ULTRASTRUCTURAL LOCALIZATION OF ANTIBODY IN DIFFERENTIATING PLASMA CELLS*

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The localization of antibodies in cells of the plasmacytic series was established at the light microscope level, first indirectly by comparative immunological and histological studies (11) and then directly by the binding of cellular antibodies with microscopically visible antigens (bacteria) (22) and by immunofluorescent (8, 9) and radioautographic (3) techniques. Since these early studies, there has been an impressive number of investigations of plasma cells (see reviews by Nossal, reference 18, and Feldman, reference 12) which have confirmed and extended the original observations. More recently, de Petris et al. (10) revealed the ultrastructural site of antibody in plasma cells with a direct immunoferritin technique. They used ferritin as an antigen to immunize rabbits, then exposed formaldehyde-preserved lymph node cells to ferritin and obtained specific antigen-antibody precipitates. The distinctive structure of ferritin permitted the localization of this antigen-antibody complex in the cisternae of the endoplasmic reticulum and the perinuclear space in young plasma cells.

Enzymes have recently been used as antigen to immunize rabbits and to trace the distribution of anti-enzyme antibodies in differentiating plasma cells at the light microscope level (2). In this study, we have used this approach to localize antibody at the ultrastructural level. Horseradish peroxidase was employed as an antigen to hyperimmunize rabbits and to trace the distribution of the antibody to peroxidase in differentiating plasma cells, from the earliest appearance of antibody in hemocytoblasts 2 days after the booster injection of antigen through its accumulation in well-developed plasma cells 3–5 days after injection. Like ferritin, horseradish peroxidase is a good antigen as judged by the high titers of precipitating antibodies which are obtained. As in the ferritin technique of de Petris et al. (10), this is a direct method which precludes the necessity of conjugating the antigen with an electron-opaque label and, therefore, penetration of antibody-containing cells by the antigen is more readily attained. The site of bound antigen is then revealed by the cytochemical pro-

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cedure developed by Graham and Karnovsky (13) for peroxidase activity which produces an electron-opaque reaction product.

Material and Methods

Antigen.—Horseradish peroxidase, 0.6 RZ, was obtained from Boehringer und Soehne (Mannheim, Germany). Doses of 10 mg in 0.5 ml of physiological saline were administered at each injection.

Immunization.—1 yr old rabbits weighing 2.5–3 kg were used. The first injection of peroxidase was given intradermally in a hind footpad with 0.3 ml of complete Freund's adjuvant. During the next 2 wk, the rabbits received one intramuscular and two or three intravenous injections. For the demonstration of antibody, two booster injections, one intramuscular and one intravenous, were administered on the same day 2–4 wk later, and the animals were killed 2–5 days after the last injection. For the demonstration of antigen, a single intravenous booster injection was administered and the animals killed after 4, 8, and 24 hr.

The spleen was excised, cell imprints were made on slides for light microscopy, and 0.5–1 mm^3 blocks were fixed for electron microscopy.

Light Microscopy.—Two methods were employed: (*a*) Slides containing the dried cell imprints were fixed in 60:40 alcohol:ether, exposed to a solution of 0.5 mg/ml of peroxidase in phosphate-buffered saline (PBS) for 45 min at room temperature, rinsed in PBS, and the sites of bound peroxidase were revealed by the Nadi reaction (20); (*b*) 1 mm^3 blocks of spleen fixed 1 hr in 2.5% glutaraldehyde were embedded in gelatin and 1–2 μ sections cut in a cryostat with a Porter-Blum microtome (4), mounted on slides, and exposed to peroxidase as above. The site of bound peroxidase was revealed by incubation for 10 min at room temperature with the substrate used for electron microscopy (see below) followed by 10 min in 2% osmium tetroxide in 0.1 M phosphate buffer.

Electron Microscopy.—Fixation of 0.5–1 mm^3 blocks of splenic tissue was carried out at 3°C with constant mild agitation. Paraformaldehyde, 1% and 4%, for 1 min to 4 hr, and glutaraldehyde, 1.25% and 2.5%, for 1 min to 1 hr, both in 0.1 M phosphate buffer at pH 7.3, were compared as fixatives. The best preservation of both antibody and ultrastructure was obtained with 2.5% glutaraldehyde for 1 hr and this procedure was followed for all observations reported here. After fixation, the tissue was washed overnight in the buffer at 3°C on the agitator. It can be stored in cold buffer at least 1 month without loss of antibody-antigen coupling. Subsequent reactions were carried out either directly on the blocks of tissue or on 40 μ thick frozen sections of these blocks. Ultrastructural preservation was better in the blocks, but penetration of the reagents was somewhat better in the sections.

For the demonstration of antibody, the tissue was exposed to peroxidase, 0.5 mg/ml of 0.1 M phosphate buffer at pH 7.3, for 1 hr at 3°C with constant agitation, washed in three changes of the buffer for a total of 5–10 min, and refixed in 2.5% glutaraldehyde for 15 min to assure firm binding of the antigen to the tissue (17). The fixative was removed with three changes of buffer for 30 min, and the tissue was incubated for 30 min at room temperature in 3 mg of 3,3′-diaminobenzidine (tetrahydrochloride) per 10 ml of 0.05 M tris-HCl buffer at pH 7.5 plus 0.01% hydrogen peroxide (13). After a brief wash in buffer or distilled water, the tissue was postfixed in 2% OsO_4 in 0.1 M phosphate buffer at pH 7.3 for 1 hr, dehydrated in alcohol, and embedded in Epon.

Two types of controls were employed. On the one hand, tissues containing antibody to peroxidase, 2–5 days after the final injection of antigen, were incubated in the diaminobenzidine-hydrogen peroxide substrate without preliminary incubation with peroxidase. On the other hand, spleens from unimmunized rabbits and from animals immunized with γ-globulin were treated with both peroxidase and its substrate. Both types of controls were negative, except for endogenous peroxidase activity.

For the demonstration of antigen in spleens of rabbits killed 4, 8, and 24 hr after the final injection of peroxidase, the tissues were incubated in the substrate alone for 30 min and 2 hr, then postfixed in osmium tetroxide and embedded in Epon as above.

Ultrathin sections were examined without staining or they were stained either for 30 min in Karnovsky's lead or for 20 min in 4% uranyl acetate followed by 10 min in lead. Electron micrographs were taken with an Elmiskop I at 80 kv with 50 μ objective apertures.

OBSERVATIONS

The peroxidase which becomes coupled with the antibody to peroxidase in the splenic cells remains active and, after exposure to 3,3'-diaminobenzidine and hydrogen peroxide or to the Nadi reagent, electron-opaque and colored reaction products are formed, respectively, at the site of the antibody.

Light Microscopy.—Cell imprints stained by the Nadi reaction (Fig. 1) gave a rapid survey of the relative numbers and the types of cells which contained antibody. Few cells contained antibody on the second day after the booster injections and these consisted chiefly of large blasts and a few immature plasma cells. Antibody-containing cells were most numerous on the 3rd day, and the entire spectrum of the plasmacytic series was present. The number of cells fell off on the 4th and 5th days, and these were chiefly immature and mature plasma cells. Thin frozen sections exposed to the diaminobenzidine and peroxide substrate confirmed the observations in cell imprints and, in addition, revealed the distribution of the antibody-containing cells in the splenic tissue. Positive cells were in the red pulp surrounding the lymphatic nodules (Fig. 2), hence blocks of splenic tissue were selected for electron microscopy that contained red pulp contiguous to the cortex of the nodules.

