AN ELECTRON MICROSCOPE STUDY

OF LYMPHATIC TISSUE IN RUNT DISEASE

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ABSTRACT

The thymus, spleen, and lymph nodes were studied in runt disease induced by a graft of
intravenously injected homologous splenic cells into newborn rats and mice. Adult Long-
Evans cells (70×10^6) were injected into Sprague-Dawley rats. Adult DBA cells (7×10^6)
were injected into C57BL/6 mice. Runted rats were sacrificed at 14 to 28 days of age; mice
at 10 to 20 days. The thymic cortex is depleted of small lymphocytes. Those remaining are
severely damaged and phagocytized. Evidence of damage includes swelling of mitochondria,
myelin figure formation, margination of chromatin, and sharp angulation in nuclear con-
tour. Large numbers of macrophages are present. Epithelial-reticular cells which envelop
small cortical blood vessels are often retracted, with the result that the most peripheral
layer in the thymic-blood barrier suffers abnormally large gaps. Lymphocytes of the peri-
arterial lymphatic sheaths of spleen and of the cortex of lymph nodes are reduced in number
and damaged. Vast numbers of plasma cells and many lymphocytes are evident throughout
lymph nodes, in the periarterial lymphatic sheaths, and in the marginal zone and red pulp
of the spleen. Plasma cells are of different sizes, the larger having dilated sacs of endoplasmic
reticulum. Lymphocytes are small to medium in size. They contain, in varying quantity,
ribosomes and smooth membrane-bounded cytoplasmic vesicles approximately 350 to 500
A in diameter. Most plasma cells and lymphocytes are damaged and many of these are
phagocytized. Many lymphocytes in lymph nodes, however, show no evidence of damage.
Reticular cells and other fixed cells of the connective tissues seldom appear affected. Thus,
the major cell types reacting in runt disease are lymphocytes, plasma cells, and histiocytes
or macrophages. It appears, therefore, that both the delayed and immediate types of sen-
sitivity play a part in this disease.

INTRODUCTION

Runt disease is an experimental process induced
by the injection of immunologically competent
homologous cells into an animal, typically a new-
born, unable to destroy such cells promptly (6,
7). The injected cells constitute a graft which
reacts against the host. The newborn host, its
immunological powers emerging, in turn reacts
against the injected homologous cells. The disease
caused by these reactions is characterized by a
marked retardation in size and weight, dermatitis,
and diarrhea (6, 34). The organs associated with
immunological reactions are affected: lymph nodes
and the spleen are enlarged and the thymus is
atrophied. The lymphocytes of the white pulp,
lymph nodes, and thymus are depleted, but small
free cells proliferate in spleen and lymph nodes

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and account for the increase in size of these organs (7).

Though the proliferating cells in the spleen and lymph nodes appear to include hemocytoblasts and endothelial or reticular cells, their cell type is not clearly known. The present work is an electron microscope study of the spleen, thymus, and lymph nodes in runt disease. It has as its objectives to obtain more information on the structure of the cell types characteristic of this process and to discover other cytological and histological changes not resolvable by light microscopy.

MATERIALS AND METHODS

The Production of Runt Disease

Runt disease was produced in Sprague-Dawley rats by the intraperitoneal injection of 65 to 75 × 10^6 spleen cells from adult Long-Evans (Hooded) rats into animals less than 24 hours old. Experimental details have been described earlier by Aisenberg *et al.* (1). Runt disease was produced in C57BL/6 black mice by the intravenous injection of 4 to 8 × 10^6 adult DBA/1 spleen cells into animals less than 24 hours old. The details of these experiments have been published by Russell (30). Inbred mice were obtained from the Jackson Memorial Laboratory, Bar Harbor, Maine. All animals were observed daily, weighed three times a week, and sacrificed when moribund or when presenting evidence of advanced runt disease. In rats this occurred 14 to 28 days after the injection of spleen cells; in mice, 10 to 20 days.

The Preparation of Tissues for Electron Microscopy

Cervical lymph nodes, spleen, and thymus were removed under anesthesia or from a freshly killed animal. In some instances inguinal or abdominal nodes were also taken. The tissue was fixed in osmium tetroxide buffered to pH 7.4 in collidine, dehydrated in a graded series of ethanol, and embedded in Araldite. Details of these procedures are given elsewhere (41). The blocks were sectioned in a Porter-Blum microtome (Model M1), and sections mounted on bare 300-mesh copper grids were stained with lead and studied in a Siemens Elmiskop I.

Thicker sections (1 to 3 μ in thickness) were cut on a Porter-Blum microtome, mounted on glass slides, stained with 1 per cent toluidine blue in borate buffer, and studied by light microscopy.

OBSERVATIONS

The findings described occur in rats. Changes in mice are similar.
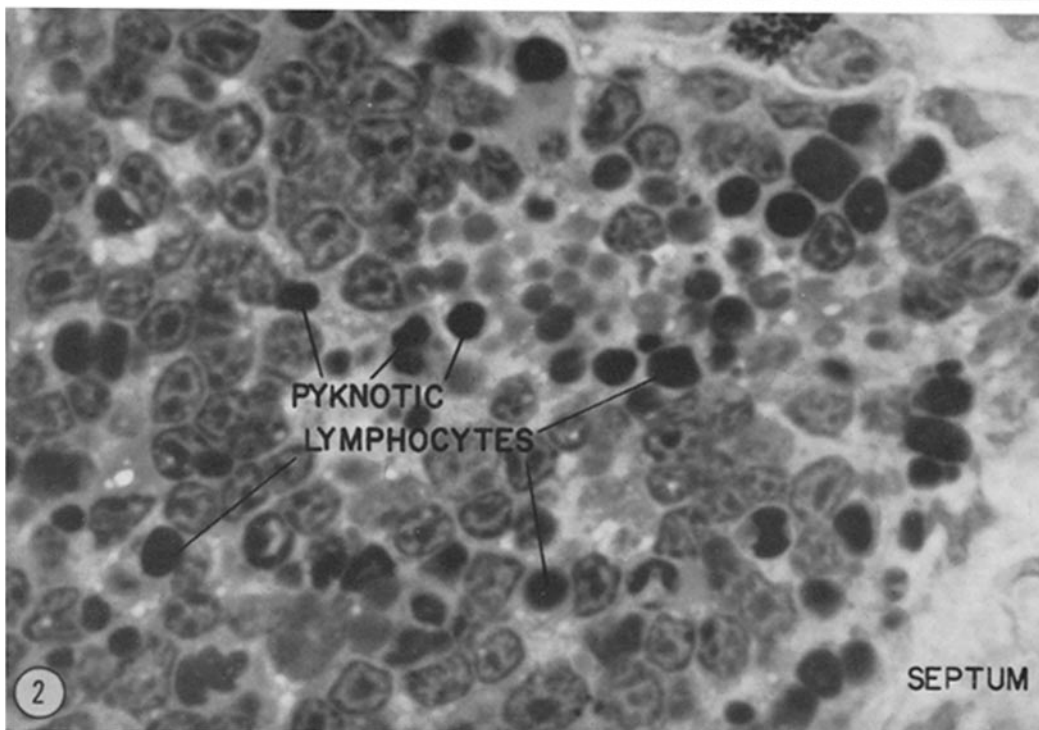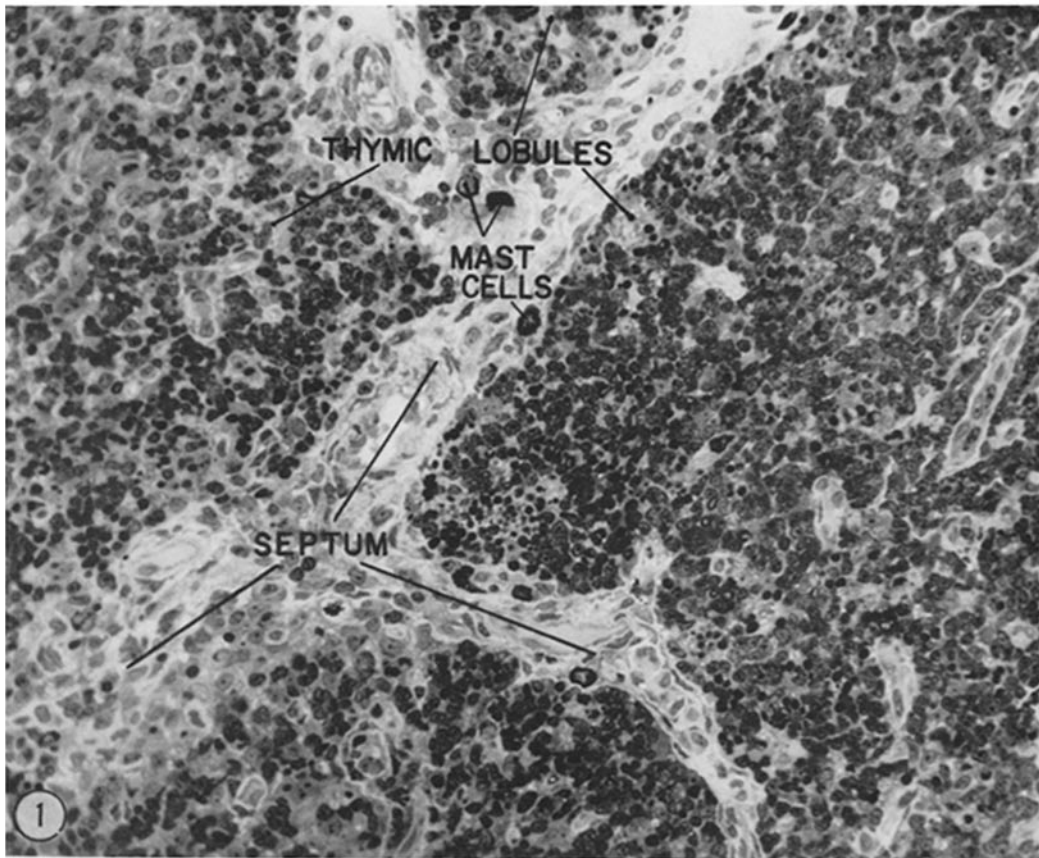
Thymus

