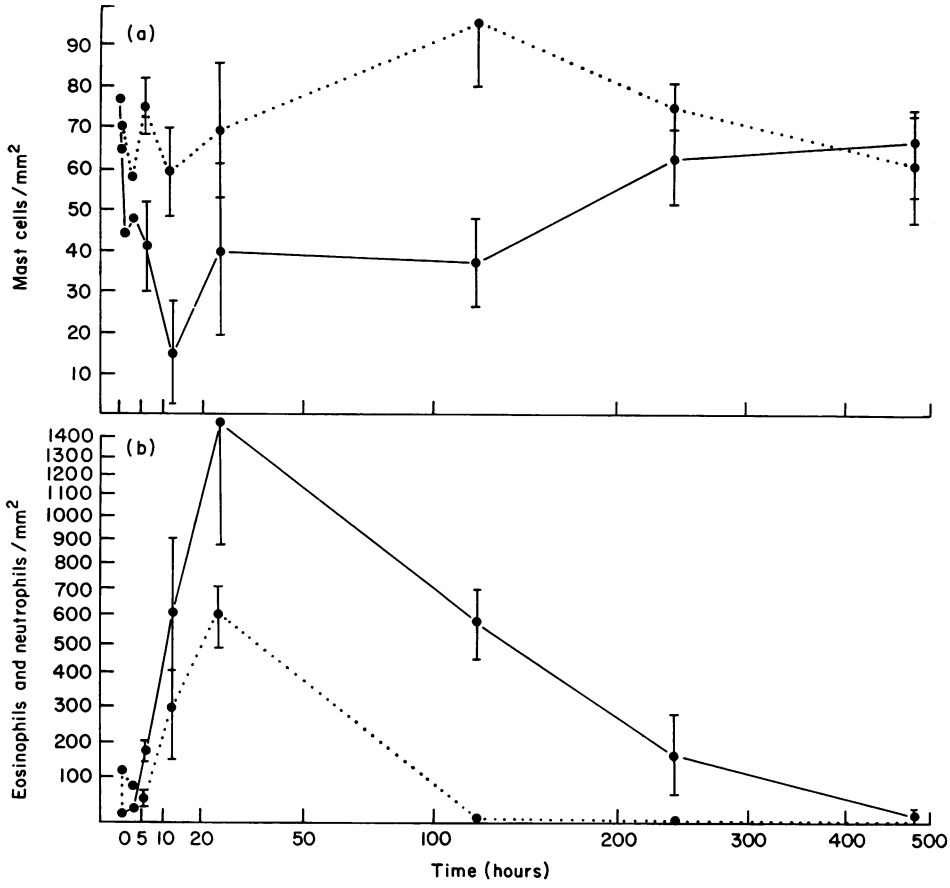

FIG. 9. Mast cells, eosinophil and neutrophil granulocytes in the thyroid. (a) Mast cells in animals receiving anti-thyroglobulin serum (——) and anti-Freund's adjuvant serum or 0·9 per cent saline (.....). (b) Eosinophil (——) and neutrophil (.....) granulocytes in animals receiving anti-thyroglobulin serum. Vertical bars indicate standard deviation (SD) of the observations. SD are omitted the first four time-intervals.

Thereafter there was a slow increase, and between 10 and 20 days after injection the level of mast cells in the controls was reached. In the control animals no systematic variation was found. Statistical analysis revealed no significant difference between the two control groups, neither concerning mean nor standard deviation of observations. Consequently the control groups were combined. The mean value of this combined group was significantly different from the mean value of the observations in the experimental animals (t = test; $P = 0{\cdot}02$).

Although the figures for the number of neutrophil and eosinophil granulocytes were rather low and variable over the first 3 hours, counting seemed to confirm the general impression that there was a preponderance of neutrophils. Later on eosinophils dominated, and there were over twice as many eosinophils as neutrophils at 12 and 24 hours. Still later the inflammatory infiltrates consisted almost exclusively of eosinophil granulocytes. It is also interesting to note that maximum granulocyte infiltration appeared to occur somewhat later than maximum mast cell reduction.

DISCUSSION

The anti-thyroglobulin serum used in the present study must be considered to be of high specificity. The antigen was purified in three steps using different physio-chemical methods. Rabbits boosted with this preparation also had antibodies against guinea-pig serum which had to be absorbed out. Lack of reaction against extracts of kidney, liver and spleen indicated organ specificity.