Electron Microscopy.—Control preparations for antibody, both those containing antibody to peroxidase that were treated only with the substrate, diaminobenzidine and peroxide, and those containing other antibodies or none that were treated with both peroxidase and its substrate, revealed only the endogenous peroxidase activity. This activity of the spleen resides in erythrocytes, inclusions in macrophages and leukocytic granules. In spleens containing antibody to peroxidase which were exposed to both peroxidase and its substrate, a positive peroxidase reaction occurred in the endogenous sites and also in some, but not all, of the cells in the plasmacytic series. All of these positive reactions were obtained only at the surface of the tissue blocks to a depth of 4–5 cells. This may be related to the depth of penetration of the peroxidase molecules, but longer (24 hr) exposure to peroxidase did not alter the results. The degree of fixation may be the important factor since glutaraldehyde fixes rapidly but penetrates slowly. In spite of this limitation, sections tangential to the surface of the block contain large numbers of reactive cells (Fig. 3). The only advantage of using 40 μ frozen sections of the 1 mm^3 blocks, prepared by the method of Holt and Hicks (14), over the immersion of entire blocks in the reagents is that many blocks can be sampled at one time. This is useful for a

preliminary survey because even in active spleens some areas of the red pulp are free of antibody-containing cells. Freezing, however, produces some structural damage to the tissue; therefore, the cells illustrated here are from blocks of tissue.

As is well known, the terminology relating to the plasmacytic series varies with different authors, and that used here will be essentially that employed in an earlier study (15).

As was described by light microscopy, utilizing a variety of techniques (3, 11, 15), the most primitive cell to contain antibody is the hemocytoblast, a cell characterized by its large electron-lucent nucleus, extensive and complex nucleolus, and cytoplasm rich in free ribosomes but poor in ergastoplasmic lamellae. The antibody is present primarily in the perinuclear space (Fig. 4); this seems to be the initial site of antibody synthesis. The rare ergastoplasmic cisternae in this cell sometimes also contain antibody, but often do not. The Golgi apparatus even in these early blast cells, may be filled with the reaction product (Fig. 4).

The subsequent differentiation of these cells is characterized primarily by the gradual development of the ergastoplasm or ribosome-associated endoplasmic reticulum. In the earliest of the differentiating cells, which we here distinguish from hemocytoblasts by the term plasmablast, the cisternae of the ergastoplasm remain flattened. The perinuclear space continues to contain antibody, and all of the developing ergastoplasm appears positive (Fig. 5); in fact, the continuity of the endoplasmic reticulum is particularly well illustrated by its content of antibody (Fig. 6).

In the next phase of cell differentiation, resulting in the immature plasma cell, the endoplasmic reticulum is more extensive and its cisternae, instead of being flattened, become dilated so that in sections they appear as a collection of ribosome-studded vesicles. At this stage antibody is usually (Fig. 7) but not always present in the perinuclear space; when present, it is sometimes irregularly distributed instead of filling the space entirely. Similarly, not all of the ergastoplasmic cisternae are positive. There is usually an intermingling within the cytoplasm of cisternae that are intensely or moderately positive with those which are nonreactive for peroxidase (Figs. 12 and 14). The latter are distended like those containing antibody, and they contain a coarse granular material which is less dense than the reaction product of peroxidase (Fig. 13). In immature plasma cells undergoing mitotic division, antibody-containing vesicles are abundant (Fig. 12). Higher magnification electron micrograph show that antibody is restricted to the space between the membranes of the nuclear envelope and of the endoplasmic reticulum (Fig. 15). Attempts to discern it associated with ribosomes in noncounterstained sections were not successful.

As the plasma cell matures, the ergastoplasmic cisternae become increasingly distended (Fig. 8). Not all mature plasma cells in a given spleen contain antibody; positive and negative plasma cells occurred side by side (Fig. 3). Two types of antibody distribution were observed in mature plasma cells. In both

cell types, the perinuclear space is free of antibody. In some cells, antibody remains within the ergastoplasm where it tends to accumulate into spherical masses, often very large (Russell bodies?) and always studded with ribosomes (Fig. 9). The remainder of the ergastoplasm then contains little or no antibody and is in the form of smaller vesicles or even flattened cisternae; nevertheless, a considerable amount of nonreactive, coarsely granular material persists. In other cells, the antibody appears to escape the confines of the endoplasmic reticulum and to be present throughout the cytoplasm (Fig. 10). The nuclei are intact and antibody-free, but they are sometimes pushed to one side of the cell and indented by the mass of intensely peroxidase-positive material with which the cytoplasm is engorged.

Throughout plasma cell development, the Golgi apparatus usually contains antibody (Figs. 3, 4, and 14), but an occasional cell has been found in which the Golgi apparatus appears to be antibody-free (Fig. 7). When present, it occurs chiefly in the interior of some or all of the large flattened sacs of the lamellar portion and only rarely in the associated small vesicles and large vacuoles.

No antibody could be identified in small lymphocytes in the cortex of lymph nodules or in extracellular spaces.

Antigen could not be found in any of the spleens with the method employed.

DISCUSSION

Previous work by immunodiffusion techniques has demonstrated that enzyme-antibody complexes always possess catalytic activity, and this property has been used to characterize these complexes (24). On the basis of the same principle, a technique has recently been described for the localization of anti-enzyme antibodies in immunocompetent cells at the light microscope level (2). It has now been possible to determine the site of anti-enzyme antibody at the electron microscope level. Since horseradish peroxidase remains strongly reactive after combining with its specific antibody, and since one of its reaction products is electron-opaque (13), we have determined the ultrastrutural localization of antiperoxidase antibody in the spleens of hyperimmunized rabbits. We did not attempt to localize the antibody in the primary response, because the cells involved in antibody synthesis are the same (15) as those in the secondary response and they are so few in number that the sampling problem for electron microscopy becomes very great. An advantage of using peroxidase as an antigen to localize antibody is that small amounts of enzyme can generate a large quantity of reaction product. Furthermore, peroxidase is a smaller molecule than the heretofore utilized ferritin (10), with a molecular weight of 40,000 instead of 650,000, hence it can penetrate cells more readily. A disadvantage, however, is that no quantitation is possible such as that reported by de Petris et al. (10) with ferritin.