The major thymic change is a depletion in number of small cortical lymphocytes which may be so marked that the separation of cortex and medulla is lost (Fig. 1). Moderate to large numbers of pyknotic cortical lymphocytes may be present (Fig. 2). By light microscopy these are hyaline, densely stained, basophilic cells whose nucleus may be sharply angulated (Fig. 2). Under the electron microscope these lymphocytes contain marginated chromatin, swollen mitochondria, and myelin figures. Many of them are phagocytized. They are often sectioned poorly, showing knife marks, ridges, and other artifacts not present in surrounding cells (Figs. 3 and 4). In places, apparently empty space, presumably edema fluid, is present between scattered lymphocytes.

An increased number of free macrophages similar to the macrophages or histiocytes of the connective tissues is present, primarily in the cortex (Fig. 3). These are large cells containing large folded nuclei, one or more nucleoli, voluminous cytoplasm, fat droplets, and phagocytic inclusions.

FIGURE 1   Thymus. Broad connective tissue septa separate four lobules of the organ. Several small blood vessels and mast cells lie in the septa. In the cortex of the lobules many small, uniformly dark cells are present. These are small thymic lymphocytes. See Fig. 2. Toluidine blue. × 400.

FIGURE 2   This is a higher power photomicrograph of an area from the thymus in Fig. 1. The cortex of a lobule is present, enveloped on the right by a septum. Note the homogeneous structures which may be dense, but vary in density. They also vary in size, but most of them are small. These are pyknotic thymic lymphocytes, representative examples of which are shown in the succeeding electron micrographs. The large nuclei with clumped chromatin represent the nuclei of macrophages and of epithelial-reticular cells. Toluidine blue. × 1850.