In an earlier investigation (Godal and Kåresen, 1967a) we have shown that rabbit antiserum against guinea-pig thyroglobulin may induce an acute inflammatory reaction in the thyroid of guinea-pigs. Based on observation of tissues stained with the May–Grünwald and Giemsa method, the granulocytes were suspected to be eosinophils. Sharp *et al.* (1967) obtained similar results, and using the Dominici staining method they found that the infiltrates consisted almost exclusively of eosinophil granulocytes.

In the present investigation, using two different specific staining methods for eosinophils, counting results confirm that this cell-type at most time intervals is the predominating cell-type of the infiltrates. However, it was found at variance with the results of Sharp *et al.* (1967), that neutrophil granulocytes were also present at the earliest time intervals. This predominance of eosinophil granulocytes is a rather peculiar phenomenon. The experimental situation in the present study should not be far from that seen in the passive direct Arthus reaction. If one accepts that thyroglobulin is normally present in the interstitium of the thyroid, as is strongly suggested from the following study in this series (Kåresen and Godal, 1969), and indirectly through the discovery of thyroglobulin in the lymph from the region (Daniel, Pratt, Roitt and Torrigiani, 1967a, b), the antigen was located outside the vessel wall and passively transferred antibodies inside. The morphological picture, however, differs in many aspects from that seen in an Arthus reaction. In the Arthus reaction the eosinophils represent an extremely small proportion of the cells present in the early inflammatory infiltrate which mainly consists of neutrophil granulocytes. Gradually eosinophils increase in numbers, reaching a maximum about 48 hours after onset of reaction. They are, however, never the dominating cell type. Furthermore, in the Arthus reaction there is an influx of mononuclear cells commencing at 8 hours and being the dominating feature by 24 hours (Cochrane, 1965).

It is also interesting to note that the intravenous injection of rabbit anti-thyroglobulin sera from different pools resulted in different morphological pictures. In the study of Sharp *et al.* (1967) the infiltrates consisted almost exclusively of eosinophil granulocytes. We found in the present study a mixture of eosinophil and neutrophil granulocytes, and in a previous study (Godal and Kåresen, 1967a) mononuclear cells were observed 5 days after the intraperitoneal injection of rabbit anti-thyroglobulin serum.

This variation in the morphology of the infiltrates may be explained by some sort of selective chemotaxis (Keller and Sorkin, 1968). Four factors must be considered to be of great significance in this connection: the nature of the antibodies and the antigen, as well as the possible involvement of complement and mast cells.

In case of serum induced experimental thyroiditis, heterogeneity of the antibodies directed against thyroid components may give rise to serum pools of different biological activity. Such a heterogeneity could be due to variation in antigen preparation and immunization procedures. The presence of antibodies against components of the thyroid other than thyroglobulin may be an explanation of our previous observation of a delayed, but prominent, mononuclear reaction (Godal and Kåresen, 1967a) since the antigen was

not of particularly high purity in that experiment. However, in the case of Sharp *et al.* (1967) as well as in the present study, the thyroglobulin preparations used for immunization were highly purified. Thus a difference in the preparation of antigen does not seem to be likely as an explanation for the higher percentage of neutrophil granulocytes in the present study as compared to that of Sharp *et al.* (1967). There still remains the possibility that variation of the immunization procedures which were used, gave rise to antibodies of different quality. As an attempt at clarifying this problem, separation of sera into different globulin classes should be tried.

However, it seems unlikely that differences in the quality of the serum pools can be the sole explanation for the differences in the morphology of the inflammatory infiltrates seen in serum induced experimental thyroiditis and the Arthus reaction. Typical Arthus reactions have been provoked by different sera and different antigens. One feature is, however, unique to our model, namely the antigen. Thyroglobulin is a glycoprotein which contains nearly 10 per cent carbohydrate of which approximately 1 per cent is sialic acid (de Groot, 1965). Cohen and Sapp (1963) and Chapman and Clark (1968) have demonstrated the eosinotactic effect of polysaccharides. Chapman and Clark (1968) also demonstrated the eosinotactic effect of sialic acid and complex material containing this substance such as blood group substances, gastric mucin and other epithelial mucopolysaccharides. In their studies the eosinophil response seemed to be directed towards carbohydrate moieties against which there already existed antibodies of blood group types. Whether there was such a cross-reactivity present or not, was not tested, however. In the case of Cohen and Sapp (1963) no cross-reacting antibodies against the carbohydrates used, could be found. In our case antibodies seem to be necessary as no leucocyte infiltration is normally found in the thyroid, although thyroglobulin appears to be present in the interstitium. The possibility that the structure of the antigen in the antigen–antibody precipitate could be the factor deciding the character of the inflammatory reaction should be considered.

