LOCALIZATION OF ANTIBODIES IN PLASMA CELLS BY ELECTRON MICROSCOPY*

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PLATES 57 TO 61

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Fluorescent antibody and autoradiographic techniques (1, 2) permit the demonstration of antibody in the cytoplasm of cells of the plasmocytic series (1–3). Details of the intracellular distribution of antibody are not resolvable by these techniques. These details can be revealed, however, by electron microscopy, if antibodies can be marked with a suitable label.

Several investigators have realized the possibility of using ferritin as a "marker" for electron microscopy, on account of its characteristic structure, clearly recognizable with the electron microscope (4). An important advance in the development of immuno-electron microscopy was made by Singer (5, 6) who described a method for conjugating ferritin molecules with gamma globulins. His technique was recently employed (7, 9, 11) to localize different antigens either on the surface or within cells. A like technique may be employed for the intracytoplasmic localization of antibodies *e.g.*; by using an antigen conjugated with ferritin.

The present paper reports the results of an electron microscopic investigation, using ferritin as marker, aimed at revealing the distribution of a specific antibody in the cytoplasm of plasma cells from an immunized animal. Instead of Singer's technique, however, we adopted the simpler, though more limited, procedure of studying the distribution of antiferritin antibodies directly, by treating lymph node cells from immunized rabbits with the antigen (ferritin). This method was chosen for several reasons:

1. Work with ferritin, carried out in our laboratory during the past 3 years, has shown it to be a good antigen, as judged by the high serum titres of precipitating antibodies and by the extensive plasma cell reaction in the lymph nodes of the immunized animals.

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2. The use of ferritin as antigen eliminates the need for conjugation procedures, thus reducing to a minimum the risk of alterations being induced in the reacting materials. For example, although the conjugation process probably does not alter the specificity of the conjugated antibody (6), it may modify markedly its ability to precipitate with the corresponding antigen (12). The efficiency of the ferritin-antiferritin reaction may therefore be expected to be greater than that of a conjugated antibody or conjugated antigen system.

3. Cells containing antiferritin antibodies can be prefixed with formalin, since this substance does not alter appreciably the specificity and reactivity of these antibodies (see Materials and Methods).

4. When a molecule of ferritin combines with a molecule of antibody, the marker, *i.e.* the electron-opaque core of the ferritin molecule, is separated from the specific site of the antibody by a distance of under 30 A. Therefore the ferritin-antiferritin system may be expected to yield the maximum resolution available using ferritin as electron microscopic label; *i.e.*, a resolution greater than that likely to be obtained with conjugated antibodies (6) or conjugated antigens.

For the correct interpretation of the data it is essential that there be no ambiguity due to the impossibility of distinguishing antigen introduced into the cells as a marker, from antigen which might be present in the same cells from previous immunizing injections. Prior studies, however, clearly indicated that significant amounts of ferritin were never present in the plasma cells of rabbits hyperimmunized with ferritin. Control sections were examined also in the present experiments, to ascertain whether ferritin was retained in the plasma cells from previous immunizing injections.

From a technical point of view, the chief obstacle to the labeling of antibodies by means of ferritin lies in the difficulty of introducing it into the cells, since cell membranes appear impermeable to ferritin and molecules of similar dimensions. It is essential therefore to produce some discontinuities in the cell membranes by suitable procedures (8, 10, 11). In the present case this objective was achieved by a technique analogous to that employed in the preparation of sections for immunofluorescence microscopy. Slices of lymph nodes were fixed in buffered formalin and subsequently frozen, care being taken to minimize damage arising from the formation of intracellular crystals. Sections were cut in a cryostat, incubated with ferritin, washed, and finally processed for electron microscopy. This method affords a considerable degree of preservation of tissue organization as well as of cell morphology. Isolated lymph node cells were also treated according to the method of Rifkind *et al.* (11).

Materials and Methods

Antigens.—(*a*) Crystalline horse ferritin (FT) was prepared from horse spleen according to the method of Granick (13). (*b*) Polysaccharide antigen H37-RA from *Mycobacterium tuberculosis* was obtained from Difco Laboratories, Inc., Detroit. (*c*) Human gamma globulin fraction II was obtained from Mann Research Laboratories, New York.

Immunization.—Adult rabbits were immunized by a series of 3 to 4 weekly injections, each of about 2 mg of FT, without adjuvants, in both hind foot-pads. The antigen was mostly in a soluble form. A booster injection was given 2 to 4 weeks after the last injection and the animals were bled and sacrificed 4 to 5 days later. The serum antibody titres determined by standard precipitin and hemoagglutination tests (14), were of the order of 0.5 to 1.0 mg/ml. Popliteal lymph nodes were excised and treated as described below. Control rabbits immunized with Difco H37-RA antigen were similarly treated.

Electron Microscopy.—

Method 1: Slices of lymph nodes from rabbits immunized with either FT, or H37-RA antigen, or from non-immunized controls were fixed for 1 or 2 hours at 0 to 4°C in 4 per cent formaldehyde buffered at pH 7.2 according to the method of Holt and Hicks (15) or Millonig (16). Either fixative gave a satisfactory degree of preservation of cell structures. Nevertheless with the former the nuclear membrane was often damaged or detached from the cytoplasm. After fixation the tissue was washed in buffer and frozen. Several procedures were tried with variable results. Rapid freezing in a dry ice–isopentane mixture resulted in a fair degree of cell preservation, but gave rise to the frequent formation of large crystals. The final procedure adopted was the following. The slices of tissue were soaked in a 10 per cent (*v/v*) solution of dimethyl sulfoxide (DMSO) in phosphate buffer. They were then frozen slowly in an expanded polystyrene box containing solid carbon dioxide, following an empirical procedure described by Dougherty (17). DMSO was chosen because recent studies (17, 18) have shown that it increases markedly the survival of frozen cells, probably by reducing the formation of intracellular ice crystals. The FT-anti FT reaction tested *in vitro* in the presence of up to 20 per cent (*v/v*) DMSO was qualitatively unchanged. After 1 hour (final temperature inside the box roughly −55°C), the frozen tissue was rapidly transferred to a carbon dioxide chest for storage. Further steps were carried out 1 to 4 days later, as convenient.