THYMIC LOBULES

MAST CELLS
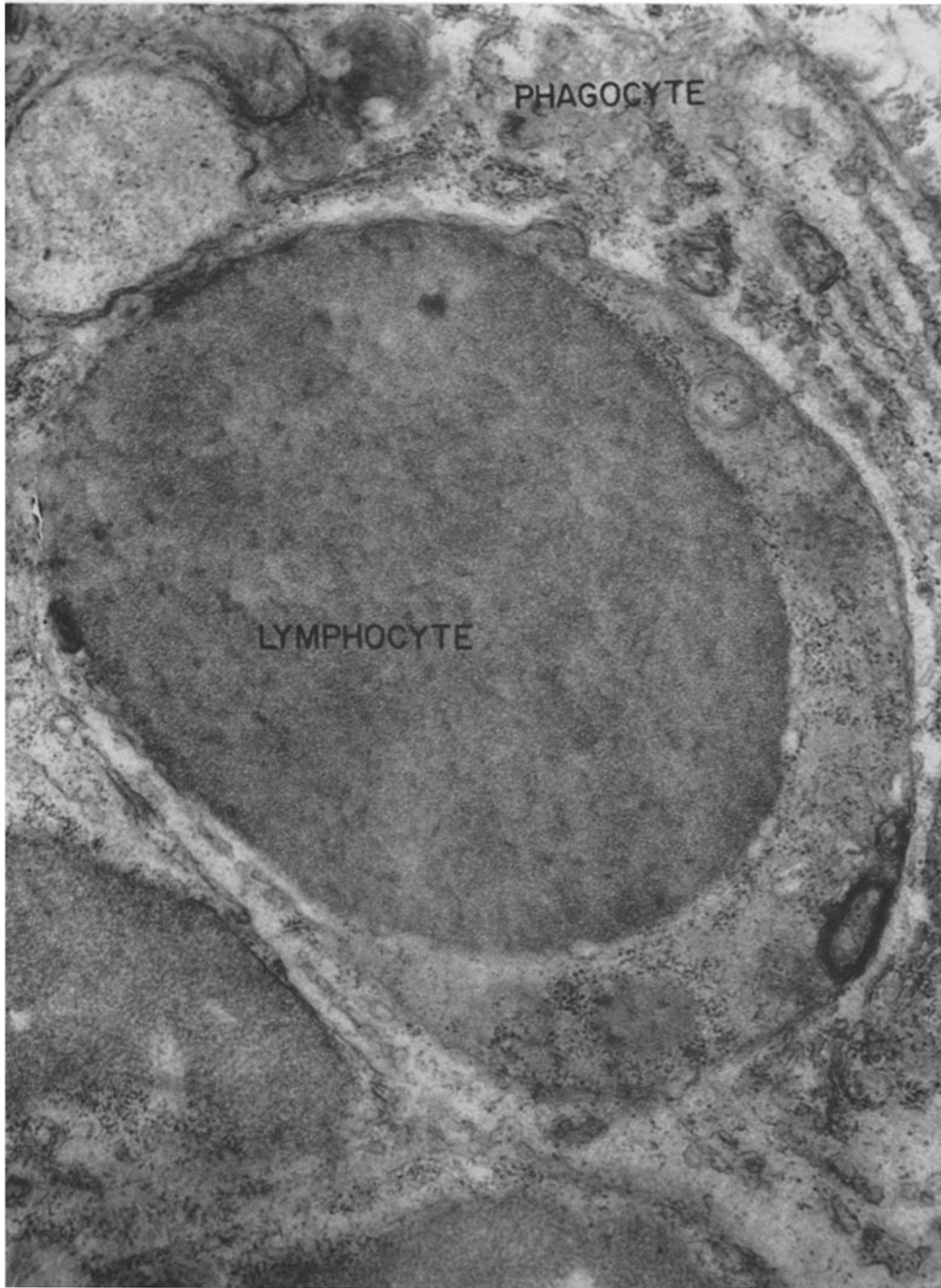
SEPTUM

PYKNOTIC LYMPHOCYTES

SEPTUM

FIGURE 3 Thymus. A phagocytized thymic lymphocyte is present in the thymic cortex. The nucleo-plasm has a fine, uniform texture, and is heaped up as a result of sectioning, an appearance typical of damaged thymic lymphocytes. The nucleus is at one pole of the cell. The cytoplasm contains some clear vacuoles, and the ribosomes, as occurs frequently in runt disease, are somewhat larger than normal. The cytoplasm of the phagocyte surrounds the phagocytized lymphocyte. $\times$ 37,000.
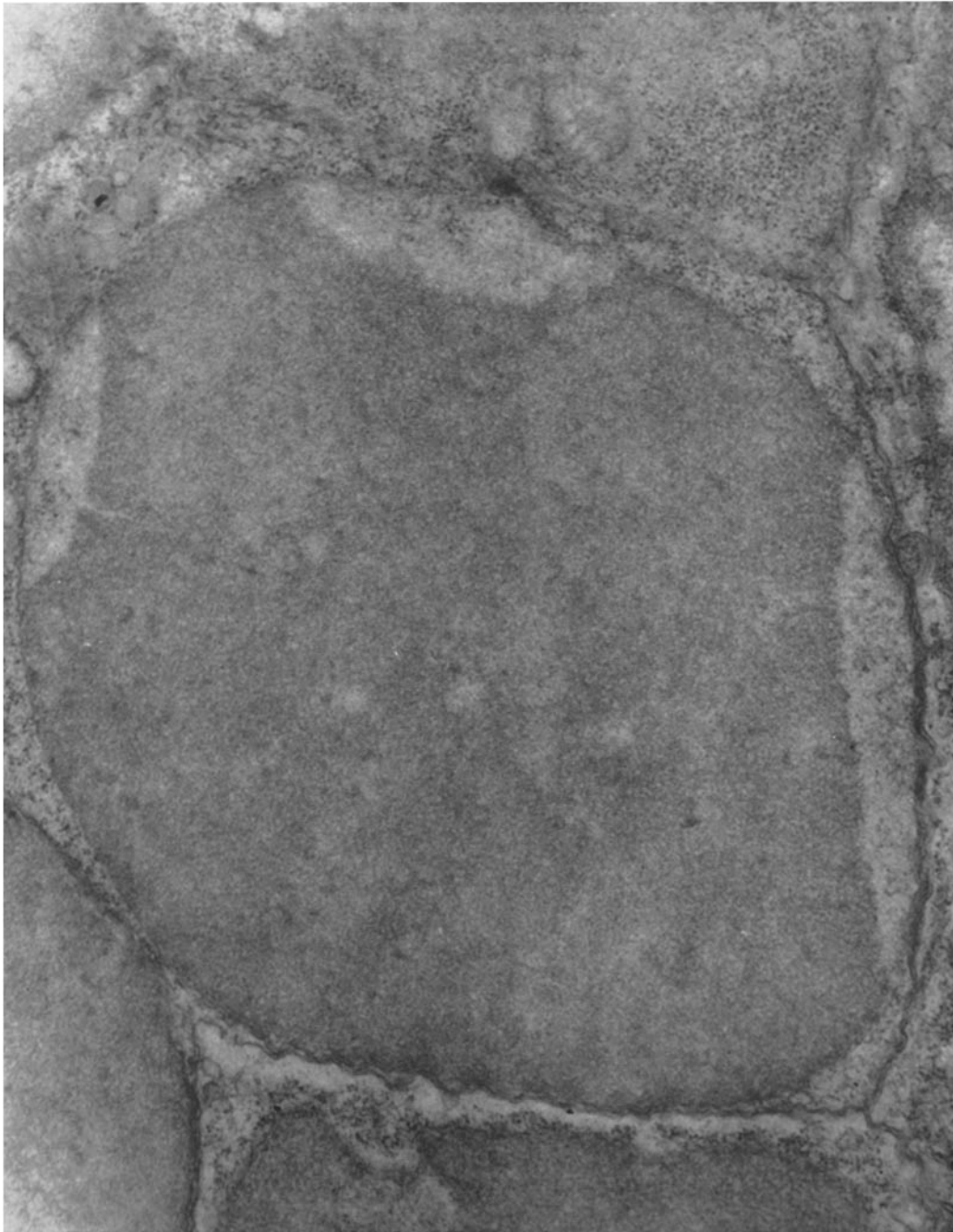
FIGURE 4 Thymus. The nucleus of this damaged lymphocyte has undergone characteristic changes. These include angulation, indistinctness of nuclear membrane, and a tendency to homogenation of chromatin. The surrounding lymphocytes, only parts of which are visible, have undergone the same change. × 40,000.

They contain moderate numbers of mitochondria, many free ribosomes, some granular endoplasmic reticulum, and a small but well developed Golgi zone (Figs. 18 and 19).

The epithelial cells of the thymus may be unaltered. But the epithelial-reticular cells, which normally are highly branched cells, may be less stellate, their processes apparently retracted. The extended processes of epithelial-reticular cells typically envelop the fine cortical blood vessels and thereby form the most peripheral adventitial element (40). These processes may be absent in runt disease, with the result that the endothelium and surrounding extracellular connective tissue are the only mural elements in capillaries (Fig. 5).

The interlobular connective tissue septa may contain plasma cells and mast cells, as in normal thymus (Fig. 1).

Lymph Nodes

Lymph nodes in runt disease consist of a rather uniform tissue, with no clear separation into cortex and medulla, provided with a moderate number of blood vessels and made up of free cells, of varying size and affinity for basic dye, scattered among the fixed reticular cells (Figs. 6 to 9).

As shown by electron microscopy three cell types account for almost all of the free cells. They are plasma cells, lymphocytes, and histiocytes. Plasma cells are very numerous. Their salient cytological characteristic as seen by electron microscopy is an endoplasmic reticulum studded with ribosomes, the so-called rough-surfaced endoplasmic reticulum (Figs. 10 to 15). In some sections the nucleus appears central (Fig. 14). The sacs of endoplasmic reticulum contain a moderately dense granular material and are slightly distended. In places in the cytoplasm the endoplasmic reticulum may be distended to form dilated sacs (Figs. 10 to 12). The cells contain few mitochondria. Many of the smaller plasma cells are damaged and presumably dead: they contain myelin figures; their mitochondria are swollen, without cristal detail; their nucleus is angulated, and their chromatin sharply marginated (Figs. 13 to 15). Such cells are often phagocytized (Fig. 14). Ribosomes associated with the endoplasmic reticulum clearly account for the demonstrable basophilia of these cells in thick sections (stained with toluidine blue) successive to thin ones. But many small free cells, whose plasma cell nature is evident by electron microscopy, may not be definitively identified as plasma

cells by light microscopy. They may lack the marked nuclear polarity, cartwheel pattern of chromatin, and large cytocentrum typical of plasma cells. They appear simply as free cells whose outstanding feature is marked cytoplasmic basophilia (Figs. 7 to 9). They may contain cytoplasmic vacuoles which correspond to the dilated sacs of endoplasmic reticulum discernible by electron microscopy.