In this connection it may be pertinent to comment on the repeated demonstration of an eosinotactic effect of antigen–antibody complexes (Litt, 1961; Cohen, Sapp and Gallia, 1963). No attempts seem to have been made to study the influx of both eosinophils and neutrophils at the same time. As studies *in vitro* (Keller and Sorkin, 1968) and *in vivo* (Cochrane, 1965) indicate also that neutrophil granulocytes are attracted by antigen–antibody complexes, it seems likely that such a simultaneous study is necessary in the search for specific eosinotactic substances.

Although the antibodies against thyroglobulin used in the present study are complement binding *in vitro*, the role of these serum factors in the development of the inflammatory infiltrate remains unknown. Neither has their role in eosinotaxis and in the uptake of antigen–antibody complexes in eosinophils been studied by other investigators. They are, however, involved in the Arthus reaction (Cochrane, 1965), and are participants in both chemotaxis (Keller and Sorkin, 1968) and immune phagocytosis (Gigli and Nelson, 1968) in *in vitro* models using mixed leucocyte populations. The role of complement in the present model deserves further study.

We have observed degranulation of mast cells in the thyroid after injection of anti-thyroglobulin serum. Mast cells of different species and also in the guinea-pig (Boreus and Chakravaty, 1960), are known to contain histamine and to release this substance by degranulation. Histamine has been suggested as an eosinotactic substance (Archer, 1963; Parish and Coombs, 1968). Others, however, have found no eosinotactic effect of histamine (Litt, 1962; Cohen and Sapp, 1965). On the other hand eosinophil granulocyte gra-

nules from rats have been shown to contain a mast cell disrupting substance (Archer and Jackas, 1965). To further complicate the picture it has been found that neutrophil granulocyte granules from rabbits and guinea-pigs contain a mast cell degranulating substance (Keller, 1968; Sherer and Janoff, 1968). One must, therefore, agree when Archer and McGovern (1968) say that the present status seems to be that any relation between eosinophils and histamine release remains obscure. But it is also known that antigen–antibody reactions when one of the reagents is reversibly adsorbed to the mast cell surface may lead to degranulation (Humphrey and Mota, 1959). An indication of the involvement of degranulation of mast cells in the development of the inflammatory infiltrate of our model may be found in the counting results, as maximum mast cell degranulation seems to occur 12 hours before maximum granulocyte infiltration. Thus mast cells may play a role in the development of the eosinophilia of the infiltrates in the present model.

Different investigators have observed the ability of eosinophils to take up antigen–antibody complexes (Archer and Hirsch, 1963; Sabesin, 1963; Litt, 1964). In the present study strongly PAS-positive vesicles were seen in the granulocytes at 12 hours and later after injection of anti-thyroglobulin serum. This brick-red PAS-positivity is characteristic for thyroglobulin; other substances displaying an equally strong reaction such as glycogen, mucin, etc., are not likely to be present in the interstitium of the thyroid. Furthermore, as will be presented in a following paper, also rabbit γ-globulin is found in the granulocytes (Kåresen and Godal, 1969). Thus it seems likely that both antigen and antibody, are phagocytosed, most probably as antigen–antibody complexes. However, also neutrophil granulocytes are known to phagocytose and catabolize antigen–antibody complexes, and in the Arthus reaction are instrumental in ridding the damaged vessel of the antigen (Cochrane, Weigle and Dixon, 1959). As both eosinophils and neutrophils are found in the present study, a possibility is that both cell types have this function in the present model. In our opinion the fact that both of them are known to phagocytose antigen–antibody complexes, and the observation that both seem to have PAS-positive vesicles, favour this hypothesis.

Other functions considered to be associated with eosinophils are phagocytosis of antigen (Roberts, 1966) as well as the breakdown of cells containing template-RNA necessary for antibody synthesis and the transport of this material to macrophages which are then thought to transform to plasma cells (Speirs and Speirs, 1964). None of these suggestions obtain any support from this study. As macromolecules are known to equilibrate between intravascular and extravascular space during the first 24 hours (Nakamura and Weigle, 1967), rabbit γ-globulin must also have been present in the thyroid interstitium of animals receiving anti-Freund's adjuvant serum. However, no inflammatory response was seen in the thyroids or in other organs of these animals. Neither could any sign of phagocytosis of eosinophils by other cell-types be found in the experimental animals.