Sections 10 μ thick were cut in a cryostat at a temperature below −20°C, immediately covered with a solution of 1 to 6 mg/ml of FT in 0.1 M phosphate buffer, or phosphate-buffered saline, or distilled water and then incubated for 30 minutes at room temperature. (Incubation in distilled water, followed by further incubation in FT in buffer, was tried in some experiments in an effort to reduce the solubility of antibodies in the cells. *In vitro* experiments had shown that, under analogous conditions, a non-specific FT containing precipitate formed also with sera from animals not immunized with FT. These precipitates, however, dissolved completely on subsequent treatment with buffer. The results with distilled water were not, however, better than those obtained using buffers.)

The floating sections were washed for about 30 minutes in several changes of 0.1 M phosphate buffer or phosphate-buffered saline, postfixed in Palade's fixative (19) for 1 hour at room temperature, rapidly dehydrated through a graded series of alcohols, and embedded in araldite (20). Polymerization was carried out at 80°C for 48 hours. The incubation, washing, postfixation, and embedding stages were carried out alternatively: (*a*) on glass slides, as for the embedding of monocellular layers or (*b*) in test tubes, centrifuging the sections after the washings and treating the resulting pellet by standard methods. Method *a*, although more tedious, gave a better preservation of cell morphology and whole tissue organization and also permitted the sections to be orientated for ultramicrotomy. The tissue was stained with 2 per cent (*w/v*) uranyl acetate during dehydration in absolute alcohol. Thin sections were cut with a Servall Porter-Blum ultramicrotome and examined with a Siemens elmiskop I electron microscope. To obtain added contrast, many sections were poststained with 5 per cent (*w/v*) uranyl acetate in 50 per cent alcohol.

Small fragments of the same lymph node slices used for the marking experiments were taken as controls, fixed for 2 hours with buffered formalin, postfixed with Palade's fixative, dehydrated, and embedded by standard methods.

Method 2: After the publication of the investigation on myeloma cells by Rifkind *et al.* (11), an experiment was carried out, for purposes of comparison, employing the procedure described by these authors. Lymph node cells were isolated in Hanks' solution, briefly fixed in isotonic, buffered formalin at pH 7.2 (for 10 minutes), frozen in a CO_2-isopentane mixture, and thawed in isotonic phosphate-buffered saline containing 3 mg of FT per ml. After 30 minutes incubation, the cells were washed three times with buffered saline, postfixed with Palade's fixative, and treated as above.

Slices of the same lymph nodes were fixed for brief periods of time, as described above, and then treated according to Method 1.

RESULTS

Characteristics of AntiFT antibodies and Effect of Formalin Treatment.—Precipitin curves obtained with the sera of the experimental animals were similar in shape to those given by Mazur and Schorr (21) for the same horse FT–rabbit antiFT system. With the sera of our rabbits, however, the valence of the antigen was markedly lower (of the order of 10). This difference might be due to the less efficient procedure of immunization employed by us, with consequent differences in the spectrum of antibodies produced.

Experiments were carried out to determine the effect of formalin, used in the preparation of the tissue, on the specificity of the FT-antiFT reaction. The specificity of this reaction in the presence of formalin appeared to be unchanged. Rabbit serum or gamma globulin solutions remained clear on standing for 3 or more hours in the presence of 4 per cent formaldehyde. When solutions containing (*a*) FT and (*b*) rabbit antiFT gamma globulins, either in the presence of 4 per cent formaldehyde, or dialysed against buffer after 2 to 3 hours treatment with the same, were mixed in suitable proportions, precipitation occurred. No precipitate was formed if the same steps were repeated using solutions of FT and gamma globulins from rabbits immunized with human gamma globulins.

The precipitating power of the antiFT antibodies was nevertheless impaired to some extent by treatment with formalin. The inhibiting effect of formalin on antigen-antibody precipitation was more evident with low concentrations of antibodies. Sera, inactivated at 56°C, were mixed with FT solution, either directly in the presence of 4 per cent formaldehyde or after 2 to 3 hours' treatment with the same in the cold, followed by dialysis against buffer or saline. In the case of sera having antibody concentrations of the order of 1 mg/ml or more, a specific, irreversible precipitate formed rapidly (in a few minutes), although less rapidly than with untreated serum. The amount of protein precipitated was not inferior to that obtained with untreated serum. With the lowering of antibody concentration, the amount of specific precipitate obtained after formalin treatment decreased out of proportion until no precipitate was formed at very low antibody titres even after prolonged storage in the cold.

Antisera made non-precipitating by formalin treatment, freed of excess

formalin by dialysis, still gave normal titres of conditioned hemoagglutination with FT-sensitized tanned sheep red cells. The observed partial inhibition in the precipitation of antiFT antibodies (more marked in antibody excess), recalls that reported for other antibodies after formalin treatment (22). A more complete serological investigation was not possible on account of the limited amounts of sera available. Nevertheless, the data obtained were adequate for the purposes of the present investigation.

Electron Microscopy.—

Lymph nodes of rabbits immunized with ferritin: Fig. 1 is a low power micrograph showing lymph node cells treated by Method 1. The thin section was cut in a plane parallel to that of the 10 μ cryostat-cut section. A good degree of preservation of cellular topography was maintained. The cells treated by this method, as well as isolated cells frozen by Method 2 (Figs. 2 and 7), appeared fairly well preserved. As a rule lymphoid and plasma cells were better preserved than macrophages and large reticular cells. There was no evidence of cellular deformation caused by the presence of large intracellular or extracellular crystals. At higher magnifications, nevertheless, small discontinuities in the surface and cytoplasmic membranes were often visible. This kind of damage might result from the formation of small crystals during freezing; a comparison with tissue frozen, but not subjected to incubation with FT and subsequent washings suggested, however, that it might also be partly due to the treatment following freezing.

The lymph nodes examined in these studies (excised 4 to 5 days after the booster injection), contained very few plasmoblasts, practically all the cells of the plasmocytic series being typical young plasma cells (Fig. 1). The nucleus of these cells was rounded or oval, with the chromatin clumped in irregular masses. A well developed nucleolus, with visible nucleolonema, was usually present. Nuclear pores were often visible, connecting the cytoplasm to the lighter nuclear areas. The rough component of the endoplasmic reticulum appeared well developed, its cisternae being usually moderately dilated and often arranged in parallel arrays. The cisternae occupied less than 50 per cent of the cytoplasm. Mature plasma cells with fully distended ergastoplasmic sacs were relatively infrequent. As a rule the cisternae contained a fairly dense flocculent material. Small concentric membranous formations ("myelin figures") 0.1 to 0.4 μ in diameter were occasionally present within cisternae. A large number of free ribosomes were scattered throughout the ground cytoplasm. Besides typical mitochondria, some plasma cells contained lysosome-like structures; *i.e.*, vacuoles with dense contents delimited by a single membrane. The Golgi apparatus was well developed, with a great many dilated vacuoles, vesicles and, less often, flattened membrane-bounded profiles. Some vacuoles occasionally exhibited moderately dense contents.

