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

## II. STUDIES WITH FLUORESCENT ANTIBODY TECHNIQUE

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**Summary.** The localization of rabbit γ-globulin was traced in the thyroids of guinea-pigs receiving rabbit anti-guinea-pig thyroglobulin serum intravenously. The fluorescent antibody technique was used. Rabbit γ-globulin was found in the interstitium and pericapsular region ¼ hour after the injection, and could be seen there for 24 hours. Five, 10 and 20 days after injection the interstitium was free of specific green-fluorescence. The first sign of specific fluorescence, associated with the epithelial cells, was seen as rounded droplets of fluorescent material after 1 hour. Thereafter, these droplets could be found, increasing in size, at all time intervals later studied. Twenty-four hours after injection green-fluorescent material was seen in some of the follicular lumina. However, most of these did not display any specific fluorescence. Granulocytes showing specific fluorescence were first observed 1 hour after injection, but this phenomenon was most evident at 12 and 24 hours. Both eosinophil and neutrophil granulocytes were found in the infiltrates, but it could not be decided whether both of these cell types were involved in the uptake of rabbit γ-globulin. The findings were discussed in relation to the previous reported observation of strongly PAS-positive material in the granulocytes. Together the observations suggested the uptake of both antigen and antibody, most probably as an antigen–antibody complex. The relevance of the observations in relation to thyroid physiology, as well as to experimental thyroiditis after active immunization and to chronic nonspecific human thyroiditis is discussed.

## INTRODUCTION

In previous communications we have described the development of an acute transient thyroiditis after the injection of rabbit anti-thyroglobulin serum into guinea-pigs (Godal and Kåresen, 1967; Kåresen and Godal, 1969). Characteristic of this inflammatory reaction is the high percentage of eosinophil granulocytes in the infiltrate. At most time intervals there were over twice as many eosinophil as neutrophil granulocytes present. Strongly PAS-positive material was found in the granulocytes which indicated that thyroglobulin had been taken up by these cells.

In the present study, the distribution of rabbit γ-globulin in the thyroid has been traced by use of fluorescent antibody technique. A preliminary report on these results has been published (Kåresen, 1968).

## EXPERIMENTAL METHODS

The tissues used in this study came from the same animals which were used for the study presented in the previous paper of this series. The methods of gel precipitation and immunoelectrophoresis were described there (Kåresen and Godal, 1969). The experimental protocol with time relationships and numbers of animals examined at each time interval is shown in Table 1.

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.

*Production of guinea-pig anti-rabbit γ-globulin serum*

In order to avoid antibodies cross-reacting with guinea-pig γ-globulin, guinea-pigs were used for the production of anti-rabbit γ-globulin antibodies.

Rabbit γ-globulin was isolated by gel filtration on Sephadex G-150 (Fig. 1). Fractions 42–44 were precipitated with 1·33 M $(NH_4)_2SO_4$ and digested by pepsin according to Osterland, Harboe and Kunkel (1963).

The final digested material at a concentration of 1 mg protein/ml was mixed carefully with equal volumes of Freund's complete adjuvant (Hyland Laboratories). This preparation was used for immunization. Four 3 months old female guinea-pigs, strain Pon-Sff-c, were immunized by six intracutaneous injections, one in each footpad and two in the back. Each animal received approximately 0·15 ml of the antigen–adjuvant mixture.

All animals responded after 4 weeks by producing an antiserum specific for rabbit γ-globulin as tested by gel precipitation and immunoelectrophoresis (Fig. 2). Two animals were boosted and bled a second time. Their sera still reacted only with rabbit γ-globulin, one at a dilution of 1 : 50 (50-precipitating units). This serum was employed for labelling with fluorescein-isothiocyanate (FITC).

*FITC-labelling of guinea-pig anti-rabbit γ-globulin antibodies*

The procedure advocated by Brandtzaeg (1967) was followed. γ-Globulin was fractionated from the guinea-pig antiserum by 'batch' absorption with Sephadex A-25 and $(NH_4)_2SO_4$ precipitation at 50 per cent saturation. The γ-globulin fraction was exposed to FITC (Baltimore Biological Laboratories) at pH 9·5 for 1 hour using 20 μg FITC/mg of protein. Unbound fluorochrome was removed by gel filtration on Sephadex G-25.

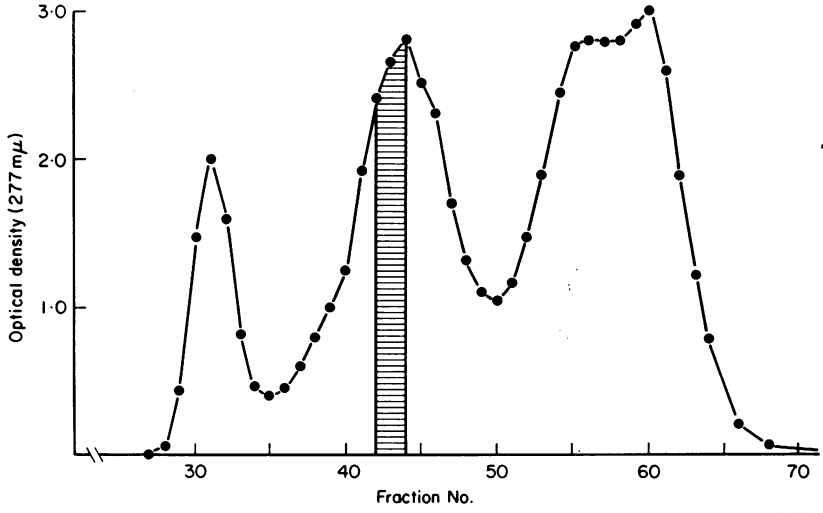


FIG. 1. Fractionation of rabbit serum on Sephadex G-150. Flow rate 13·4 ml/hr. Eluant: 0·07 M Tris–EDTA buffer and 1 M NaCl. Fraction volume: 5 ml. The hatched column indicates fractions used for further purification of rabbit γ-globulin.