Large plasma cells have a somewhat larger nucleus and more voluminous cytoplasm than smaller ones. But in large cells the sacs of the endoplasmic reticulum are often widened, in contrast to the flattened sacs in small plasma cells (Fig. 15). The sacs contain an amorphous granular material of the same sort found in the smaller cells. The larger cells are less basophilic than smaller cells, presumably because their ribosomes are more widely dispersed. Indeed, their cytoplasm may be tigroid, probably owing to the separation of zones of ribosomes by the material filling the sacs of the endoplasmic reticulum. Many of the larger cells are damaged or dead, but the proportion is considerably less than for the smaller plasma cells.

The larger cells, which are clearly of the plasma cell type as observed by electron microscopy, are, by light microscopy, large moderately basophilic free cells whose nature cannot be ascertained. They may even be taken for substantive fixed elements because the nucleus may have the same appearance as that of a fibroblast or of a reticular cell, and the cytoplasm may be drawn out and so closely pressed upon extracellular connective tissue as to be unresolvable from it by light microscopy.

Lymphocytes of large and medium size are present in small to moderate number among the plasma cells. These differ from plasma cells primarily in the absence of an endoplasmic reticulum. They contain ribosomes in moderate amounts scattered through the cytoplasm (Figs. 16 and 17). No polyribosomal pattern is evident. They may also contain very small vesicles, about 300 to 500 A in diameter (Fig. 16). Their Golgi zone is small and they contain few to moderate numbers of mitochondria. Their chromatin may be clumped but lacks the cartwheel distribution present in small plasma cells. Like plasma cells, many lymphocytes may be damaged and phagocytized (Fig. 17). But many appear normal (Fig. 18) and some may be observed in mitosis. By light microscopy lymphocytes are moderately to slightly
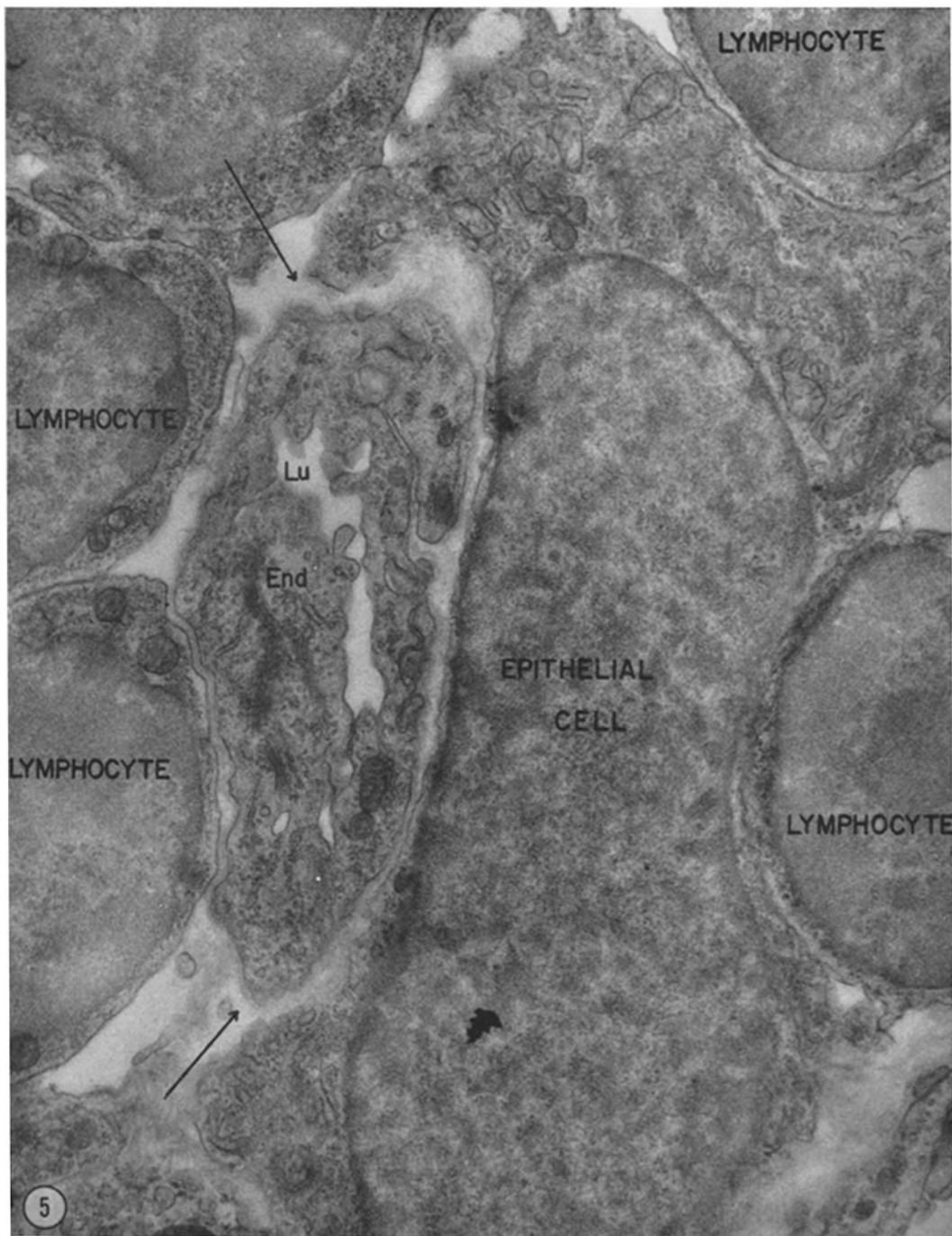
FIGURE 5 Thymus. A cortical capillary is present in the left center of the field (lumen labeled *Lu*). The endothelium (*End*) is surrounded by a variable thick layer of extracellular connective tissue, and this in turn is partly enveloped by a large epithelial cell (labeled). Normally the envelopment of a capillary by an epithelial-reticular cell is more nearly complete. Here the cell's cytoplasmic processes appear to have been retracted, enclosing the vessel only to the points of the arrows. Portions of lymphocytes are also present here. On the right the epithelial cell intervenes between the capillary and the lymphocytes. On the left the lymphocytes lie directly against the basement membrane of the capillary. × 25,500.

basophilic free cells which may be indistinguishable from the larger plasma cells described above.

Histiocytes are present throughout lymph nodes. They are 15 to 30 micra in diameter. They have large folded nuclei, one or more nucleoli, and voluminous cytoplasm. The cytoplasm contains fat droplets, moderate numbers of mitochondria, many free ribosomes, some granular endoplasmic reticulum and some lysosomes, occasional fibers, and small but well developed Golgi zones (Figs. 17 to 19). An ectoplasmic zone, lobulated by indentations of plasma membrane, is often visible. These cells may be phagocytic, and as such may be recognized as *macrophages*. A considerable amount of phagocytosis is present in runt disease, and the most frequently phagocytized structures are plasma cells. A surprisingly high proportion of these phagocytized cells are found virtually intact within the phagocyte.