In the lymph node preparations incubated with FT, a large number of plasma

cells (in some preparations up to 20 to 50 per cent) contained FT, which appeared localized mostly within the cisternae of the endoplasmic reticulum. The pattern of distribution of the FT was practically identical irrespective of whether Method 1 or 2 was used. The concentration of FT used for the incubation did not appear to be critical in the range tested. The higher concentrations (5 to 6 mg protein per ml) perhaps gave a greater degree of tagging.

Details of plasma cells obtained by Method 1 (frozen sections cut in a cryostat), are shown in Figs. 3–6. A large number of FT molecules were visible inside the cisternae of the ergastoplasm. They were distributed evenly or in small aggregates, and often stood out against a background of electron-opaque material comparable in density to that normally present within cisternal profiles. FT molecules were present also in the perinuclear space (Figs. 2, 5, and 6). The appearance of the aggregates was practically indistinguishable from that of the aggregates often present in the extracellular space, and of the FT-antiFT precipitates described by Mercer (23). They were, therefore, interpreted as specific precipitates of FT with the antibodies present inside the cisternae. The concentration of FT in the tagged cisternae was evaluated by direct counts on thin sections. It varied from cell to cell, but ranged usually between 1000 and 4000 molecules/μ^2 of section, averaging about 3000 in the most uniformly labeled cisternae. Assuming an average section thickness of 600 A, the concentration of intracisternal antigen was estimated to be 1.6 to 6.6 $\times$ 10^{16} molecules/cm^3 (5.0 $\times$ 10^{16} in the most uniformly labeled cisternae).

The extracisternal cytoplasm usually displayed only a few isolated molecules of FT, but at times it contained aggregates like those observed in the cisternae. Since these aggregates occurred generally in close relation to discontinuities in the cisternal membranes, they probably represented antigen-antibody precipitates between free FT molecules and gamma globulin molecules escaped from the damaged cisternae. In the best preserved cells, only few FT molecules were present outside cisternal profiles.

As a rule not all the cisternae, in any one cell, were labeled with FT (Figs. 4 and 5). Thus, for example, heavily tagged cisternae could often be found adjacent to others completely devoid of FT; the latter, however, usually contained small aggregates of the characteristic fairly dense, flocculent material. In several plasma cells cut across by the cryostat knife, practically all the cisternae which opened to the exterior, and the adjacent ones, were labeled with FT, while cisternae deeper within the cells were either labeled or not.

There was no obvious relationship between FT molecules and the ribosomes scattered in the cytoplasm. The clusters of free ribosomes, often present in plasma cells, were generally devoid of FT. The relationship between membrane-bound ribosomes and FT molecules was even harder to establish, in view of the possibility that antibody molecules might have escaped from the cisternae. Thin sections which were not poststained with uranyl acetate were helpful in

clarifying this point. In such sections the molecules of FT could be distinguished clearly even when overlying the ribosomes (Figs. 5 and 6). From a close inspection of such sections it was concluded that the vast majority of ribosomes associated with the FT-labeled cisternae, were untagged. Only occasional molecules of FT were in contact with the ribosomes.

The smooth component of the endoplasmic reticulum and the Golgi region were generally devoid of FT even in cells exhibiting heavily tagged cisternae. Nevertheless in some cases a few molecules of FT were present inside the typical "smooth" vacuoles and in the membrane-bounded profiles of the Golgi region. Moreover FT-containing precipitates were observed occasionally in the Golgi region inside vacuoles which appeared wholly or partly devoid of ribosomes (Fig. 7). The exact nature of the vacuoles in Fig. 7 is undefined. Part of the membrane delimiting the vacuoles is free of ribosomes, while the remainder appears studded with formations of much lower electron density than that of the typical ribosomes present in the same cell. These formations recall those described by Roth and Porter (24) in other cell types.

In none of the plasma cells examined in the course of these experiments did we observe molecules of FT attached to the surface membranes of the cells (Figs. 2 to 6). The same applies also to the isolated cells, tagged with FT, which were fixed and frozen by the method of Rifkind *et al.* (11). The mitochondria as a rule were unlabeled. Only a few mitochondria (mostly damaged ones), occasionally contained some FT molecules; they probably represented an artifact. The nuclei were generally devoid of FT. A few isolated molecules were visible at times in the lighter nuclear areas.

Lymph nodes of rabbits immunized with a mycobacterial antigen: Control lymph nodes of rabbits immunized with an antigen unrelated to FT (Difco H37-RA polysaccharide antigen), were treated by the same procedures employed for the FT-immunized rabbit lymph nodes. A large number of plasma cells were examined in the thin sections of these lymph nodes; some of these were cut across by the cryostat knife thus favouring the entry of FT into them. After incubation with FT in either buffer or distilled water, the plasma cell did not contain molecules of FT either within the cisternae or in the ground cytoplasm. Only occasional isolated molecules of FT could be observed in some cells.

Control observations on lymph nodes of rabbits immunized with FT: In order to establish whether molecules of FT, introduced by previous immunizing injections, were still present in the plasma cells prior to incubation with FT, fragments of the original lymph nodes were fixed and embedded directly by standard methods. A detailed report of the observations will be published elsewhere, together with the results of previous work on lymph nodes of animals immunized with FT. In the present context it suffices to say that, on close examination, no FT molecules were found in the majority of the plasma cells

observed. Occasional plasma cells, however, contained a few molecules of FT either enclosed in dense lysosome-like structures or scattered freely in the cytoplasm. In the latter case the number of molecules present was extremely low, of the order of 1 to 5 molecules/μ^2 of section. This concentration was 100 to 1000 times lower than the concentration found in the cisternae, and was even lower than the background of FT molecules which may be observed in the cytoplasm of the labeled plasma cells after incubation with FT. FT was never observed inside the cisternae of control plasma cells. From the above observations it is reasonable to assume that practically all the FT observed in the labeled plasma cells was introduced at the time of incubation.

A large number of FT molecules were present instead, as aggregates, inside vacuoles, or free in the cytoplasm of many macrophages and reticular cells. Such observations indicated that the possible presence of antibodies in the latter cell types cannot be determined by the methods employed in the present investigation.