A major problem in immunology has been the location of antigen in cells

of lymphatic tissues in relation to the cells which synthesize antibody. Wellensiek and Coons (25) have succeeded in demonstrating the ultrastructural localization of antigen in the secondary response by using ferritin as antigen. They found ferritin within the nucleus, associated primarily with chromatin, and in the extracisternal cytoplasm not only of phagocytic cells but also of hemocytoblasts and, in diminishing quantities, immature and mature plasma cells. In this study with peroxidase as antigen, we were not able to detect the antigen in any nuclei or in the cytoplasm of plasmacytic cells. In this respect, our results resemble those of Nossal et al. (19), who found no ^{131}I-labeled flagellar antigens in plasma cells, and Cheng et al. (7) who found some cytoplasmic but no nuclear ^{35}S-labeled globulin and albumin antigens in splenic red pulp. A possible explanation is that the antigen molecule is partially broken down when it enters the primitive blast cells. An alteration of the protein moiety of ferritin would have no effect on the appearance of its ferric hydroxide core, as shown by protease digestion of ferritin,[1] but such a modification of peroxidase might inhibit its activity.

The antibody-containing cells revealed by the peroxidase-antiperoxidase antibody system are the same as those described at the light microscope level by the immunofluorescent technique (8, 9, 15), namely, those of the plasmacytic series. The ultrastructure of these cells has been described by many authors (reviewed by Feldman in reference 12) but the terminology has varied considerably. For example, in the excellent illustrations of Movat and Fernando (16), their immunoblasts are our hemocytoblasts, we are in agreement on an intermediate category of plasmablasts, their proplasmacytes are our immature plasma cells, and their plasmacytes are our mature plasma cells. Two ultrastructural investigations of the localization of specific γ-globulins in plasma cells have been reported in which ferritin was used as a marker. In one, Rifkind et al. (23) employed ferritin-labeled antibody to locate myeloma globulin in a plasma cell tumor. In the other, de Petris et al. (10) used unconjugated ferritin directly to localize antibody to ferritin in lymph nodes of hyperimmunized animals. In both studies, the specific γ-globulins were found primarily within the cisternae of the endoplasmic reticulum including the perinuclear space. Our results with antibody revealed by peroxidase activity confirm this localization. In addition, our material has permitted us to localize antibody in the progressive phases of plasma cell differentiation and, although the evidence is in the form of a series of static electron micrographs, it is possible to extrapolate to some extent to obtain a dynamic picture of antibody synthesis and storage.

A striking finding was that in all hemocytoblasts that contained any antibody at all the perinuclear space between the two membranes of the nuclear

[1] Monneron, A., and Y. Moulé. Isolement de particules ribonucleoproteiques nucleaires: correlations entre données biochimiques et ultrastructurales. Manuscript in preparation.

envelope was always completely and intensely positive, and often this was the only site of antibody in these primitive cells. This is perhaps not surprising inasmuch as in cells that otherwise contain very little ergastoplasm the nuclear envelope represents the major component of the endoplasmic reticulum (21). A few, flat, ribosome-studded ergastoplasmic cisternae are usually present in hemocytoblasts, however, and while these cisternae sometimes also contain antibody they often do not. As the cell differentiates, antibody continues to be in the perinuclear space in the plasmablasts, is variably present in immature plasma cells, and absent in mature plasma cells. Thus, one has the impression that antibody formation begins in the perinuclear ergastoplasm, then gradually shifts into the peripheral ergastoplasm. These observations suggest that some component of the nucleus may have a primary role in the initiation of antibody synthesis.

We have arbitrarily interposed a stage called "plasmablast" (unwisely, perhaps, since some authors use this term synonomously with "hemocytoblast") between the more primitive hemocytoblast and the more differentiated immature plasma cell, to indicate a numerically important population of cells in which the ergastoplasm has proliferated, but has not yet become distended, and appears to be uniformly filled with antibody. In the next morphologically distinctive stage, the immature plasma cell, in which the ergastoplasm is even more extensive and its cisternae are becoming dilated, antibody is not uniformly present in all segments of the ergastoplasm. Antibody-containing cisternae may be intermingled with antibody-free cisternae throughout the cytoplasm or the two may be more or less segregated in different parts of the cell. Rifkind et al. (23) and de Petris et al. (10), using two other methods, report a similar observation. De Petris et al. (10) suggest that this may be an artifact of unequal penetration of the ferritin, but Rifkind et al. (23) propose that it is evidence of a real functional heterogeneity. Like the latter authors, because of the close approximation of peroxidase-positive and -negative cisternae, we do not feel that this is an artifact. The peroxidase-negative cisternae are distended with material which presumably represents a synthetic product of the ergastoplasm but one that does not contain antiperoxidase antibody. Nossal (18) and Attardi et al. (1) have found plasma cells that contain two antibodies, but such cells make up only a small percentage of the cell population whereas nearly all plasma cells in our tissues were heterogeneous. This is at variance with the "one cell - one antibody" concept (5). On the third day of the secondary response, a large number of cells were found which were clearly immature plasma cells with a well-developed ergastoplasm, but which contained antibody only in the perinuclear space (Fig. 11). This might be interpreted as an indication that some young plasmacytic cells, already engaged in protein synthesis, had been stimulated to produce a new protein, antibody to peroxidase. This, however, militates against the concept of the clonal differentiation of plasma cells (5, 18) initiated by the effect of a single antigen on a primitive precursor (15). A more likely

explanation is that some parts of the ergastoplasm of these cells also contained antibody but these regions were not included in the ultrathin sections that were examined. The significance of the presence of antibody-positive and antibody-negative regions of the ergastoplasm remains to be determined. It is possible that the peroxidase-negative cisternae represent portions of the ergastoplasm in which only one of the two polypeptide chains which ultimately form the antibody is present, or both may be present but not linked.

The cisternae of the lamellar portions of the Golgi apparatus were nearly always filled with peroxidase-positive material in all antibody-containing cells, from hemocytoblasts to mature plasma cells, whereas the vacuolar components of the apparatus were usually negative. This is at variance with the observations of Rifkind et al. (23) and de Petris et al. (10) who found that the Golgi apparatus was generally devoid of ferritin even when the ergastoplasm was heavily labeled. In the acinar pancreas, another type of cell highly specialized for protein synthesis, the Golgi apparatus is thought to function in the secretory process by accumulating, condensing, and packaging the secretory products for export (6). We have not been able to detect a similar sequence of events in plasma cells. The large, spherical accumulations of antibody in mature plasma cells remain within highly distended, ribosome covered, ergastoplasmic sacs. Thus, the role of the Golgi apparatus in these cells remains to be elucidated.

The mode of excretion of antibody from the plasma cell was not detected, but presumptive evidence for both theories (23) was found. On the one hand, the accumulation of antibody into large spherical granules within the ergastoplasm suggests that the contents of these granules, like those of zymogen in enzyme secreting cells, may be excreted from a viable and persisting cell, possibly via a transient communication between the ergastoplasm and the cell membrane. On the other hand, some mature plasma cells were found in which antibody had overflowed the lumina of the ergastoplasm and Golgi apparatus to fill the cytoplasm. In some cases, the mitochondria were obscured by the intense peroxidase reaction and the nuclei, although free of antibody, were physically deformed by the massive accumulations of it in the cytoplasm. These are apparently terminal cells, the result of "suicidal differentiation," which will disrupt and thereby release their contents.