Spleen

The periarterial lymphatic sheath is normally evident and may be well developed even in infant animals. It may be somewhat depleted of lymphocytes in runt disease, and its lymphocytes damaged. In many instances, in runt disease, the lymphocytes of the periarterial lymphatic sheath are replaced by plasma cells (Figs. 20 and 21). These are large and small cells. Many appear damaged but a great number do not. Plasma cells are abundant in the marginal zone and red pulp (Figs. 22 and 23). As in lymph nodes and the periarterial lymphatic sheath, a very large proportion of small plasma cells are damaged and, in many instances, phagocytized.

Lymphocytes are present among the plasma cells in the periarterial lymphatic sheaths, marginal zone, and red pulp. Lymphocytes appear to be fewer than in the lymph nodes, and a larger proportion shows signs of damage than in lymph nodes.

Macrophages are present throughout the spleen, notably in red pulp (Fig. 23). In addition to phagocytosis of lymphocytes and plasma cells, a moderate number of erythrocytes are phagocytized and many macrophages contain pigments derived from hemoglobin.

Many erythroblasts are present in marginal zone and red pulp (Figs. 22 and 23). A few are present in the white pulp. A few erythroblasts display mitochondrial swelling, but most appear undamaged.

The reticular cells and other fixed elements are but slightly affected. Some reticular cells phagocytize plasma cells and lymphocytes.

DISCUSSION

Plasma cells and lymphocytes, and histiocytes or macrophages, are the major responsive cell types in runt disease.

The plasma cell, evidently suited to the synthesis and secretion of protein as indicated by the presence of ribosomes and by the abundance of cytoplasmic membranes (3, 10, 13, 42), is of the class of cells alike in the development of cytologic organelles, which includes the acinar cells of pancreas and salivary gland, the chief cells of the stomach, and the fibroblast, each of which synthesizes and secretes protein. Plasma cells produce and secrete antibody and are the cell types asso-

FIGURE 6 Lymph node. Cortex is below and medulla above. There is depletion of lymphocytes, and the boundary between cortex and medulla is not distinct. Small dark cells are scattered about without preferential distribution. See Figs. 7 to 9. × 400.

FIGURES 7 TO 9 Lymph node. These fields are taken from lymph nodes similar to that in Fig. 6. Several cell types are present. A group of free cells are of moderate size, and have darkly stained nuclei with clumped chromatin and deeply basophilic cytoplasm (exemplified by cells labeled *1* in Fig. 7). The cytoplasm contains clear zones (*CZ*) or vacuoles which are much less densely stained (Figs. 8 and 9). The cytoplasm may be angular and polarized in relation to the nucleus. Other cells have features similar to those in the first group, differing primarily in that the cytoplasm is less densely stained (*e.g.* cells labeled *2* in Fig. 7). Several cells in these fields are apparently severely damaged or dead, as indicated by dense round nuclei without evident texture (Figs. 7 and 9). Some nuclei contain vacuoles and some cells are dividing (cell *3* in Fig. 7 is in metaphase).