DISCUSSION

In interpreting the results of these experiments the following facts must be taken into account. Formalin fixation, essential for an acceptable degree of preservation of cell structure, does not alter substantially the solubility of gamma globulins. The latter therefore remain in solution in the plasma cells when, after thawing, these come into contact with FT. On the other hand FT apparently fails to penetrate into cells through intact cell membranes. In order, therefore, that antigen and antibody meet, discontinuities must be produced in the cell membranes without, at the same time, markedly affecting cell morphology. Rifkind *et al.* (11) achieved this by freezing and thawing isolated cells previously fixed for a brief period of time with formalin; in the present studies the same effect was obtained by cutting sections of frozen tissues.

If the two methods are compared, the former appears simpler and more convenient for the study of isolated cells. If, however, examination of isolated cells is difficult or it is important to retain tissue organization, *e.g.* to study the localization of antigen in different areas of a tissue, a method of the kind described in the present investigation would be preferred. Under the experimental conditions adopted in Method 1 (*i.e.* formalin fixation for 1 to 2 hours and use of DMSO) the cells are sufficiently well fixed to withstand subsequent contact with hypotonic solutions. Examination of the sections suggests that the penetration of the FT occurs chiefly through the discontinuities produced by the cryostat knife. Shorter times of fixation perhaps favour the penetration of labeling molecules into cell structures. Preliminary experiments combining the technique of cryostat-cut sections with shorter times of fixation, have not so far yielded good results. In view of the wide field of application of the method, efforts to obtain a convenient standard procedure are probably justified.

Whichever the procedure adopted, it is obvious that the conditions which allow the entry of FT into cells, at the same time favour the escape of antibody from cells and cell structures, in which it is stored. Antibody loss may be minimized by thawing the tissue directly in the incubation fluid. Even so, diffusion of antibody molecules within the cells may occur. For the above reasons, the occasional finding of small FT-containing aggregates displaced with respect to the original antibody site is not surprising. We consider the presence of FT aggregates in the extracisternal ground substance (and in some damaged mitochondria) to be artifacts of this kind. This view is supported by the absence of any definite pattern of distribution and by the apparent correlation between these aggregates and discontinuities in the membranes of the endoplasmic reticulum.

Even taking into account the possible sources of error, the constant finding of FT-containing precipitates within the cisternae of the endoplasmic reticulum, points to the latter as the major, if not the sole, site of storage and possibly synthesis of antibodies. This confirms the observations of several authors (25–27), which point to the microsomes as the probable site of antibody synthesis. Furthermore, in view of the biochemical and physicochemical similarities between myeloma gamma globulins and normal antibodies, our results are also in agreement with those of Rifkind *et al.* (11) on the localization of myeloma gamma globulins in the ergastoplasmic cisternae of myeloma cells.

FT-containing precipitates were normally observed also in the perinuclear space, suggesting a functional continuity between it and the rough endoplasmic reticulum, in accordance with the morphological continuity demonstrated by electron microscopy (28, 29).

Some quantitative data on the concentration of antibodies in plasma cells can be derived from the density of FT molecules within the cisternae of these cells. The concentration of FT molecules varies between 1.6 (in cisternae containing isolated aggregates) and $6.6 \times 10^{16}/cm^3$. We take a mean value of 5×10^{16} as the basis of subsequent calculations; this value refers to the most uniformly labeled cisternae; *i.e.*, those from which presumably fewest antibody molecules have escaped. A density of 5×10^{16} molecules/cm^3 corresponds to 38 mg of protein per cm^3, taking the molecular weight of apoferritin to be about 460,000. This concentration is 6 to 7 times higher than the maximum present in the incubation fluid (5 to 6 mg/ml), a finding compatible with the interpretation of intracisternal aggregates as antigen-antibody precipitates. To deduce the concentration of antibodies some assumptions must be made on the number of antibody molecules combined with one molecule of antigen in the precipitate. In a precipitate of soluble molecules, there must be less than 2 FT molecules per molecule of bivalent antibody; *i.e.*, the antibody concentration in the cisternae must be higher than 6.1 mg/cm^3 and probably not lower than double this value. These values indicate that the local concentration of antiFT antibodies in plasma cells is certainly high enough to ensure complete

precipitation with FT even after treatment with formaldehyde (see results on the effect of formalin on antibodies). Precipitin curves obtained with the sera of the experimental animals showed that an antibody concentration of this order of magnitude, as compared with the concentration of antigen in the incubation fluid, gives rise to precipitates in the zone to the left of the point of maximum precipitation. It seems probable, therefore, that more than one molecule of antibody is bound to one molecule of FT, and that localized regions of slight antibody excess may occur in the cells. We take the value of 12.2 mg/cm^3 (corresponding to a 1:1 molecular ratio between FT and antibodies) as a lower limit for the concentration of complete, *i.e.* bivalent, antibodies in the cisternae. The concentration may be higher also on account of a small proportion of apoferritin in the incubation fluid.

From the above data it is possible to deduce the average antibody content of plasma cells, making some reasonable assumptions on the dimensions of these cells. If we take 220 μ^3 as the average volume of a plasma cell (roughly corresponding to a weight of 250×10^{-12} gm, used by Berenbaum (2) for analogous calculations), and we subtract 65 μ^3 belonging to the nucleus (diameter 5 μ), the volume of the cytoplasm corresponds to 155 μ^3. These dimensions correspond roughly to those of the plasma cells in our preparations. The average volume occupied by the cisternae, estimated from our micrographs, appears to be about 37 per cent of the total, that is 57 μ^3. Using this value and an antibody concentration of 12.2 mg/cm^3, the total quantity of antibodies in a plasma cell is estimated to be not lower than 7.0×10^{-13} gm. This value falls in the same range as those calculated by Berenbaum (2) from his own and other authors' experimental data (1.25 to 37.5×10^{-13} gm). Considering that the assumptions made in our own and Berenbaum's calculations are necessarily approximate, the agreement may be considered to be very satisfactory.

Many cells labeled with FT exhibit several untagged cisternae. A possible explanation of this finding is that FT fails to penetrate into all cisternae, a situation which might occur if the latter form an extensive but not complete intercommunicating system. This would seem the most likely explanation, for example, where of two adjacent cisternae, one is heavily tagged and the other completely free of label (Fig. 4). In support of such a hypothesis is the observation that, in the plasma cells sectioned by the cryostat knife and marked with FT, as a rule all the cisternae adjacent to the cut surface contain FT-antiFT precipitates, while those further removed are often unlabeled. The above observation, while not excluding the possibility of a functional heterogeneity between different groups of cisternae, strongly suggests that the majority of, if not all, the cisternae contain antiFT antibodies. As regards the postulated "interruptions" of the ergastoplasmic system, one possibility, though not a very likely one, is that they may result from fixation artifacts. Some cases have in fact been reported, where imperfect fixation transformed a continuous system into a series of vacuoles (37).