The OD ratio (optical density 277 mμ/optical density 495 mμ of the conjugated serum) was 0·75, corresponding approximately to 8 FITC molecules per antibody molecule. The final preparation contained guinea-pig anti-rabbit γ-globulin antibodies at a concentration of 4 precipitating units.

*Histological techniques*

At the time intervals after injection shown in Table 1, the animals were killed by ether. The left thyroid lobe was removed and cut with a razor blade into two halves. One half, together with a piece of kidney, was fixed in 95 per cent ethanol and embedded in paraffin for fluorescence microscopy after the method of Sainte-Marie (1962). The other half was fixed for electron microscopy. The paraffin embedded material was sectioned at

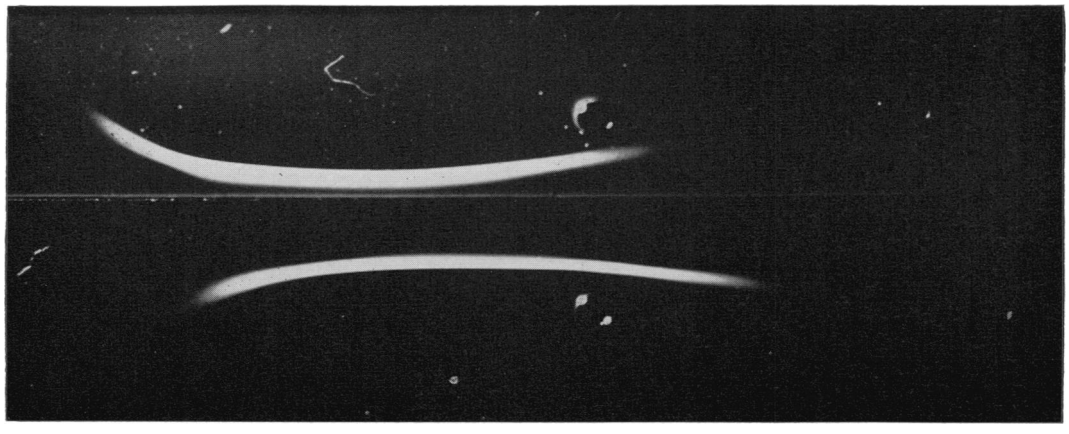

FIG. 2. Immunoelectrophoresis of normal rabbit serum (NRS) (upper well) and pepsin digested NRS (lower well) against guinea-pig anti-rabbit γ-globulin serum (trough).

6 $\mu$. After removal of the paraffin, the sections were placed in a moist chamber at room temperature and treated for ½ hour with FITC-labelled serum diluted 1 : 5 with normal guinea-pig serum. As control un-labelled guinea-pig anti-rabbit serum from the same animal as used for FITC-labelling was applied for ½ hour, and thereafter the 1 : 5 diluted FITC-labelled serum for another ½ hour. As another control the FITC-labelled serum was diluted 1 : 5 with rabbit anti-Freund's adjuvant serum without centrifugation. Thus one had an excess of rabbit $\gamma$-globulin for reaction with the guinea-pig anti-rabbit globulin antibodies before applying this serum to the sections for ½ hour. Also the sections from the animals injected with rabbit anti-Freund's adjuvant or 0·9 per cent saline, were incubated with FITC-labelled serum for ½ hour, serving as control for the specificity of rabbit anti-guinea-pig thyroglobulin antibody fixation to the thyroid.

Between the application of different sera, and at the end of the procedure, the sections were thoroughly washed in phosphate-buffered saline, pH 7·1. A mixture of polyvinyl alcohol, glycerol and phosphate buffer, pH 7·1, was used as mountant (Thomason and Cowart, 1967).