SUMMARY

Antibody was localized by electron microscopy within differentiating and mature plasma cells of the spleens of hyperimmunized rabbits. Horseradish peroxidase was used as antigen. Intracellular antibody to peroxidase was revealed in glutaraldehyde-fixed tissue by coupling it with its antigen and then revealing the sites of peroxidase activity cytochemically. Antibody first appears in the perinuclear space of hemocytoblasts where it persists through differentiation into immature plasma cells, but it disappears from this site in mature plasma cells. Concomitant with the development of the ergastoplasm, anti-

body accumulates in many but not all of its cisternae. Antibody is present in the lamellar portion of the Golgi apparatus in all phases of plasmacytic differentiation. Mature plasma cells exhibit two types of antibody distribution, a concentration into large spherical intracisternal granules or an overflowing into all parts of the cytoplasm.

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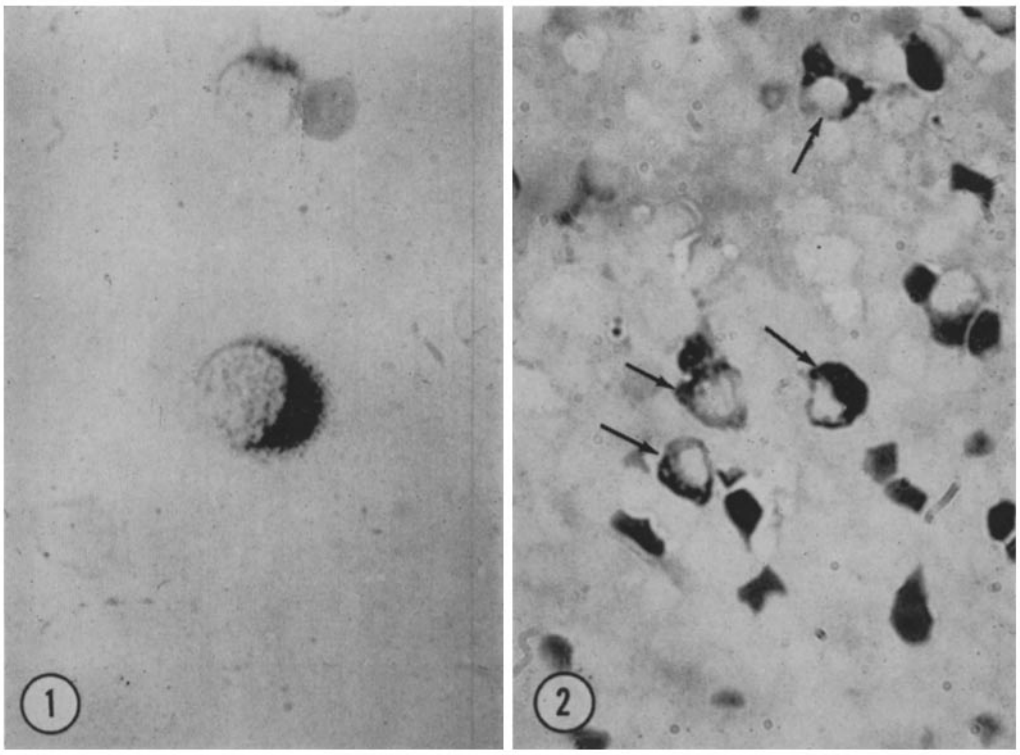
EXPLANATION OF PLATES

All of the photomicrographs illustrate the localization of antibody to horseradish peroxidase in cells of rabbit spleens 2–4 days after the last injection of antigen. The fixed cells were incubated with peroxidase to obtain the coupling of this enzyme to its antibody in the cells. The site of peroxidase was then revealed cytochemically by the Nadi reaction (Fig. 1 only) or by incubation with 3,3-diaminobenzidine and hydrogen peroxide, followed by postosmication.

PLATE 12

Fig. 1. Immature plasma cell in a cell imprint (Nadi reaction). The cytoplasm appears filled with antibody. Since this is a whole cell, antibody is also seen in the thin layer of cytoplasm over the nucleus. The particulate form of antibody probably corresponds to the distended vesicles of the endoplasmic reticulum which are visible in electron micrographs. × 900.

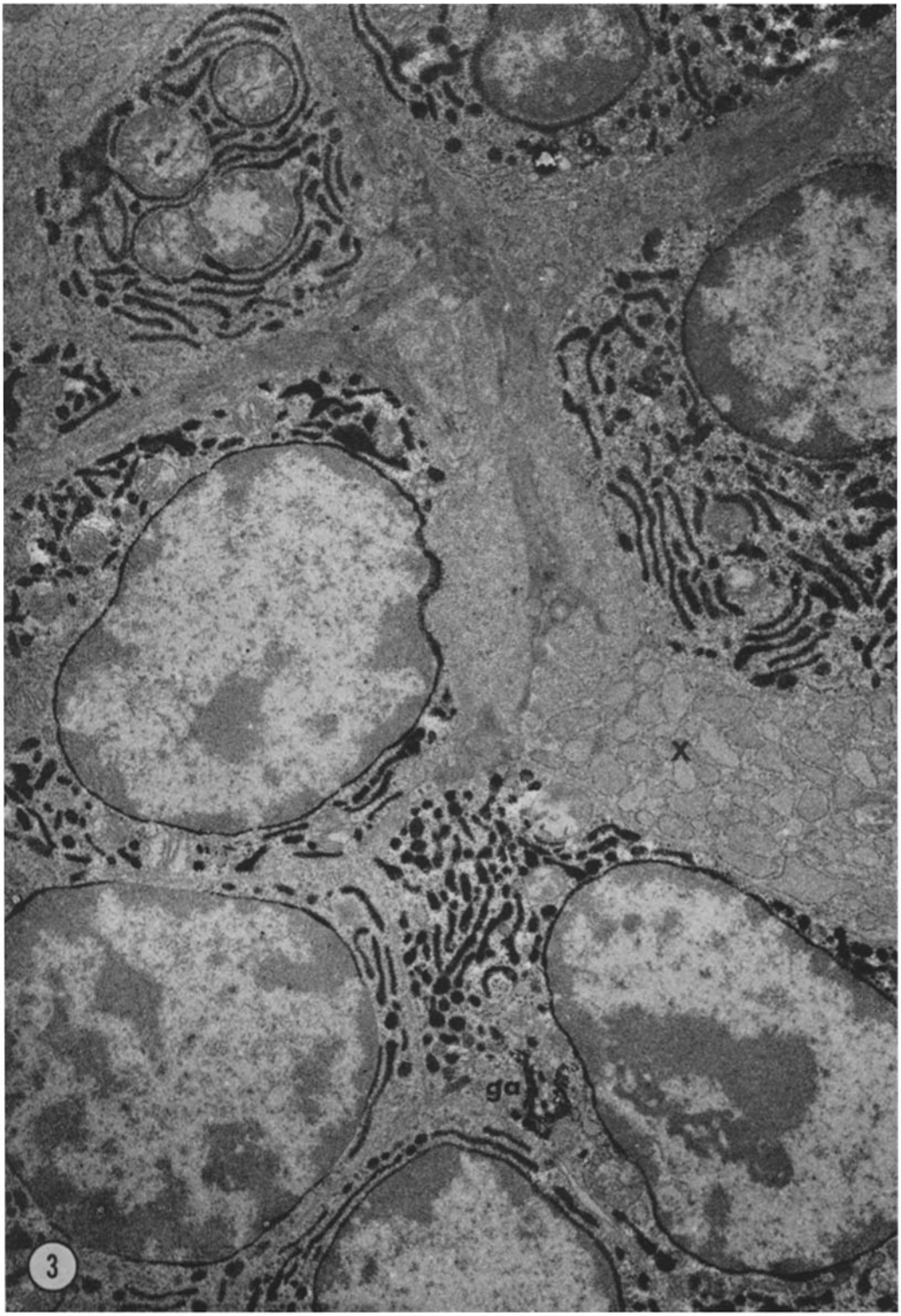
Fig. 2. Four antibody-positive immature plasma cells (arrows) in a 1–2 μ thick frozen section of spleen red pulp (diaminobenzidine and hydrogen peroxide substrate). The cytoplasm is intensely but irregularly stained. Endogenous peroxidase activity is also evident in the erythrocytes. × 900.