Although some micrographs suggest that the Golgi region may contain some antibody, it appears completely devoid of label in the majority of plasma cells. If the components of the Golgi complex are assumed to be largely independent, or communicating with each other and with the ergastoplasmic cisternae only for short intervals of time, the difficulty encountered by FT molecules in penetrating the intact membranes of each isolated component, together with the possibility that only part of the vacuoles may in fact contain antibody, might be sufficient to explain the generally observed absence of tagging in this region. It has been suggested that the Golgi apparatus is implicated in the secretion of antibodies (30). Our data, while not incompatible with this interpretation, do not allow any conclusions to be reached on this point. We found no evidence, on the other hand, in favor of the existence of direct communications between the cisternae of the rough endoplasmic reticulum and the extracellular space. If such communications do in fact exist, they must be extremely rare and functional for only very short periods of time, because, as a rule, FT fails to enter plasma cells with intact plasma membranes.

In our preparations we were unable to demonstrate a special affinity of FT for the ribosomes of plasma cells. The ribosomes associated with the membranes of the endoplasmic reticulum are probably sites of antibody synthesis (31). Occasional molecules of FT were found on ribosomes, but the number of such tagged ribosomes may be accounted for by purely casual contacts with the molecules of FT dispersed in the cytoplasm. The actual reason for this absence of association cannot be determined on the basis of the present experiments. It might be due to one of the following reasons: (*a*) only a small fraction of the ribosomes is associated with active antibody molecules; (*b*) the antibody molecules still attached to the ribosomes do not have the proper tertiary structure necessary for the reaction with the antigen; (*c*) the different polypeptide chains which form the complete antibody molecule (32–34) are manufactured on different ribosomes and subsequently assembled, with acquisition of the capacity to react with the antigen in the interior of the cisternae; (*d*) the antibodies still attached to the ribosomes are unable to take part in a specific precipitate with the antigen and therefore bind the antigen reversibly. Some of these possibilities are open to further investigation with immuno-electron microscopy.

A striking observation is the complete absence of FT molecules from the surface membrane of all plasma cells prepared by either method (1 or 2). This may be relevant to the postulated existence of cell-bound antibodies in lymph node cells (35, 36). FT molecules in close association with plasma membranes were seen instead in myeloma cells by Rifkind *et al.* The discrepancy between the two sets of observations cannot be explained at present: it may be related to differences between the two types of cells examined, to the different nature of the reactive site (antigenic sites in the case of the myeloma proteins and antibody sites in our experiments), or to technical reasons (*e.g.* the use of FT

as against FT-conjugated gamma globulins). Experiments are in progress to investigate further this interesting point.

SUMMARY

The localization of antibody in the interior of plasma cells of lymph nodes of rabbits hyperimmunized with ferritin was studied by electron microscopy. The cells were incubated with the antigen (ferritin) which was allowed to penetrate into the cells by suitable methods. Antigen-antibody precipitates were localized in the cisternae of the endoplasmic reticulum and in the perinuclear space. No evident association was found between ferritin and ribosomes or ferritin and outer cell membrane. Cells from control animals immunized with an unrelated antigen, incubated with ferritin, exhibited no labeling. From direct counts of ferritin molecules in plasma cells, a lower limit was evaluated for the antibody concentration in the endoplasmic reticulum (12.2 mg/cm^3) and for the total antibody content of a plasma cell (7.0×10^{-13} gm).

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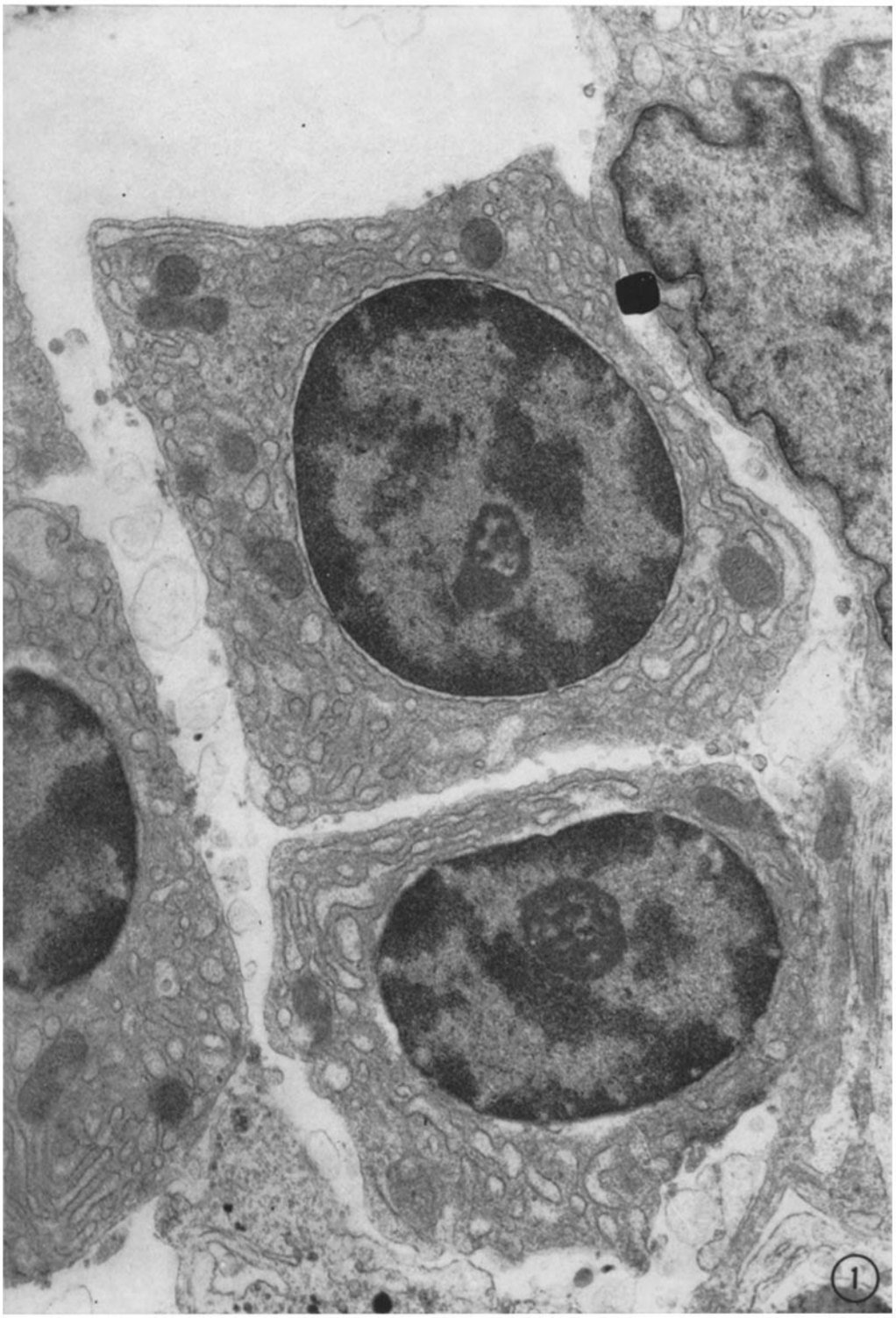
EXPLANATION OF PLATES