Most of the free cells in these fields are of the above types. Toluidine blue. × 1850.
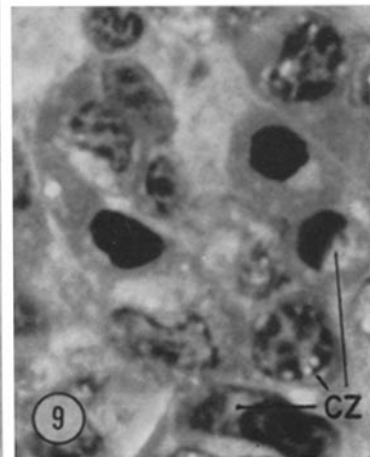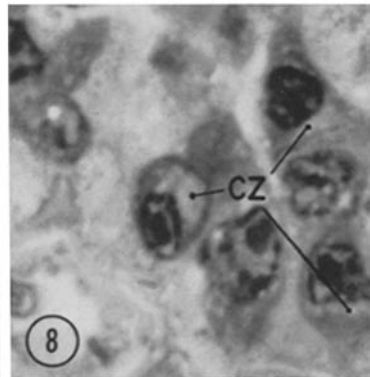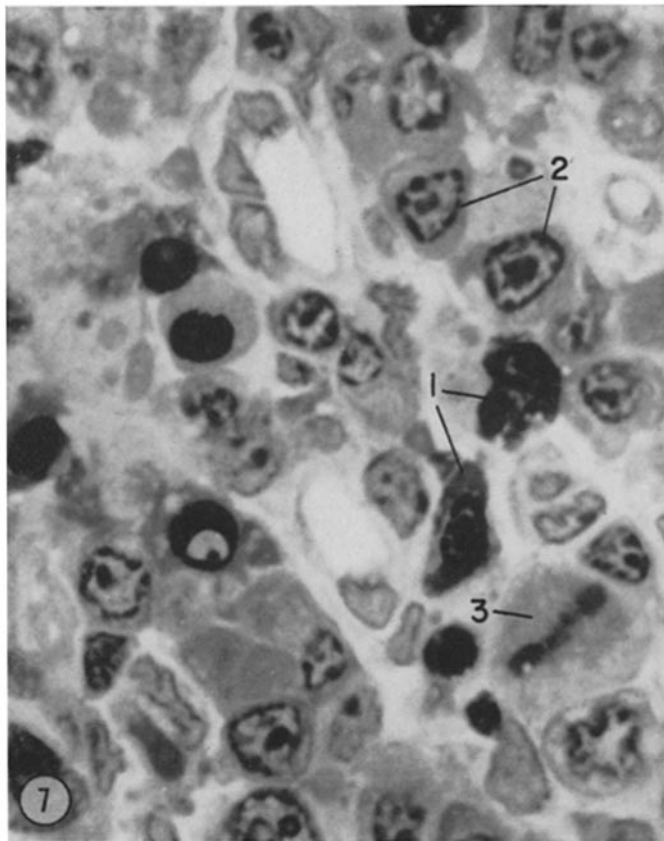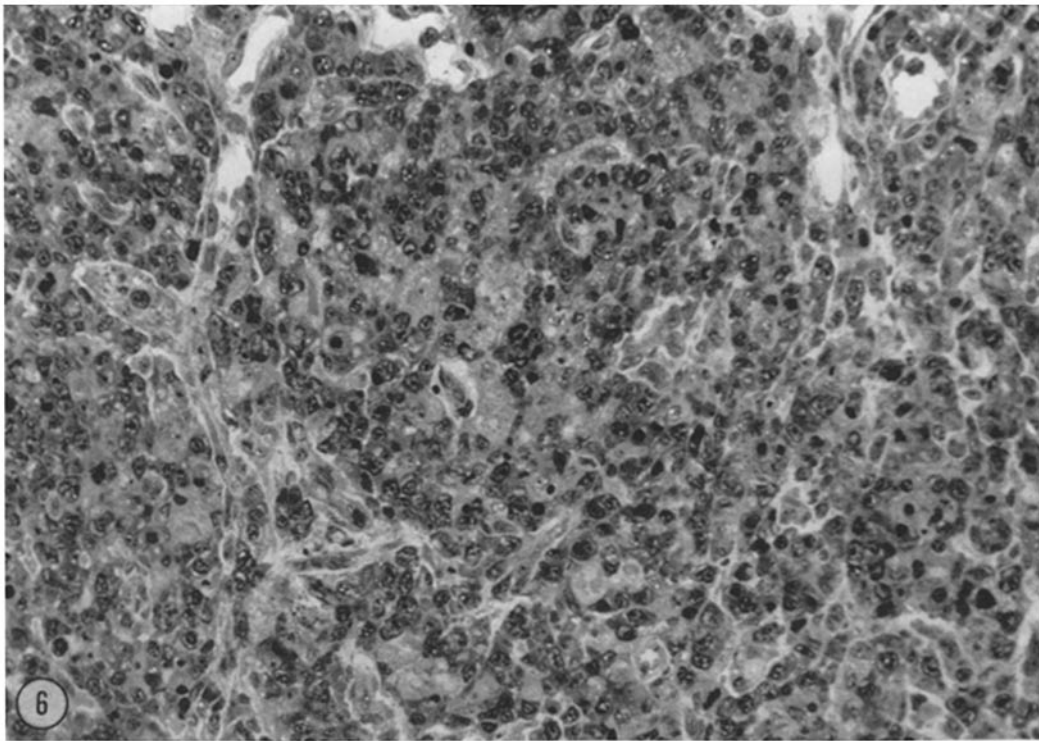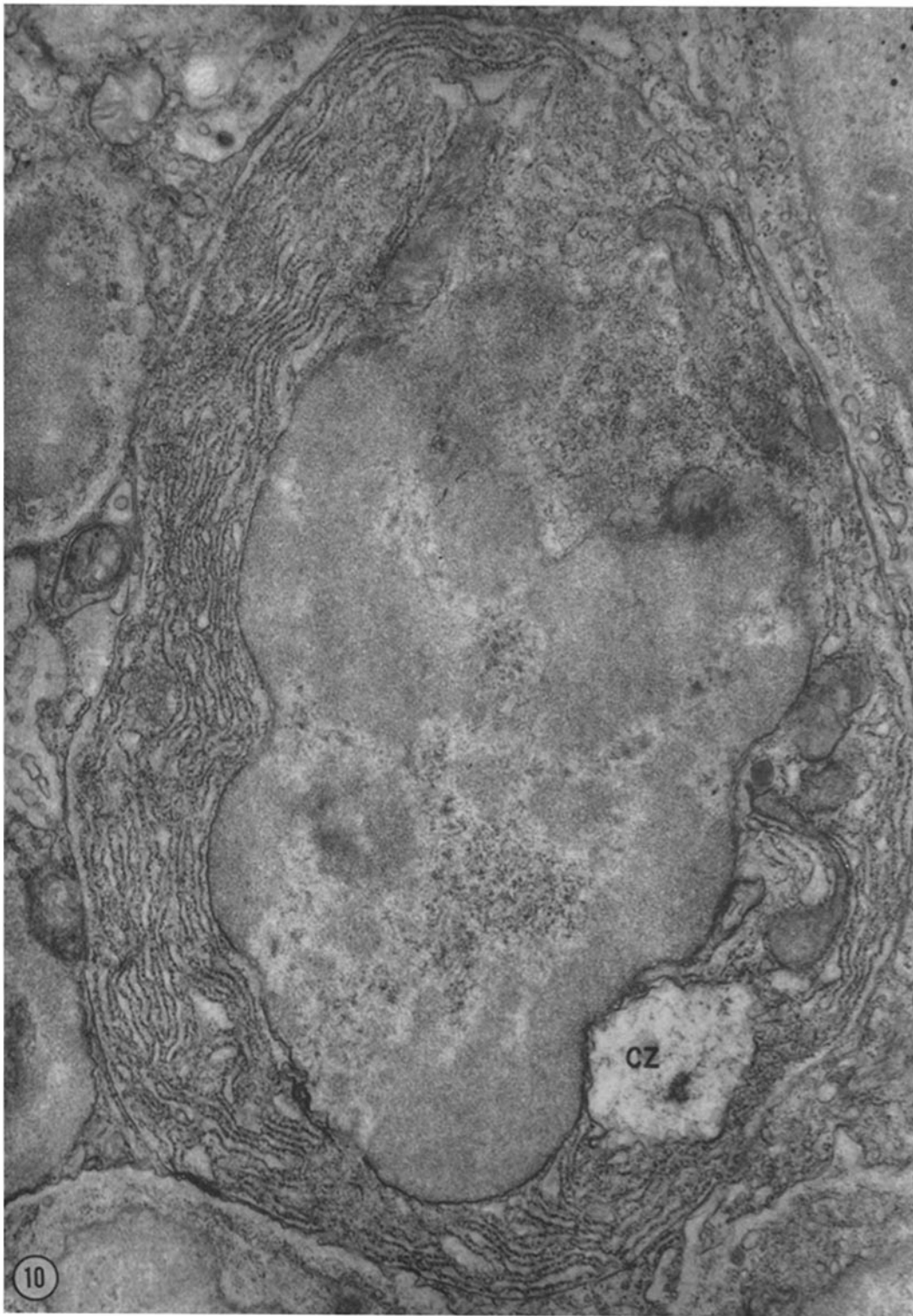
FIGURE 10   Lymph node. A plasma cell occupies most of the field. Sections of several mitochondria are present. Note the dilated portion of the endoplasmic reticulum in the right lower part of the cytoplasm (*CZ*). This corresponds to the clear areas in the cytoplasm in the light micrographs of the preceding plates. Nuclear eccentricity is not marked in this section. × 40,000.