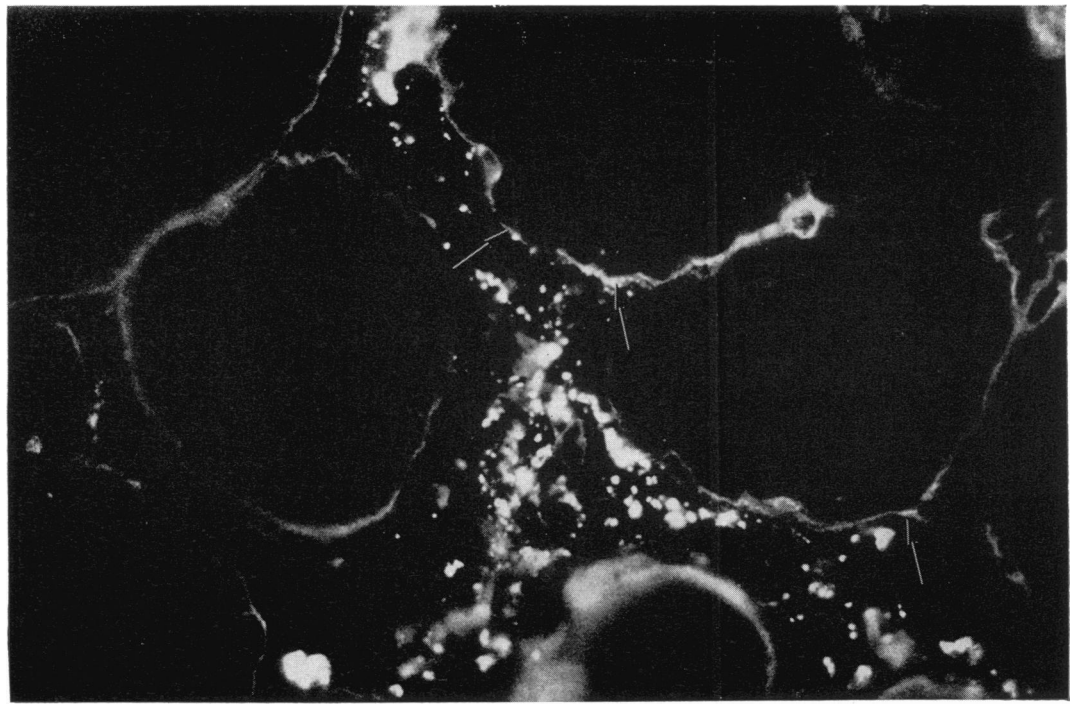

FIG. 3. Section from the thyroid of a guinea-pig treated by the injection of rabbit anti-guinea-pig thyroglobulin serum, incubated with FITC-conjugated guinea-pig anti-rabbit $\gamma$-globulin 'serum' for ½ hour and killed ¼ hour after injection. Note granular aggregates of fluorescent material in the interstitium, and the thin fluorescent rings surrounding the basal portion of the follicles (arrows). ×670.

*Microscopy*

A Zeiss Ultraphot II fitted with a HBO 200-W/4 mercury lamp was used, equipped either with a darkfield condenser of type Ultracondenser 1,2/1,4 or with a vertical illuminator type II/Fl. As exciter filters UG1 and BG 38 were used, and as barrier filter '41' which transmits wavelengths from 410 m$\mu$ and upwards.

*Photography*

Anscocrom 500 daylight high-speed colour film was used. Time of exposure varied from 15 to 40 seconds. Black-and-white copies were made from the colour film.

## RESULTS

### THYROIDS OF ANIMALS TREATED WITH ANTI-THYROGLOBULIN SERUM

A quarter of an hour after injection of the antiserum, the bright-green fluorescence characteristic of FITC was seen when sections were incubated with FITC-conjugated guinea-pig anti-rabbit γ-globulin (FGpARG) 'serum'. This fluorescent material was found in the interstitium of the thyroid, in some areas of the section as granular aggregates, in others mostly as a fine network (Fig. 3). A tendency to a more intense staining along the periphery of the follicles gave the appearance of a thin green-fluorescent circle surrounding the basal portion of the follicle (Fig. 3). This phenomenon and also the interstitial precipitates were most evident ½ hour after injection. Between 1 and 24 hours, fluorescent material was found scattered among the granulocytes in the interstitium, but the fluorescent rings surrounding the follicles were no longer apparent. From 5 to 20 days after injection no interstitial fluorescence was seen.