PLATE 57

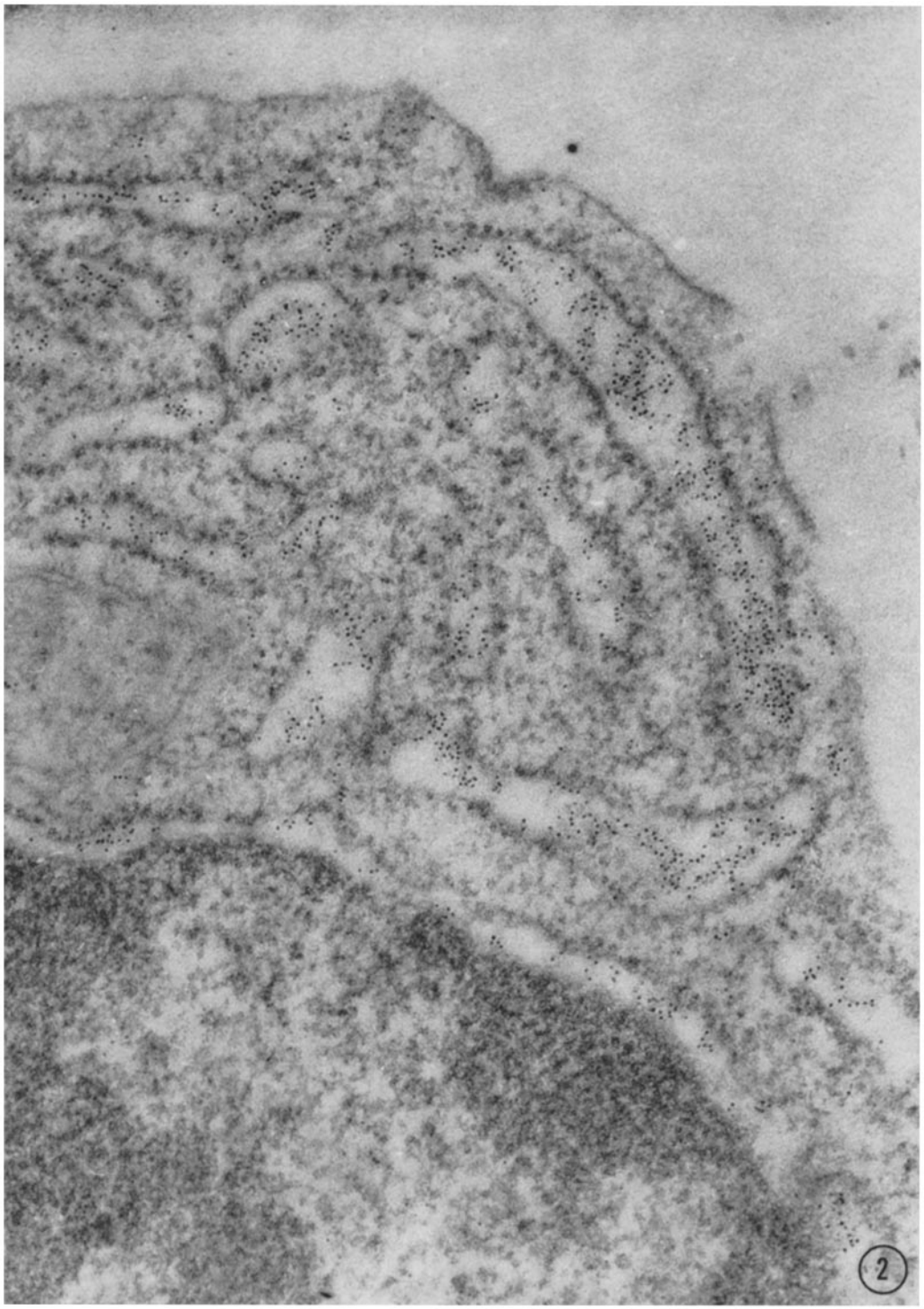
FIG. 1. A low power micrograph of plasma cells incubated with ferritin (Method 1). Part of a fibrocyte is visible at the upper right. This thin section was cut parallel to the cryostat-cut section. Tissue organization and cell structure are well preserved. Ferritin cannot be recognized at this magnification. × 15,000.



(de Petris *et al.*: Localization of antibodies in plasma cells)

PLATE 58

FIG. 2. Particular of a plasma cell prepared by Method 2 (isolated cells). Ferritin is concentrated in the cisternae of the endoplasmic reticulum and in the perinuclear space. A few molecules of ferritin are scattered in the remaining ground cytoplasm. No ferritin is visible on the outer cell membrane. × 82,000.