(Leduc et al.: Antiperoxidase antibody in plasma cells)

PLATE 13

Fig. 3. Electron micrograph of a group of antibody-containing immature plasma cells. The extreme electron opacity of the peroxidase reaction product makes the site of antibody stand out clearly. Antibody is present in the perinuclear space and most of the ergastoplasmic cisternae of all the cells and one positive Golgi apparatus (ga) is in the plane of section. The presence of a nonreactive mature plasma cell at X suggests that some plasma cells were synthesizing antibody to some antigen(s) other than peroxidase. × 7500.

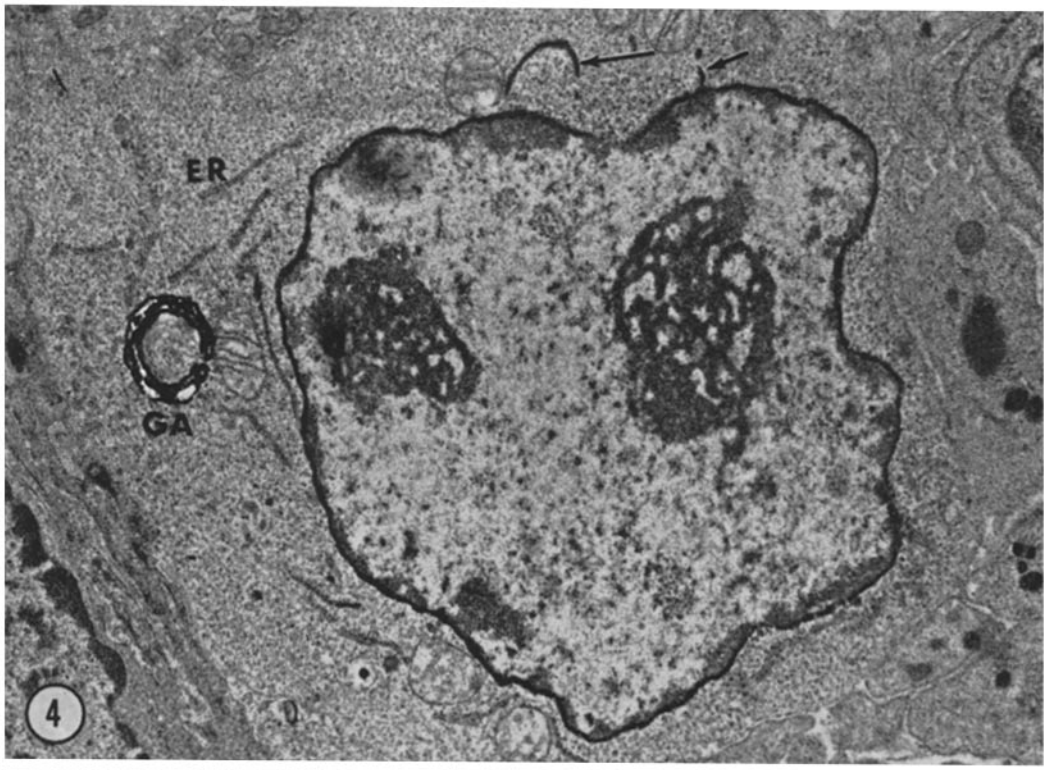


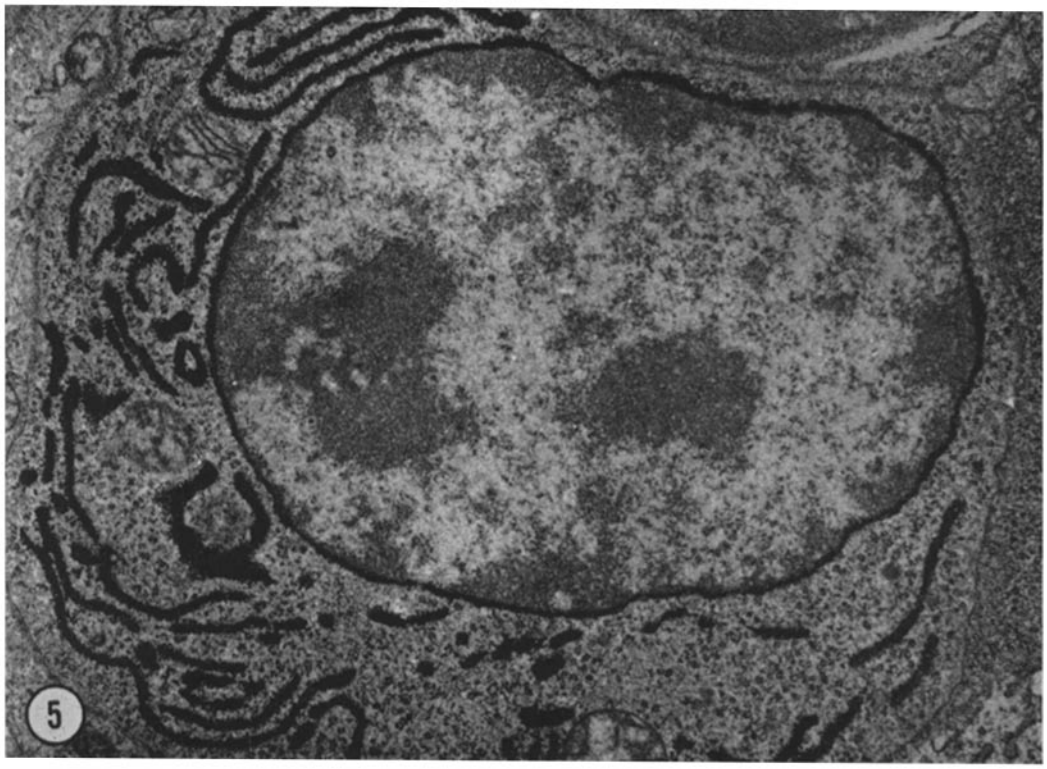
(Leduc et al.: Antiperoxidase antibody in plasma cells)

PLATE 14

Fig. 4. A typical hemocytoblast in which antibody is present in the perinuclear space, two segments of the ergastoplasm (arrows) and the Golgi apparatus (ga). Several ergastoplasmic lamellae (er) are present that do not contain demonstrable antibody. × 14,000.