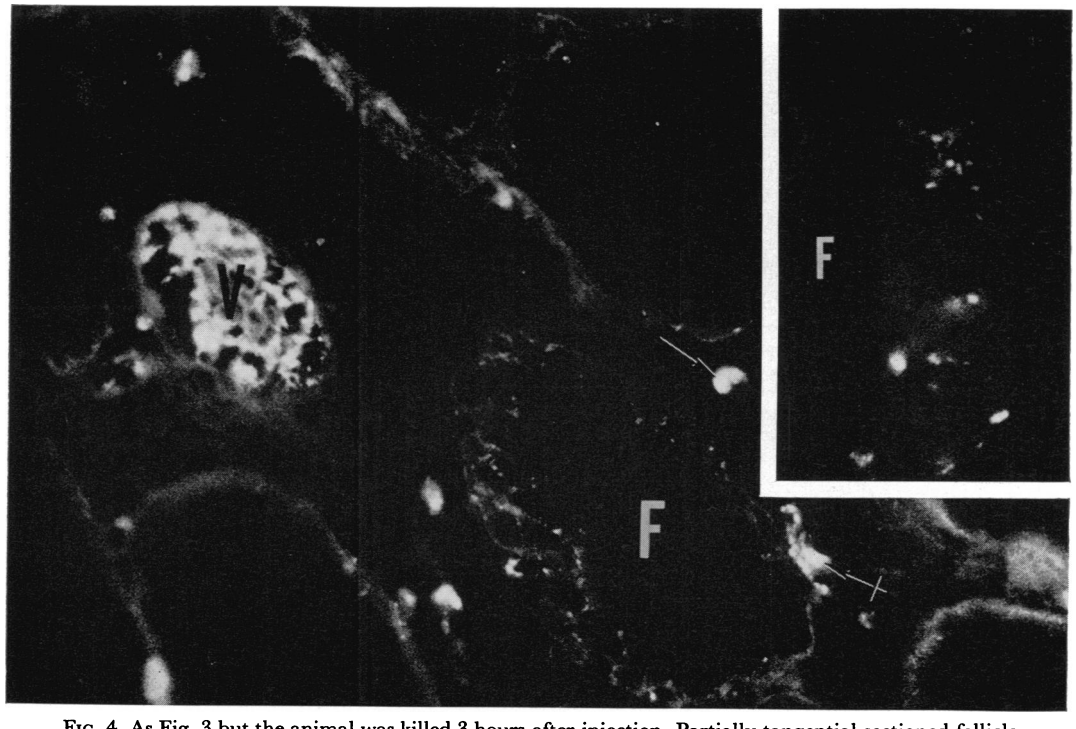


FIG. 4. As Fig. 3 but the animal was killed 3 hours after injection. Partially tangential sectioned follicle with multiple small fluorescent vesicles can be seen (F). In the upper left-hand corner a blood vessel with large amounts of fluorescent material appears (V). A strongly autofluorescent eosinophil granulocyte can be seen (arrow), and in the lower right-hand corner a rather large cell with a long cytoplasmic protrusion and fluorescent granules can be found (cross-barred arrow). × 670. Inset: Section from the same animal at a higher magnification. Part of a follicle with apparently intracellular vesicles can be seen. Luminal side of the follicle (F). × 1010.

In areas where the tissue surrounding the thyroid was intact, intense green-fluorescence was seen in a rather broad pericapsular zone during the first 6 hours after injection. Thereafter there was such a heavy infiltration of granulocytes in this region that it could not be decided whether all fluorescent material found in this region was intracellular or not.

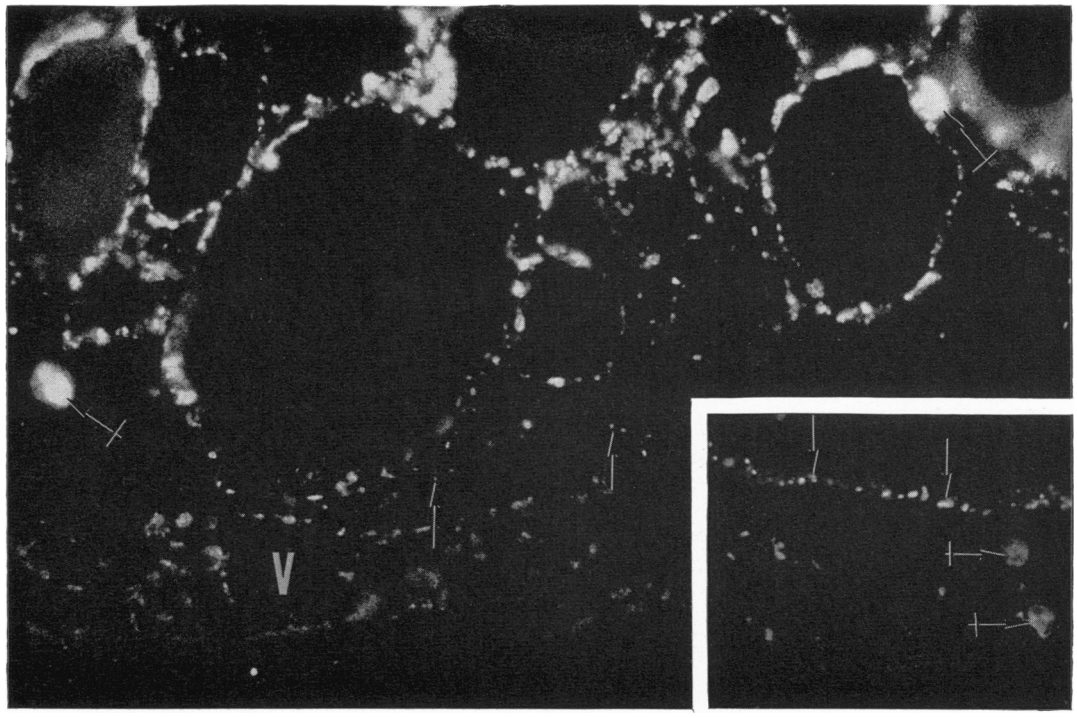


FIG. 5. As Fig. 3 but the animal was killed 10 days after injection. Rather large intraepithelial vesicles can be seen in all follicular cells. In the lower part of the picture a large thin-walled vessel is found, apparently a small vein (V), with specific fluorescent material in the lumen and as small vesicles in the vessel wall (arrows). A few autofluorescent eosinophil granulocytes can also be seen (cross-barred arrows). ×336. Inset: Part of another vessel with more evident vesicles in the wall (arrows). In the lumen faintly autofluorescent neutrophil granulocytes (cross-barred arrows) and small aggregates of specific fluorescent material can be seen. ×670.

One hour after injection, the first sign of intra-epithelial fluorescence was found. This localization was, however, more evident after 3 hours. Small rounded droplets of green-fluorescent material, assumed to be intracellular vesicles, were seen mainly in the basal portion of the epithelial cells, but also to a lesser degree laterally and apically (Fig. 4). The cell periphery could, however, not always be clearly defined due to the very faint blue autofluorescence of the epithelial cells. The possibility, therefore, exists that some of the rounded droplets could be located just outside the plasma membrane. The size of the vesicles increased with time. Five and 10 days after injection they seemed to occupy a large portion of the cytoplasm, giving the appearance of strings of pearls surrounding the follicular lumina (Figs. 5 and 7). Twenty days after injection there were still some large fluorescent vesicles, but now no longer in all follicles. Some were only partially affected, others seemed quite normal (Fig. 6).

Specific fluorescence was rarely seen in the follicular lumina, and only in some of the sections from animals killed 24 hours after injection. In these follicles the fluorescence was, however, rather intense and they were always infiltrated by granulocytes.