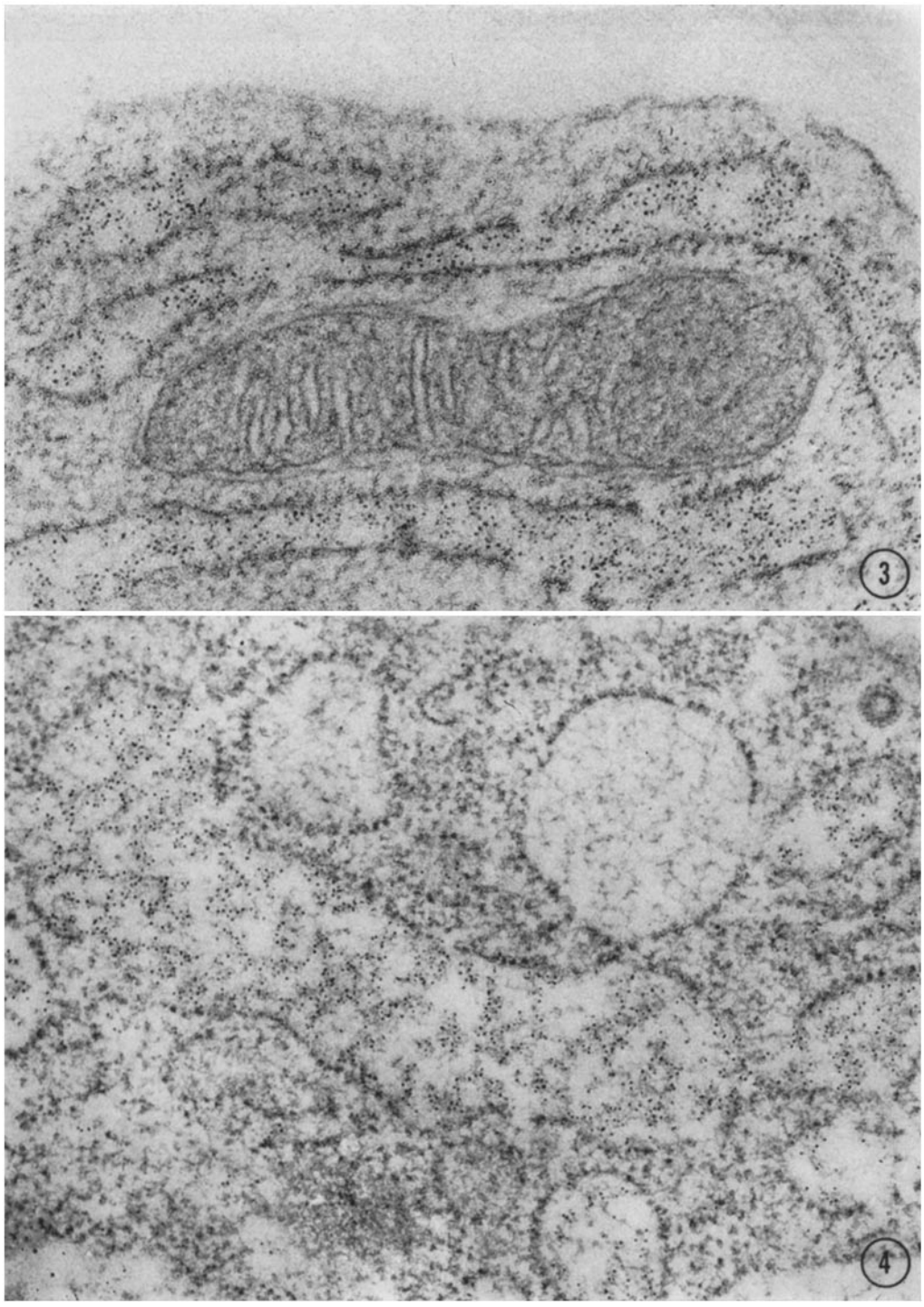


(de Petris *et al.*: Localization of antibodies in plasma cells)

PLATE 59

FIG. 3. Particular of another plasma cell with heavily tagged cisternae. Practically no ferritin is present in the extracisternal cytoplasm, in the mitochondrion and on the cell surface. Discontinuities in the membranes are artifacts resulting from the preparation of the specimen (Method 1). × 82,000.

FIG. 4. Ferritin is concentrated in some of the cisternae, while two adjacent cisternae are unlabeled. The latter contain the characteristic fairly dense flocculent material. A small vesicle is visible at the upper right, which resembles the pinocytic vesicles described by Roth and Porter (24). Similar vesicles are commonly observed in plasma cells. × 67,000.