Thus a reasonable interpretation of the present experimental model seems to be that the intravenously injected anti-thyroglobulin antibodies react with thyroglobulin in the thyroid. These antigen–antibody complexes attract granulocytes which have the function of clearing the thyroid of these complexes. Some unknown factor (or factors) is operative favouring the accumulation of eosinophils. Further evidence for this interpretation will be presented in a following paper (Kåresen and Godal, 1969).

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REFERENCES

ARCHER, R. K. (1963) *The Eosinophil Leucocytes*. Blackwell Scientific Publications, Oxford and Edinburgh.

ARCHER, G. T. and HIRSCH, J. G. (1963). 'Motion picture studies on degranulation of horse eosinophils during phagocytosis.' *J. exp. Med.*, **118**, 287.

ARCHER, G. T. and JACKAS, M. (1965). 'Disruption of mast cells by a component of eosinophil granules.' *Nature (Lond.)*, **205**, 599.

ARCHER, G. T. and MCGOVERN, V. J. (1968). 'Mast cell changes in rats with eosinophilia.' *J. Path. Bact.*, **95**, 217.

BORÉUS, L. O. and CHAKRAVARTY, N. (1960). 'The histamine content of guinea pig mast cells.' *Experientia (Basel)*, **16**, 192.

CHAPMAN, J. S. and CLARK, J. (1968). 'Chemical stimulus to eosinophils.' *Amer. J. clin. Path.*, **49**, 815.

COCHRANE, C. G. (1965). 'The Arthus reaction.' *The Inflammatory Process* (Ed. by B. W. Zweifach, L. Grant and R. T. McCluskey), pp. 613–648. Academic Press, New York.

COCHRANE, C. G., WEIGLE, W. O. and DIXON, F. J. (1959). 'The role of polymorphonuclear leucocytes in the initiation and cessation of the Arthus vasculitis.' *J. exp. Med.*, **110**, 481.

COHEN, S. G. and SAPP, T. M. (1963). 'Experimental eosinophilia. IV. Eosinotactic influences of polysaccharides.' *Exp. molec. Pathol.*, **2**, 74.

COHEN, S. G. and SAPP, T. M. (1965). 'Experimental eosinophilia. VIII. Cellular responses to altered globulins within cutaneous tissue.' *J. Allergy*, **36**, 415.

COHEN, S. G., SAPP, T. M. and GALLIA, A. R. (1963). 'Experimental eosinophilia V. Specificity of regional lymph node responses to antigen-antibody systems.' *Proc. Soc. exp. Biol. (N.Y.)*, **113**, 29.

CULLING, C. F. A. (1963). *Handbook of Histopathological Techniques*, p. 228. Butterworths, London.

DANIEL, P. M., PRATT, O. E., ROITT, I. M. and TORRIGIANI, G. (1967a). 'The release of thyroglobulin from the thyroid gland into thyroid lymphatics; the identification of thyroglobulin in the thyroid lymph and in the blood of monkeys by physical and immunological methods and its estimation by radioimmunoassay.' *Immunology*, **12**, 489.

DANIEL, P. M., PRATT, O. E., ROITT, I. M. and TORRIGIANI, G. (1967b). 'Thyroglobulin in the lymph draining from the thyroid gland and in the peripheral blood of rats.' *Quart. J. exp. Physiol.*, **52**, 184.

ERIKSEN, J. (1965). 'Immunochemical studies on some serological cross-reactions in the Klebsiella group. XII. Serological reactions with the acidic capsular polysaccharides from Klebsiella type 3 (c) as antigen.' *Acta path. microbiol. scand.*, **64**, 522.

GIGLI, I. and NELSON, R. A., JR (1968). 'Complement dependent immune phagocytosis. I. Requirements for C'1, C'4, C'2, C'3.' *Exp. Cell Res.*, **51**, 45.

GODAL, T. and KÅRESEN, R. (1967a). 'Induction of thyroiditis in guinea pigs by serum from rabbits immunized with guinea pig thyroglobulin.' *Acta path. microbiol. scand.*, **69**, 332.

GODAL, T. and KÅRESEN, R. (1967b). 'Induction of thyroiditis in guinea pigs by serum from guinea pigs immunized with guinea pig thyroglobulin.' *Acta path. microbiol. scand.*, **69**, 343.

DE GROOT, L. J. (1965). 'Current views on the formation of thyroid hormones.' *New Engl. J. Med.*, **7**, 355.

HELLWIG, C. A. (1951). 'Colloidophagy in the human thyroid gland.' *Science*, **113**, 725.