Granulocytes were seen in the interstitium and vessel lumina ¼ hour after injection of anti-thyroglobulin serum. Eosinophil granulocytes were easily identified by their strong

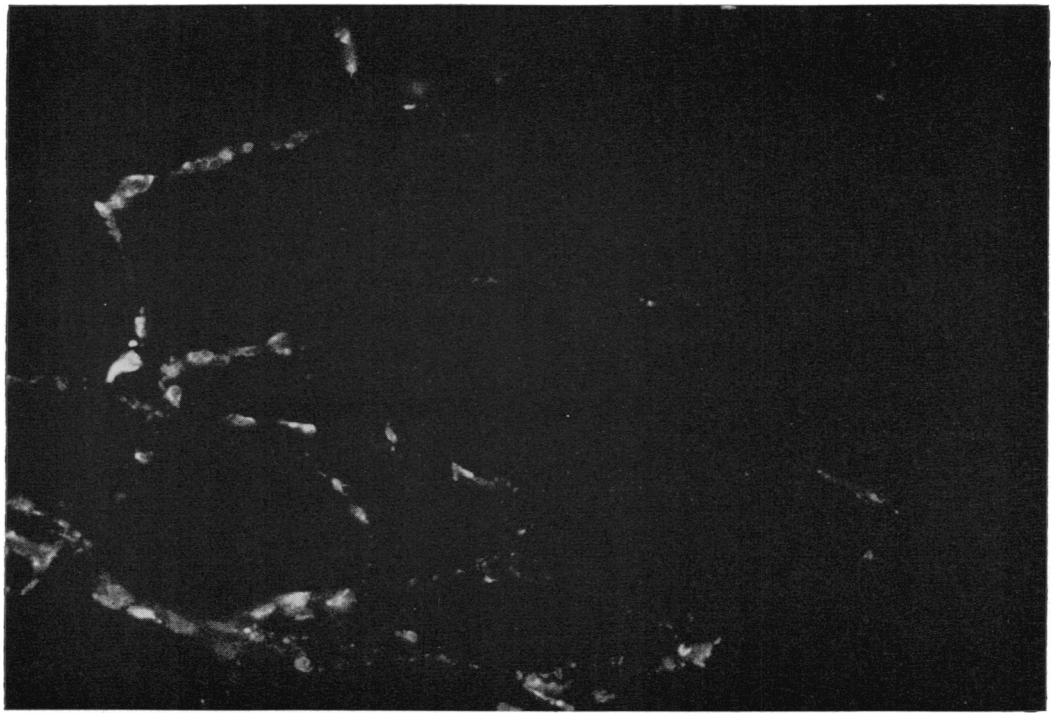

FIG. 6. As Fig. 3 but the animal was killed 20 days after injection. Large fluorescent vesicles can still be seen intra-epithelially, but now no longer in all follicles. Some follicles are only partially affected, others seem quite normal. ×670.

steel-grey autofluorescence. Neutrophil granulocytes could also be distinguished from other cells of the insterstitium, as their multilobular nuclei were outlined by the faint grey autofluorescence of the cytoplasm. No evidence of specific fluorescence was found in granulocytes in the thyroid till 1 hour after injection. Then, in a few of them rounded droplets of green-fluorescent material, assumed to be intracellular vesicles, were seen. A definite identification as neutrophil or eosinophil granulocytes was not possible as granules were not evident in cells with fluorescent vesicles. Attempts at using specific light microscopic staining methods on the sections after fluorescence microscopy were unsuccessful. The major part of the granulocytes, both eosinophils and neutrophils, did not show any sign of specific fluorescence during the first 6 hours. However, 12 and 24 hours after injection nearly half of the granulocytes contained green-fluorescent material (Figs. 8 and 9). From 5 to 20 days after injection none of the granulocytes remaining in the thyroid had any sign of specific fluorescence. However green-fluorescent material was found in pericapsular granulocytes up to 5 days after injection.

Still another cell type showing specific fluorescence was found in the interstitium of the thyroid. This was a cell several microns larger than the granulocytes and often with long cytoplasmic protrusions (Fig. 4). This cell type, containing multiple small fluorescent granules or vesicles, was seen between 1 and 24 hours after injection. There was no obvious increase or decrease in number of these cells during that time. No attempt at counting was done. They were never encountered in controls. The cells are suspected to be mast cells. However, the possibility that they may be macrophages can not be excluded. Attempts at staining the sections with toluidine blue after fluorescence microscopy were not successful.

Green-fluorescent material was seen in the lumina of blood vessels at all time-intervals studied. Ten and 20 days after injection multiple small fluorescent vesicles were seen in the walls of small veins (Fig. 5). No fluorescent material was found in the walls of other vessels at any time.

When the sections were preincubated with unconjugated guinea-pig anti-rabbit $\gamma$-globulin serum for $\frac{1}{2}$ hour and thereafter with FGpARG-'serum' for another $\frac{1}{2}$ hour, they displayed only a very weak green-fluorescence. This fluorescence was found in the same areas as when the sections were incubated directly with FGpARG-'serum'.

When the sections were incubated with FGpARG-'serum' diluted 1 : 5 with rabbit anti-Freund's adjuvant serum no specific fluorescence was seen.

### THYROID OF CONTROL ANIMALS

When sections from animals receiving rabbit anti-Freund's adjuvant serum were incubated with FGpARG-'serum', specific fluorescence was seen in the lumina of blood vessels at all time intervals studied. The intensity of the fluorescence was, however, diminished 10 and 20 days after injection. No fluorescence was seen outside the vessel lumina (Fig. 10).