FIG. 5. A more advanced stage of differentiation (plasmablast) in which the endoplasmic reticulum is more extensive, but still not distended, and is filled with antibody. × 12,500.



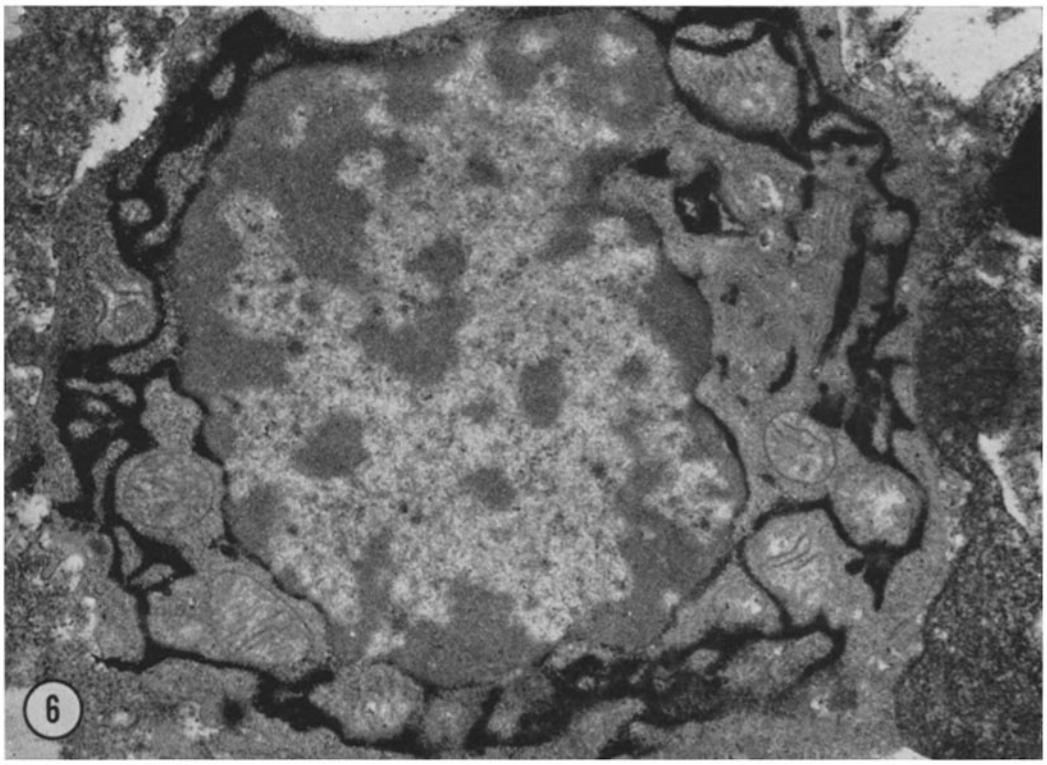


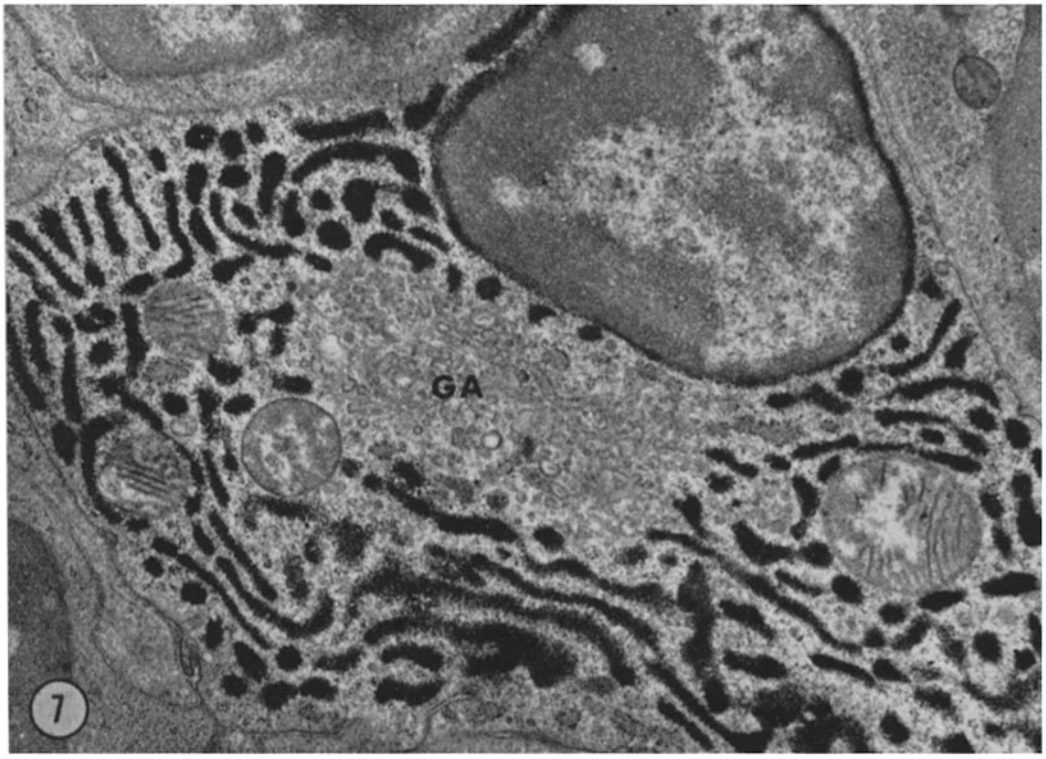
(Leduc et al.: Antiperoxidase antibody in plasma cells)

PLATE 15

FIG. 6. A numerically abundant form of plasmablast in which the antibody-enzyme reaction product reveals the continuity of the ergastoplasmic system of cisternae and the perinuclear space. × 13,500.

FIG. 7. An immature plasma cell in which antibody still fills the perinuclear space and most of the ergastoplasm, but is absent from the vesicles of the Golgi apparatus (ga) and a few of the cisternae of the ergastoplasm. × 18,400.