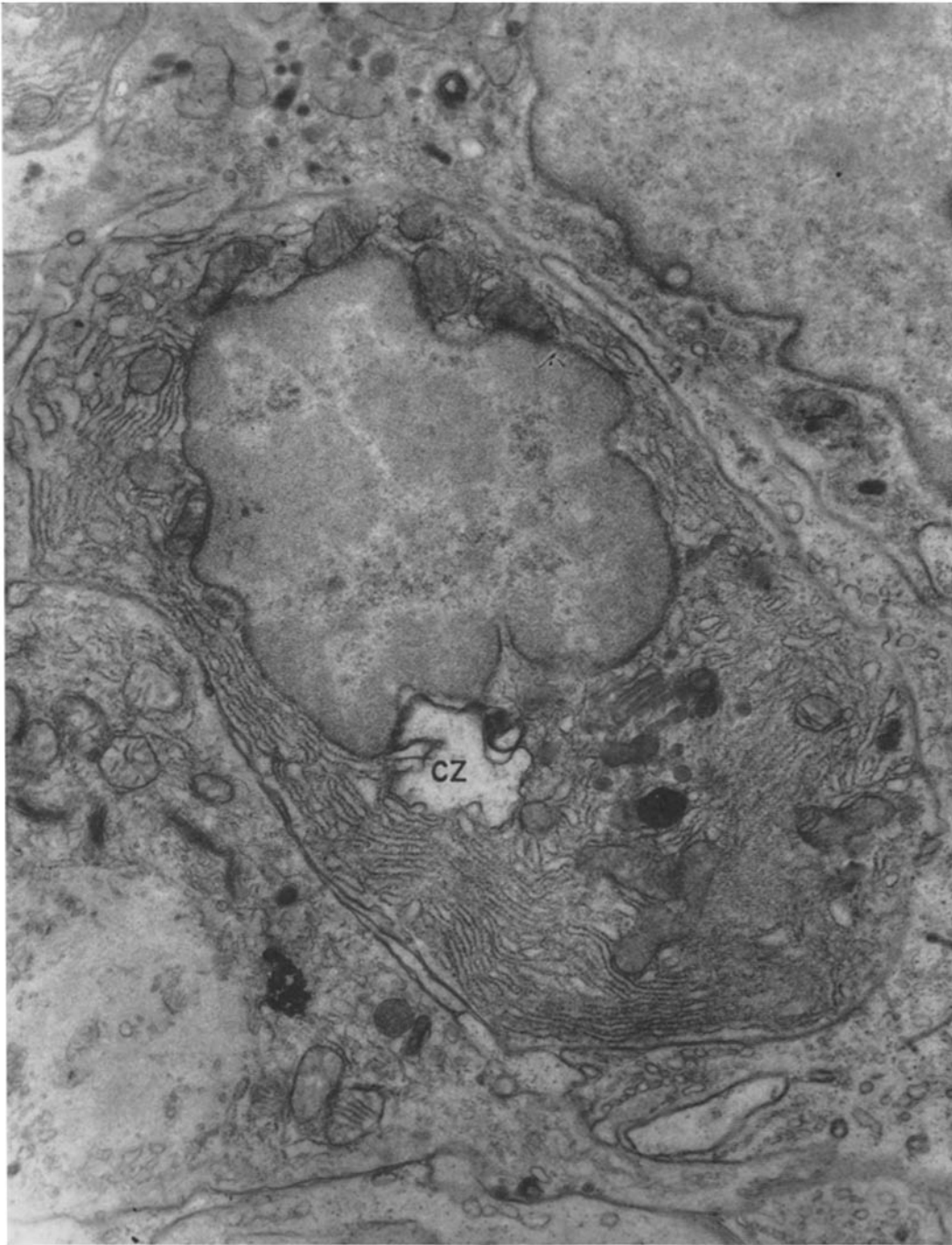FIGURE 11 Lymph node. This plasma cell shows the nuclear eccentricity characteristic of its class. Note the dilated sac of endoplasmic reticulum (*CZ*). Mitochondria and lysosomes are present. × 40,000.
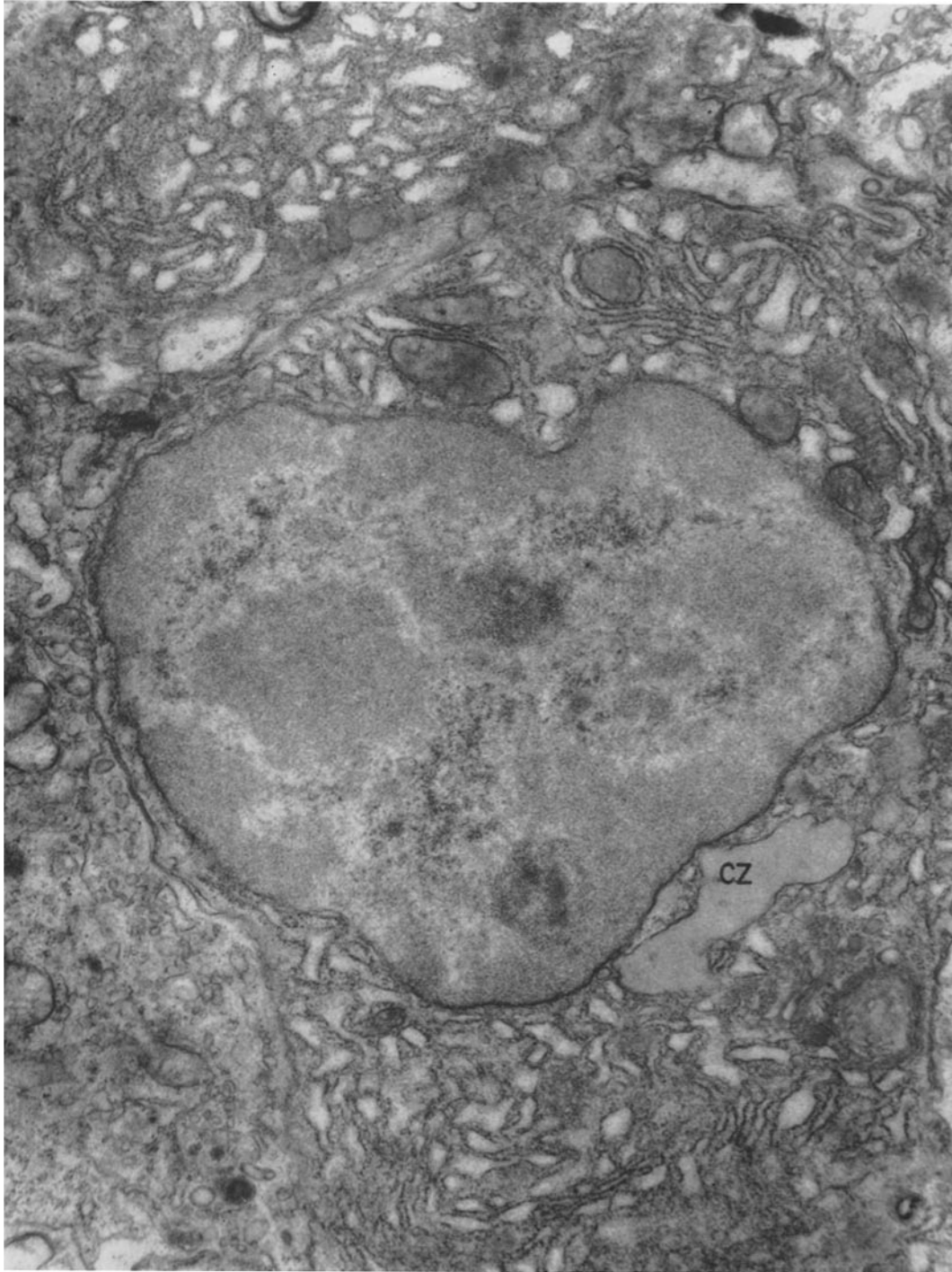
FIGURE 12 Lymph node. This large, apparently viable plasma cell contains relatively open sacs of endoplasmic reticulum. It would correspond to one of the less deeply basophilic free cells in light microscope preparations. A dilated sac of endoplasmic reticulum (*CZ*) would correspond to one of the clear cytoplasmic zones in light microscopic preparations (cells labeled *2* in Fig. 7). Above and to the left, and below and to the right, parts of the cytoplasm of other plasma cells are seen which give an impression of the abundance of these cells in this node. × 40,000.