When sections from animals injected with 0·9 per cent saline were incubated with FGpARG-'serum' no green-fluorescence was found.

### KIDNEY

Sections of kidney from animals receiving anti-Freund's adjuvant serum and anti-thyroglobulin serum had the same appearance when incubated with FGpARG-'serum'. Fluorescence was seen in the lumina of blood vessels of the cortex and of the medulla at all time intervals studied. In the glomeruli fluorescent material was found in the lumina of capillaries and in linear fashion along the basement membrane. No fluorescence was evident in tubular cells or lumina or in the interstitium of the kidney.

## DISCUSSION

The specificity of the injected rabbit anti-guinea-pig thyroglobulin serum has been discussed in a previous communication (Kåresen and Godal, 1969). The guinea-pig anti-rabbit $\gamma$-globulin serum used for FITC-labelling was specific by immunoelectrophoresis. As guinea-pigs of the same strain as the experimental animals were used for immunization with rabbit $\gamma$-globulin, the possibility of FITC-labelled antibodies reacting with blood or tissue components of guinea-pigs, should have been minimal. This was demonstrated

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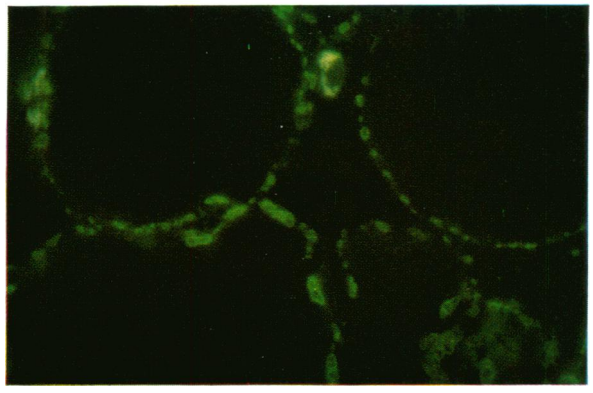

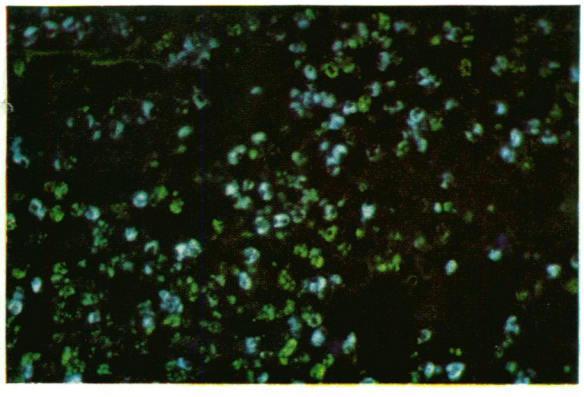

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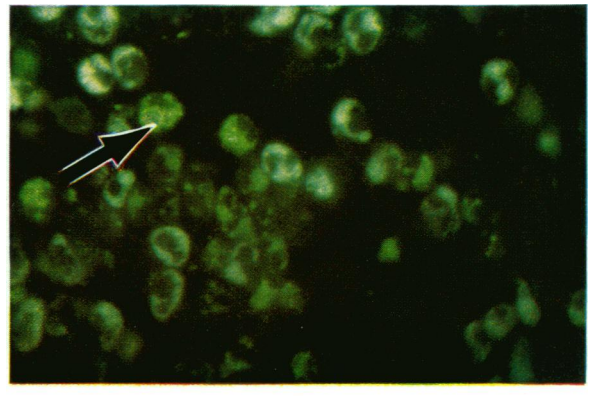

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FIG. 7. A section from the thyroid of a guinea-pig treated by the injection of rabbit anti-guinea-pig thyroglobulin serum, incubated with FITC-conjugated guinea-pig anti-rabbit $\gamma$-globulin 'serum' for ½-hour and killed 10 days after injection. The large intra-epithelial vesicles can here clearly be seen, some of them are more strongly fluorescent in the periphery than in the central portion. An eosinophil granulocyte demonstrating the steel-grey autofluorescence is found in the upper part of the picture. × 865.

FIG. 8. As Fig. 7 but the animal was killed 24 hours after injection. Granulocytes in the pericapsular region of the thyroid can be seen of which many show specific green-fluorescence. Surrounding these specific stained granulocytes, eosinophil granulocytes demonstrating only strong steel-grey autofluorescence can be found. × 350.

FIG. 9. As Fig. 7 but the animal was killed 24 hours after injection. Granulocytes in the interstitium of the thyroid can be seen of which many show specific green-fluorescence. In the upper left-hand corner a granulocyte with a rather large rounded droplet of green-fluorescent material can be found (arrow). Surrounding the specific stained granulocytes, eosinophil granulocytes demonstrating only steel-grey autofluorescence can be seen. × 865.

(*Facing p.* 870)

by the absence of green-fluorescence in the thyroids of animals receiving 0·9 per cent saline, and in the interstitium and parenchymal cells of animals receiving anti-Freund's adjuvant serum when the sections were incubated with the FGpARG-'serum'. Other evidence for specificity is the fact that eosinophil granulocytes exhibited only non-specific steel-grey autofluorescence in these sections. These cells are known to show a strong tendency to take up fluorescent material non-specifically.