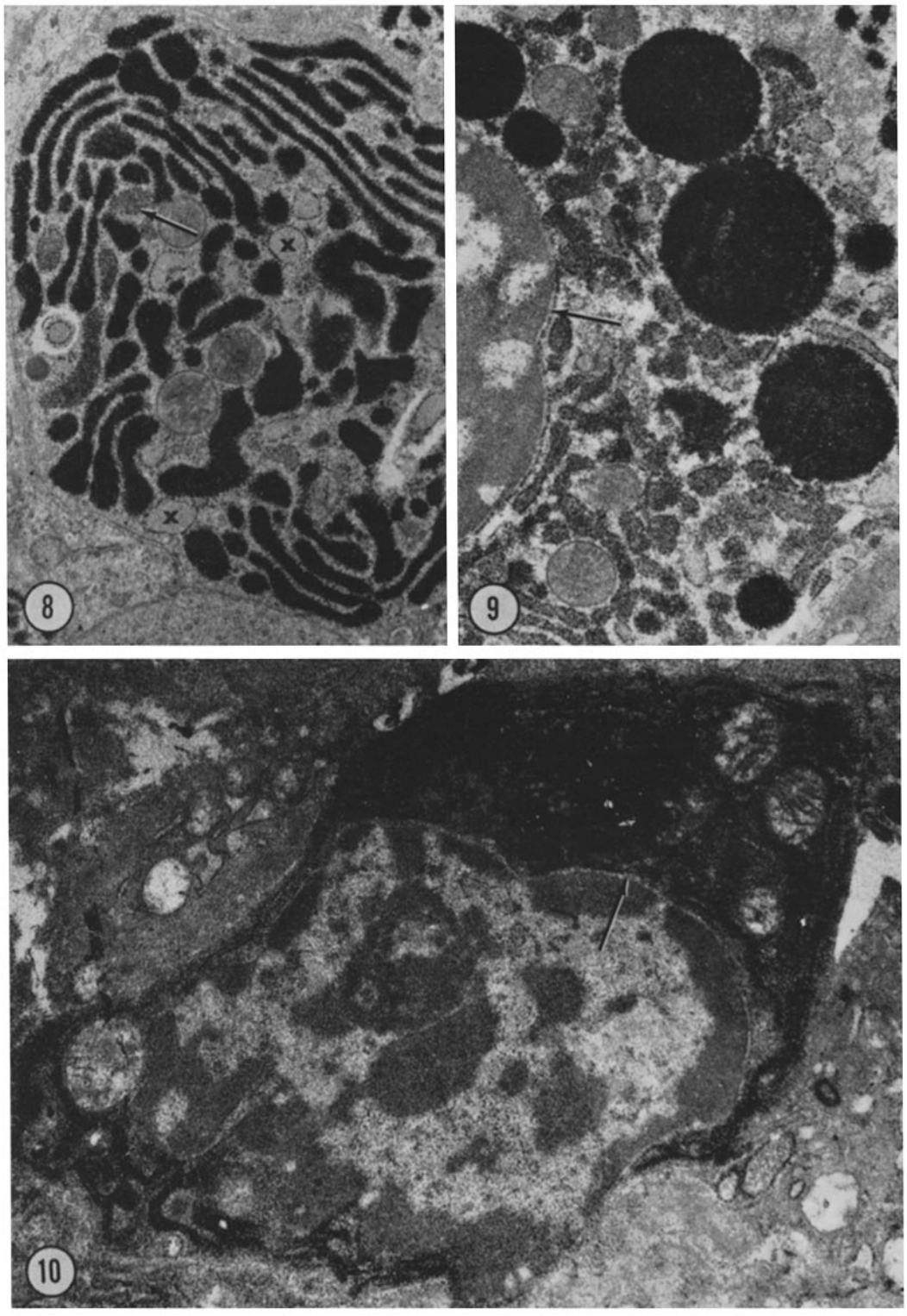
(Leduc et al.: Antiperoxidase antibody in plasma cells)

PLATE 16

FIG. 8. The cytoplasm of a mature plasma cell containing distended ergastoplasmic cisternae in which the reaction for localizing antibody is intense (uniformly black), more moderate (less dense and granular) (arrow) or absent (x). × 12,500.

FIG. 9. A portion of a mature plasma cell in which most of the antibody is concentrated into dense intracisternal granules. A small amount of reaction product is present in the rest of the more flattened cisternae, but there is none in the perinuclear space (arrow). × 15,000.

FIG. 10. A mature plasma cell in which antibody, although most concentrated in the region of the Golgi apparatus and within some ergastoplasmic cisternae, is also in the extracisternal cytoplasm. The perinuclear space (arrow) and nucleus are free of the reaction products. × 15,000.