HENRIKSEN, S. D. (1967). *Komplementbindingsreaksjonen. Kurs i Immunologisk Teknikk*. Oslo University, Medical Faculty, Oslo.

HIRSCH, J. G. (1965). 'Neutrophil and eosinophil leucocytes.' *The Inflammatory Process*. (Ed. by B. W. Zweifach, L. Grant and R. T. McCluskey), p. 246. Academic Press, New York.

HUMPHREY, J. H. and MOTA, I. (1959). 'The mechanism of anaphylaxis: specificity of antigen-induced mast cell damage in anaphylaxis in the guinea-pig.' *Immunology*, **2**, 31.

KABAT, E. A. and MAYER, M. M. (1961). *Experimental Immunochemistry*. Thomas, Springfield, Illinois.

KÅRESEN, R. (1969). 'Induction of thyroiditis in guinea-pigs by intravenous injection of rabbit anti-guinea-pig thyroglobulin serum. III. Electron microscopic study.' (In preparation).

KÅRESEN, R. and GODAL, T. (1969). 'Induction of thyroditis in guinea-pigs by intravenous injection of rabbit anti-guinea-pig thyroglobulin serum. II. Studied with fluorescent antibody technique.' *Immunology*, **17**, 863.

KARNOVSKY, M. J. (1965). 'A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron-microscopy.' *J. Cell Biol.*, **27**, 137A.

KELLER, R. (1968). 'Interrelations between different types of cells. II. Histamine-release from the mast cells of various species by cationic polypeptides of polymorphonuclear leucocyte lysosomes and other cationic compounds.' *Int. Arch. Allergy*, **34**, 139.

KELLER, H. U. and SORKIN, E. (1968). 'Chemotaxis of leucocytes.' *Experientia (Basel)*, **24**, 641.

LITT, M. (1961). 'Studies in experimental eosinophilia. III. The induction of peritoneal eosinophilia by the passive transfer of serum antibody.' *J. Immunol.*, **87**, 522.

LITT, M. (1962). 'Studies in experimental eosinophilia. IV. Determinants of eosinophil localization.' *J. Allergy*, **33**, 532.

LITT, M. (1963). 'Studies in experimental eosinophilia. V. Eosinophils in lymph nodes of guinea pigs following primary antigenic stimulation.' *Amer. J. Path.*, **42**, 529.

LITT, M. (1964). 'Studies in experimental eosinophilia. VI. Uptake of immune complexes by eosinophils.' *J. Cell Biol.*, **23**, 355.

MOSES, L. E. and OAKFORD, R. V. (1963). *Tables of Random Permutations*. Stanford University Press, Stanford, California.

NAKAMURA, R. M. and WEIGLE, W. O. (1967). In vivo behaviour of homologous and heterologous thyroglobulin and induction of immunologic unresponsivness to heterologous thyroglobulin.' *J. Immunol.*, **98**, 653.

PADAWER, J. (1959). 'A stain for mast cells and its application in various vertebrates and in a mastocytoma.' *J. Histochem. Cytochem.*, **7**, 352.

PARISH, W. E. and COOMBS, R. R. A. (1968). 'Peripheral blood eosinophilia in guinea-pigs following implantation of anaphylactic guinea-pig and human lung.' *Brit. J. Haemat.*, **14**, 425.

ROBERTS, A. N. (1966). 'Rapid uptake of tritiated antigen by mouse eosinophils.' *Nature (Lond.)*, **210** 266.

RYTÖMAA, T. (1960). 'Organ distribution and histochemical properties of eosinophil granulocytes in rat.' *Acta path. microbiol. scand.*, Suppl. 140, 86.

SABESIN, S. M. (1963). 'A function of the eosinophil: Phagocytosis of antigen–antibody complexes.' *Proc. Soc. exp. Biol. (N.Y.)*, **112**, 667.

SCHERER, J. and JANOFF, A. (1968). 'Mediators of inflammation in leukocyte lysosomes. VIII. Observations on mast cell-rupturing agents in different species.' *Lab. Invest.*, **18**, 196.

SHARP, G. C., WORTIS, H. H. and DUNMORE, B. (1967). 'The biological effects of anti-thyroid antibodies. Thyroid eosinophilia following passive transfer of anti-thyroglobulin antibody.' *Immunology*, **13**, 39.

SPEIRS, R. S. and SPEIRS, E. E. (1964). 'Cellular reactions to reinjection of antigen.' *J. Immunol.*, **92**, 540.