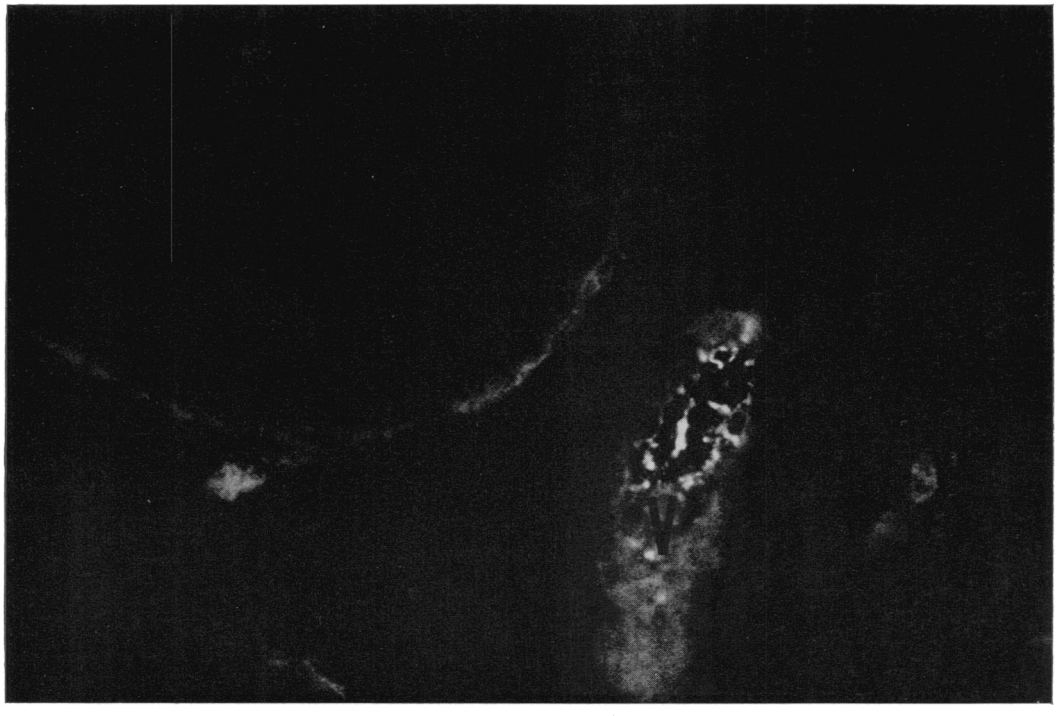

FIG. 10. A section from the thyroid of a guinea-pig treated by the injection of rabbit anti-Freund's adjuvant serum, incubated with FGpARG- 'serum' for ½ hour. The section was taken from an animal killed 1 hour after the injection. No specific fluorescence can be seen in the interstitium and follicular walls. A vessel, however, has fluorescent material in the lumen (V). ×670.

The specificity of the serum used and the absence of fluorescence outside the vessels of animals receiving anti-Freund's adjuvant serum, seem to justify the following conclusion. When fluorescent material was found outside the vessels of animals receiving anti-thyroglobulin serum, it was the result of specific immunological reactions: first, *in vivo*, between guinea-pig thyroglobulin or its subunits and rabbit anti-guinea-pig thyroglobulin antibodies; then, *in vitro*, after incubation of sections with FGpARG-'serum', these antibodies combined with the thyroglobulin–anti-thyroglobulin complexes and possibly also to free rabbit $\gamma$-globulin molecules. Although light microscopic study (Kåresen and Godal, 1969) gave no indication of any vessel wall damage, this may have occured, resulting in an increased diffusion of rabbit $\gamma$-globulin. However, diffusion cannot be the only reason for the localization of fluorescent material outside the vessels, as one would also have expected to find it in animals injected with anti-Freund's adjuvant serum.

This means that the extensive fluorescence seen in the interstitium and pericapsulary

region ¼ hour after injection, indicates the presence of thyroglobulin in these areas at that time. The possibility exists that this was due to damage of the follicular cells through the immunological event. However, as thyroglobulin has been found in the lymph from the region in other species (Daniel, Pratt, Roitt and Torrigiani, 1967a, b), it is more likely that this localization of thyroglobulin in the guinea-pig is a physiological phenomenon. This was also the interpretation of Sharp, Wortis and Dunmore (1967) who described wavy strands of specific green-fluorescence in the interstitial areas of the thyroid of guinea-pigs when they applied fluorescent goat anti-rabbit γ-globulin serum to sections from animals injected with rabbit anti-guinea-pig thyroglobulin serum. They found no fluorescence in the follicles or follicular cells. The last observation is in contradiction to ours as we observed green-fluorescent material in both follicles and in or close to the follicular cells.

Although granulocytes were present during the 1st hour, they were rather few and maximum granulocyte infiltration occurred 24 hours after injection (Kåresen and Godal, 1969). On the other hand interstitial fluorescence was most evident 15 and 30 minutes after injection. This may indicate that initially antigen–antibody complexes were formed in the thyroid, and that granulocytes were attracted secondarily by these complexes directly or indirectly through mediators such as complement or mast cells. Since strongly PAS-positive material is found in the granulocytes, indicating uptake of thyroglobulin, we have previously suggested that the role of the granulocytes may be to clear the thyroid of antigen–antibody complexes (Kåresen and Godal, 1969). In the present study the uptake of rabbit γ-globulin in the granulocytes is demonstrated. When light microscopic and fluorescence microscopic observations are combined, uptake of both antigen and antibody seems to take place, most likely as an antigen–antibody complex. Thus, further evidence supporting the above suggested role of the granulocytes seems to exist. Whether it was the eosinophil or neutrophil granulocytes which had this function, still remains uncertain, although studies using the PAS-method indicate that both may be involved (Kåresen and Godal, 1969).