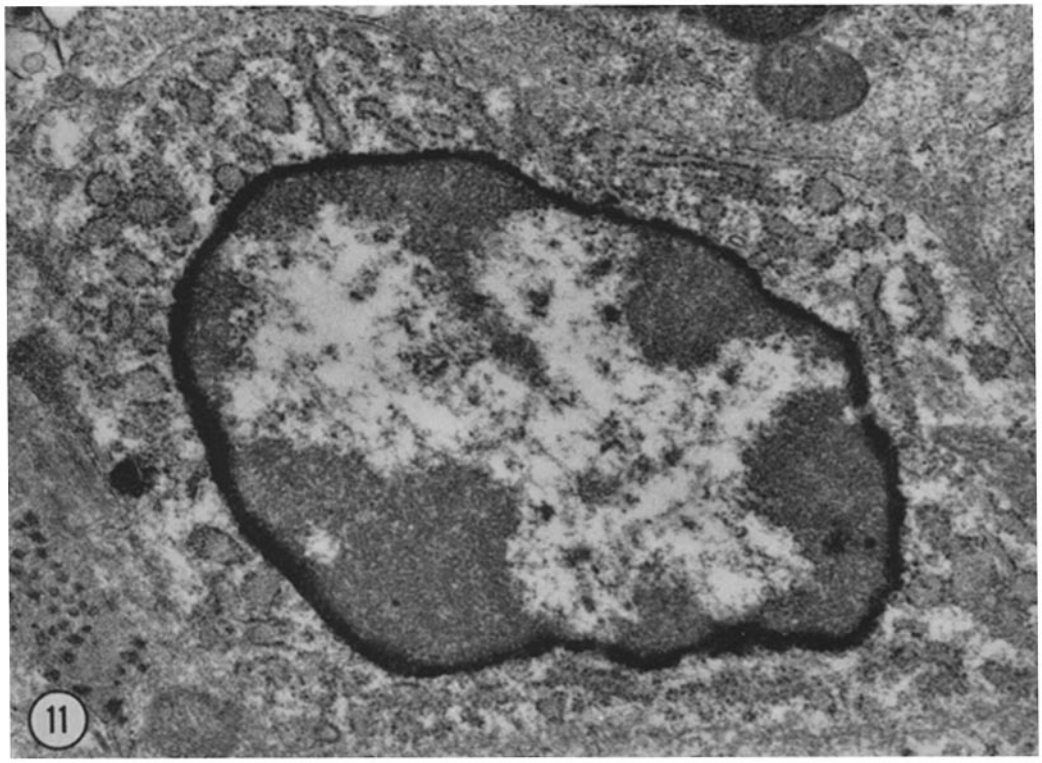


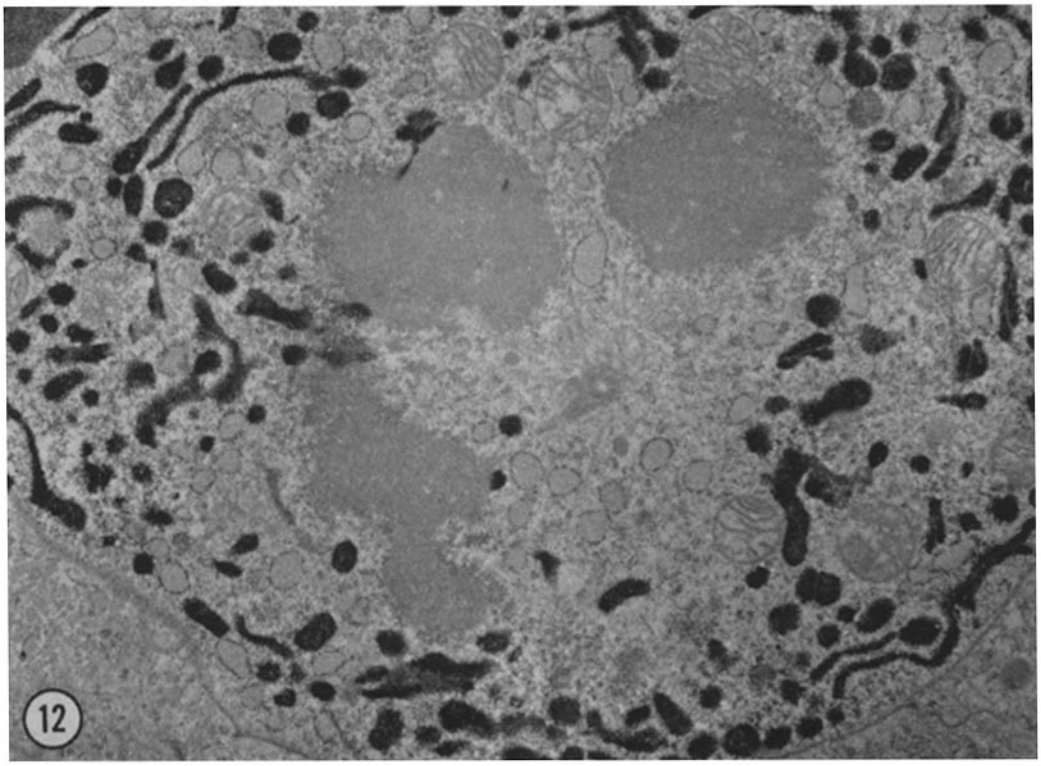
(Leduc et al.: Antiperoxidase antibody in plasma cells)

PLATE 17

Fig. 11. An immature plasma cell in which the perinuclear space is filled with antibody but the well developed ergastoplasm is antibody-free. × 25,000.

FIG. 12. An antibody-containing cell undergoing mitosis (the chromosomes are faint because this section was not counterstained). Antibody-rich and antibody-free ergastoplasmic cisternae are intermingled throughout the cytoplasm. × 14,000.





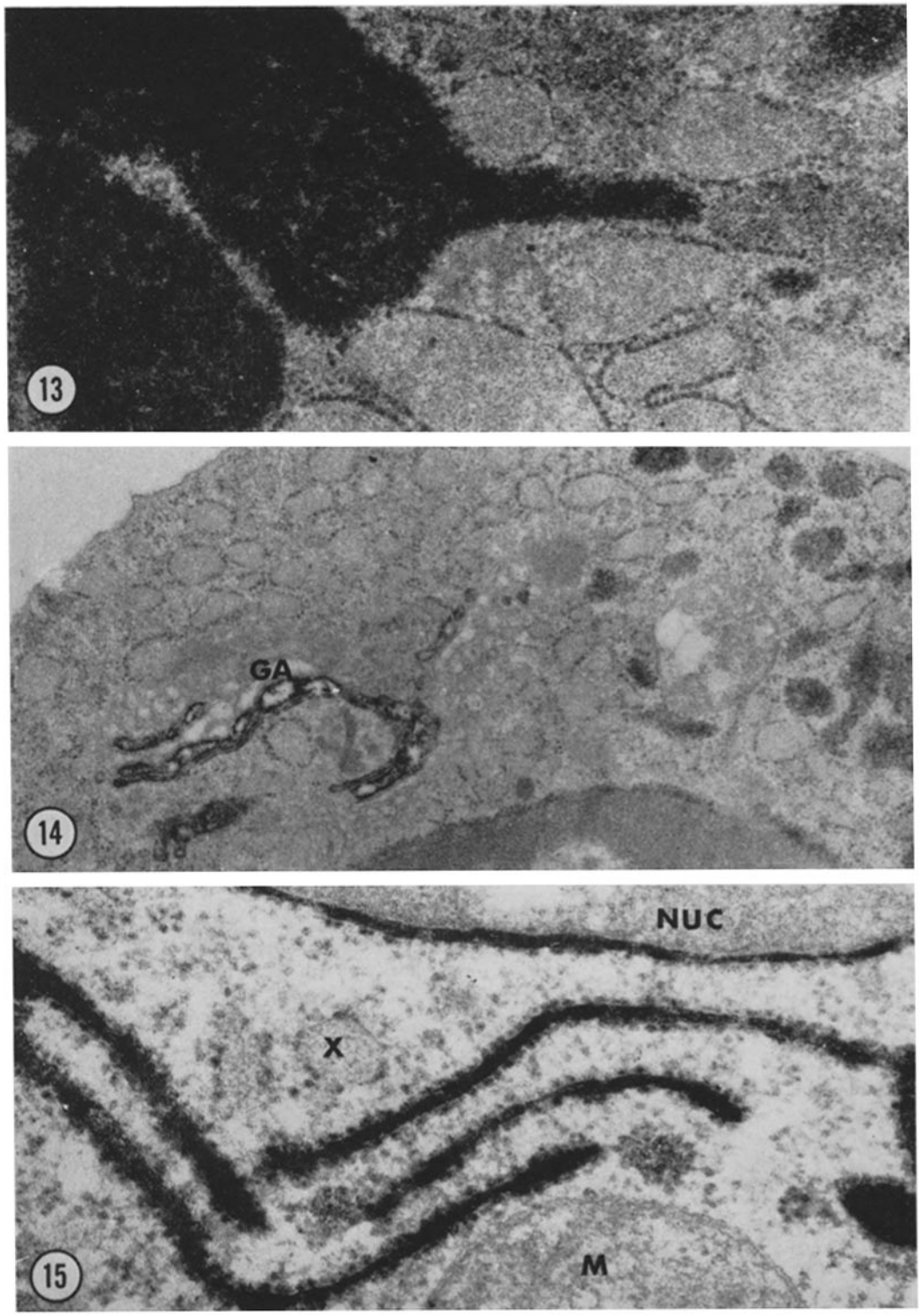
(Leduc et al.: Antiperoxidase antibody in plasma cells)

PLATE 18

FIG. 13. Cytoplasm of a mature plasma cell containing greatly distended ergastoplasmic cisternae, some of which are antibody-positive and others, antibody-free. × 40,000.

FIG. 14. An immature plasma cell in which the antibody reaction appears most intense in the lamellar portion of the Golgi apparatus (ga), moderate in the ergastoplasm at the right, and absent from the rest of the ergastoplasm and Golgi vacuoles. × 24,000.

FIG. 15. A higher magnification of a plasmablast that illustrates that the reaction product revealing the site of antibody is bounded by the ribosome-studded lamellae; (nuc) nucleus, (m) mitochondrion, (x) antibody-free cistern. × 60,000.



(Leduc et al.: Antiperoxidase antibody in plasma cells)