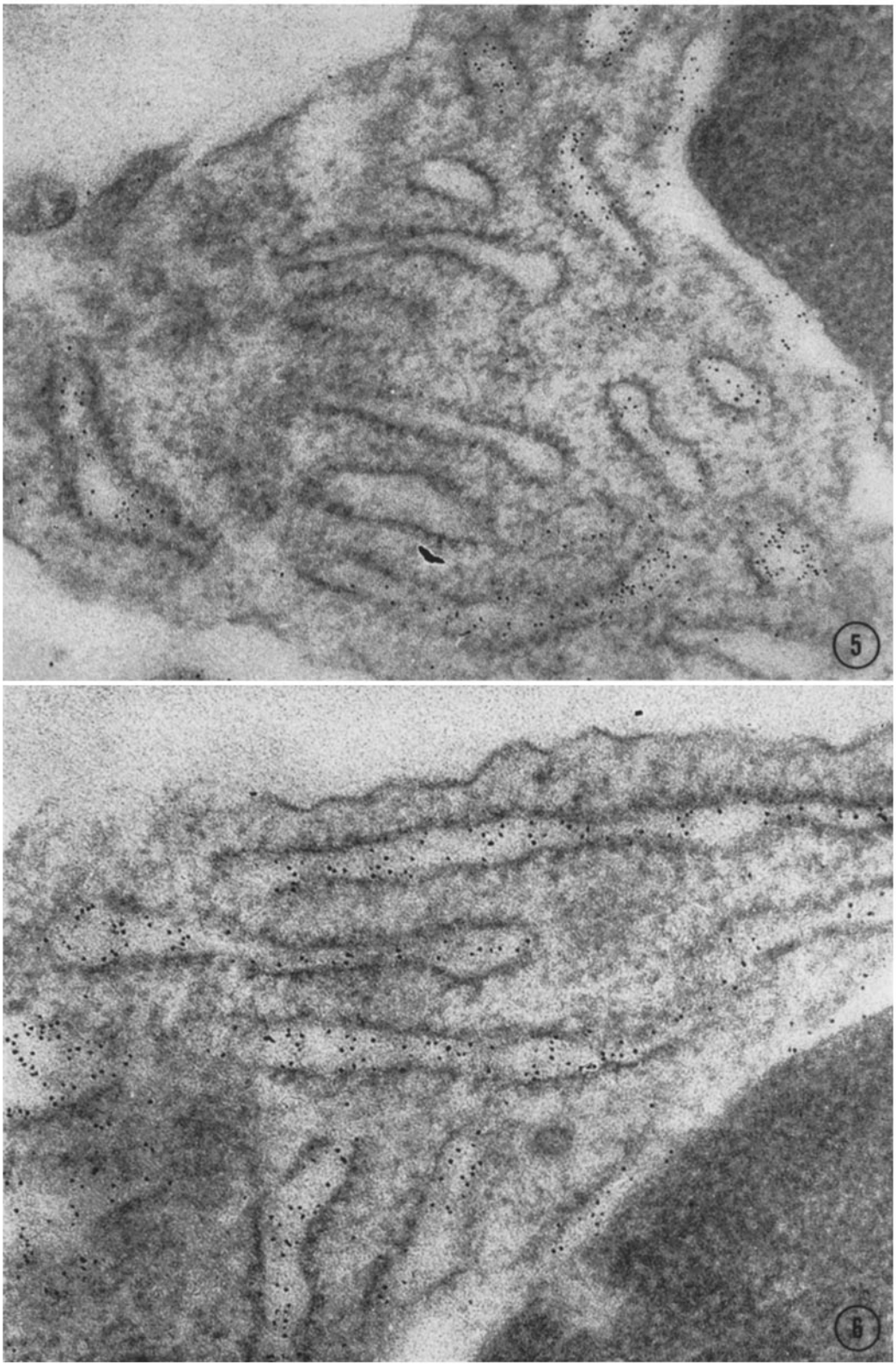


(de Petris *et al.*: Localization of antibodies in plasma cells)

PLATE 60

FIG. 5. Detail of a plasma cell prepared by Method 1. This section was not post-stained with uranyl acetate, in order to reveal ferritin molecules possibly associated with ribosomes. No obvious relationship can be noted. Some cisternae are labeled with ferritin while others are completely unlabeled. Ferritin is not present on the plasma membrane. × 92,000.

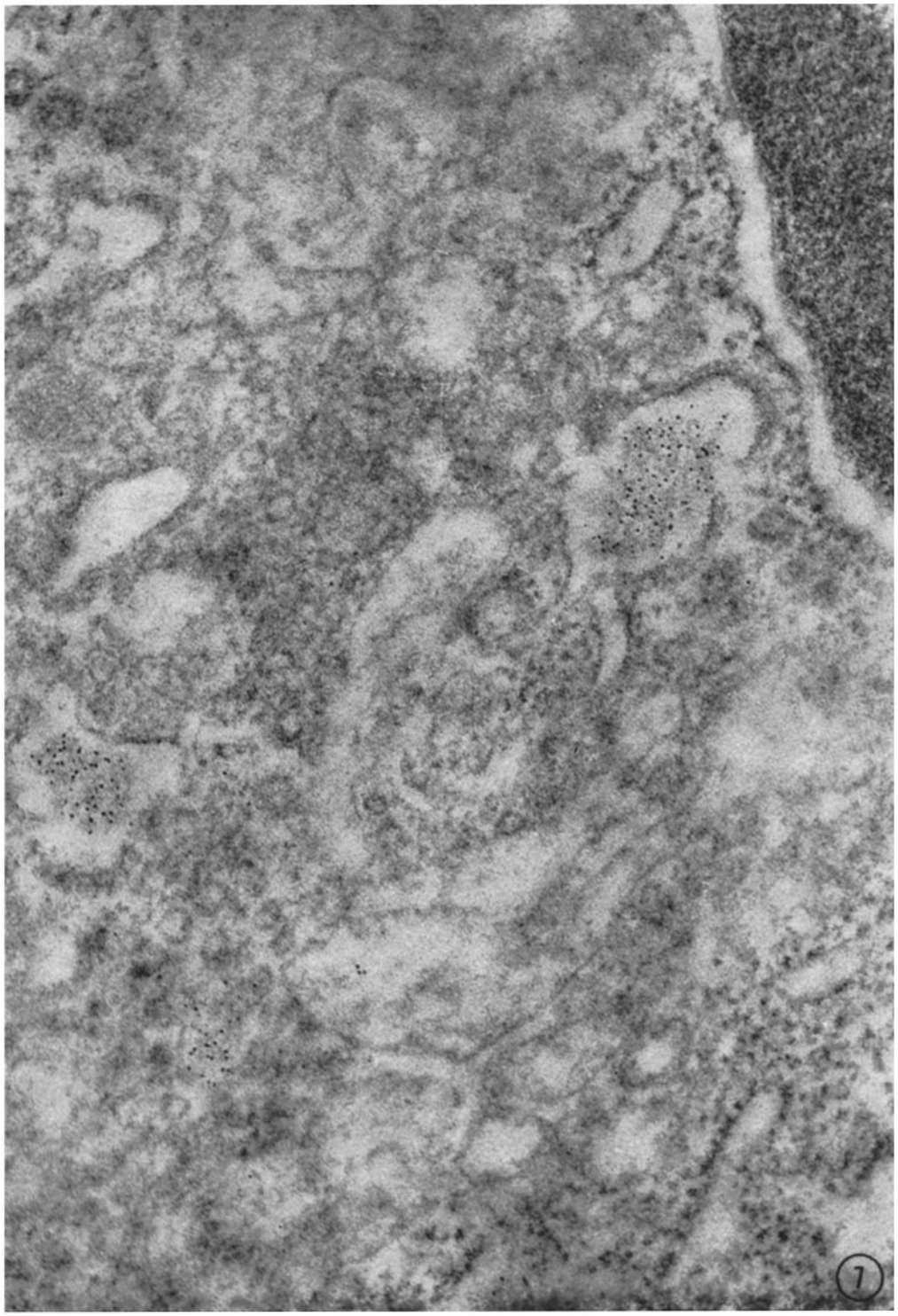
FIG. 6. Another plasma cell, treated as in Fig. 5. No obvious association is noted between ferritin and the faintly discernible ribosomes. The outer cell membrane is untagged. × 110,000.



(de Petris *et al.*: Localization of antibodies in plasma cells)

PLATE 61

FIG. 7. Golgi region of a plasma cell (prepared by Method 2). In this region ferritin is concentrated inside two vacuoles; the molecules of ferritin are visible against a background of fairly dense material. The membranes delimiting these vacuoles are partly free and partly studded with "particles" of lower electron density than that of the typical ribosomes, observed in the same field. The exact nature of these "particles" is difficult to assess because of the imperfect visibility of the membranes in this cell. They resemble the formations surrounding the three small vesicles visible at the upper left of the micrograph. A small cluster of ferritin molecules is present in the cytoplasm (possibly derived from a disrupted vacuole). × 85,000.



(de Petris *et al.*: Localization of antibodies in plasma cells)