Twenty-four hours after injection of anti-thyroglobulin serum, green-fluorescent material was seen in a few of the follicular lumina. As fluorescent vesicles were found in nearly all epithelial cells from 3 hours to 10 days after injection, this would indicate that the hindrance of uptake of γ-globulin molecules into the cells was far less than into the follicles. The most probable explanation of this phenomenon seems to be that γ-globulins of a molecular weight of 160,000 or higher, are virtually unable to pass the intercellular space, but have to penetrate the follicular cells to reach the lumina. As thyroglobulin and its subunits are known to be present in the cell, one can readily assume that antigen–antibody reactions occurred in the cell, and that anti-thyroglobulin antibodies rarely reached the follicular lumina. This interpretation is supported by recent studies of the passage of macromolecules through the intracellular space of capillaries, which indicate that the upper limit of passage may be about 90,000 molecular weight (Karnovski, 1967). On the other hand, this would suggest that thyroglobulin with a molecular weight of approximately 650,000 is not present in the interstitium due to a 'leakage' through the intercellular space, but has passed through the follicular cells.

The first sign of specific fluorescence associated with the epithelial cells was seen as rounded droplets of fluorescent material 1 hour after injection of anti-thyroglobulin serum. Thereafter droplets could be found, increasing in size, at all time intervals later studied. Due to the low resolving power of fluorescence microscopy, their identity and precise localization could not be determined. Areas such as that shown in the inset of Fig.

4 did, however, strongly suggest an intracytoplasmic localization of some or all of them. Recent observations have demonstrated that intracellular thyroglobulin is located in the rough endoplasmic reticulum (Ekholm and Strandberg, 1967, 1968), in endocytic vacuoles, apical vesicles and secondary lysosomes (Seljelid, 1967). Therefore the most probable localization of the fluorescent material seen intraepithelially in the present study seems to be in some or all of these organelles. The uptake of antibodies in the cells may have occurred through vesicle transport or by passage into the cytoplasm through defects in the cellular membrane due to antigen–antibody reactions close to these. Both these mechanisms of uptake of antibodies into cells are known to occur (Goldberg, 1963).

The rounded droplets of fluorescent material seen 10 and 20 days after injection of anti-thyroglobulin serum were far too large to represent normal cell organelles. This large size could have been due to long acting thyroid stimulator (LATS) activity in our serum-pool, leading to the formation of large colloid droplets in which rabbit γ-globulin accumulated. This does not seem to be very likely as it is difficult to obtain LATS-activity after immunization, and the most potent antigens for the production of LATS has been found to be the microsomal fraction or antigens in the cell sap (Burke, 1968). To us it seems more probable that some or all of the previously mentioned cell organelles increased in size, either through confluence or through simple expansion due to accumulation of rabbit γ-globulin in them. It is hoped that electron microscopic studies (Kåresen, 1969) will give further information on this point. The electron microscopic observations of Themann, Andra, Rose, Andra and Witebsky (1968) working with experimental thyroiditis in rhesus monkeys after immunization with a crude thyroid extract in Freund's complete adjuvant may be relevant in this connection. In addition to mononuclear cells and granulocytes partly invading the follicular cells, they observed dilation of the endoplasmic reticulum, and an increase in size and number of droplets characterized as colloid droplets. In some cells confluence of droplets was seen forming homogeneous finely granular masses. This could be the counterpart to the large fluorescent vesicles seen in the present study, thus indicating that this alteration was an antibody-mediated change, while infiltration of mononuclear cells may have been due to delayed hypersensitivity.

The initial purpose of the experiments with passive transfer of thyroglobulin antibodies was to establish the role of serum antibodies in the development of experimental thyroiditis after active immunization and in human chronic nonspecific thyroiditis. In most reports no positive correlation has been found between the titres of antibodies found in serum of individuals with these diseases and grade of thyroiditis (Godal and Kåresen, 1967). However, both γ-globulin and γ-globulin-producing cells have been observed in their thyroids by use of fluorescent antibody techniques (Koeffler and Friedman, 1964; Koeffler and Paronetto, 1965). γ-Globulin was mainly seen in the interstitium and in the colloid, but has also been noted in the epithelial cells in human thyroiditis (Mellors, Brzosko and Sonkin, 1962). Furthermore, transient infiltration of both eosinophil and neutrophil granulocytes among the dominating mononuclear cells of the infiltrates of experimental thyroiditis after active immunization, has been observed in the rabbit (Rose and Witebsky, 1956), the rat (Jones and Roitt, 1961), the rhesus monkey (Theman *et al.*, 1968) and the guinea-pig (Lerner, McMaster and Exum, 1964). As it is now established that thyroglobulin antibodies give rise to infiltrates of neutrophil and eosinophil granulocytes in the thyroid, it is not unlikely that the above cited observations might have been due to such antibodies. Furthermore, the present study indicates that thyroglobulin antibodies may give rise to morphological changes in the follicular cell corresponding to

alterations observed both in human thyroiditis (Mellors *et al.*, 1962) and in experimental thyroiditis after active immunization (Theman *et al.*, 1968). Thus it seems likely that circulating antibodies may play a part in the development of these diseases, although the transient character of the infiltrates in the present model indicates that they play no major role.

## ACKNOWLEDGMENTS

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