FIGURE 13 Lymph node. This plasma cell is markedly damaged; the most striking evidence is the highly irregular contour of the cytoplasm, myelin figure formation, and rarefied cytoplasmic zones. Additional segments of plasma cells are at the left margin and in the lower left corner. × 40,000.
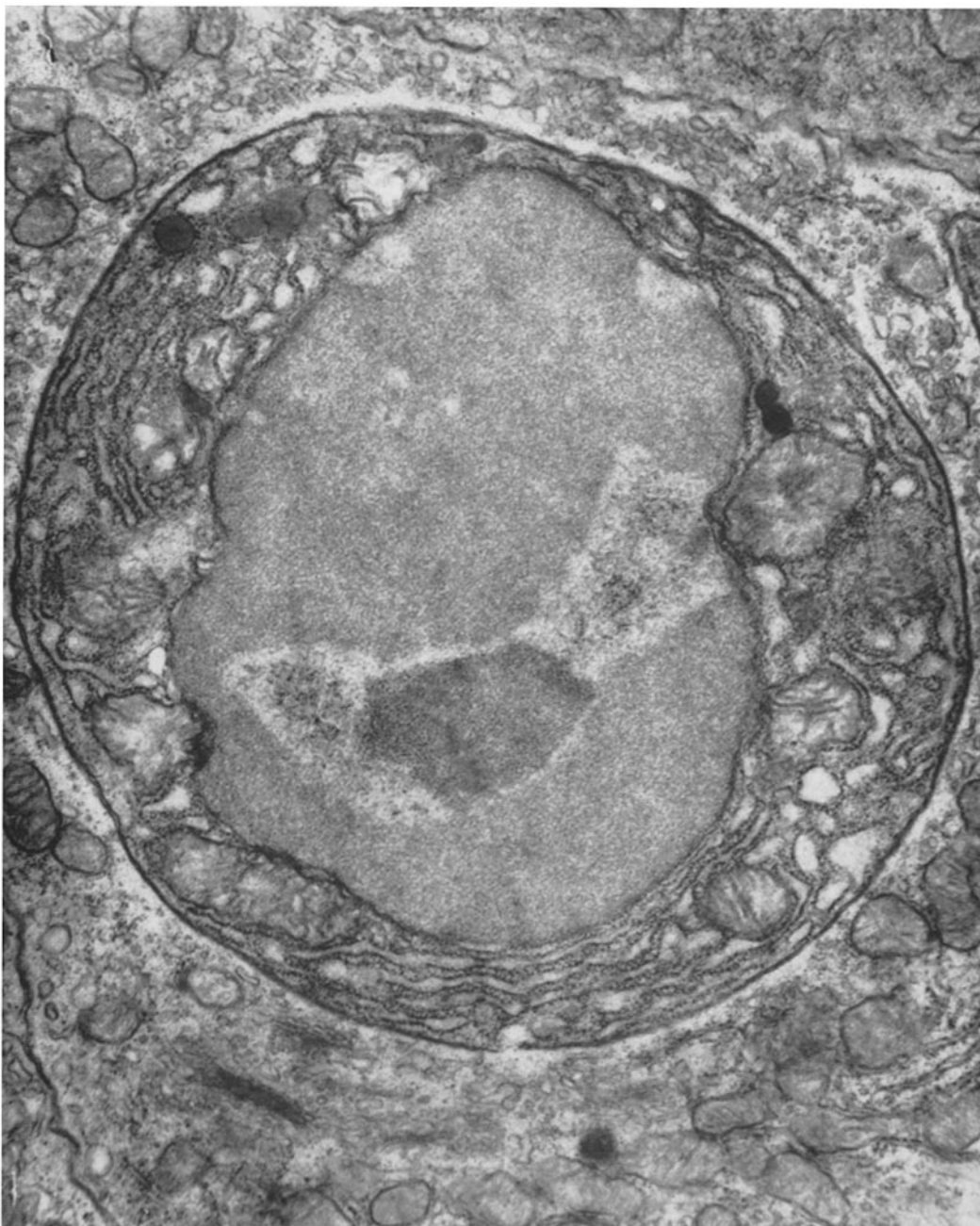
FIGURE 14 Lymph node. A phagocytized plasma cell is present here. The margination of chromatin and swelling of mitochondria are evidence of damage. The nucleus is not eccentric, and by light microscopy the plasma cell nature of this cell would not be evident. Note the abundant mitochondria and the packets of membrane in the phagocyte. × 40,000.
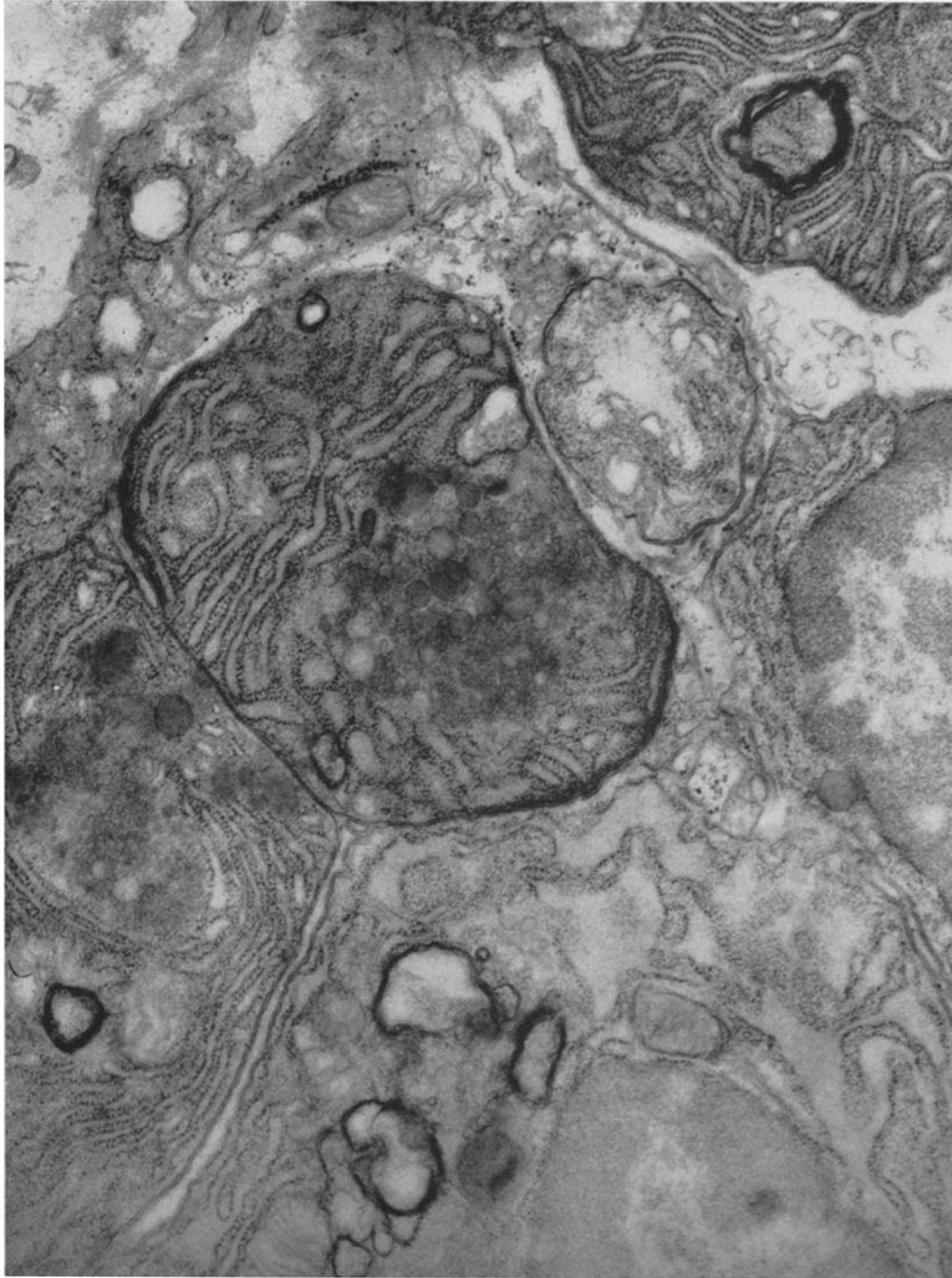
Figure 15 Lymph node. Parts of several plasma cells varying in the density of the granular endoplasmic reticulum are present here. The myelin figures and the increased density of many plasma membranes, probably a prelude to myelin figure formation, suggest that most of the plasma cells are damaged. × 40,000.
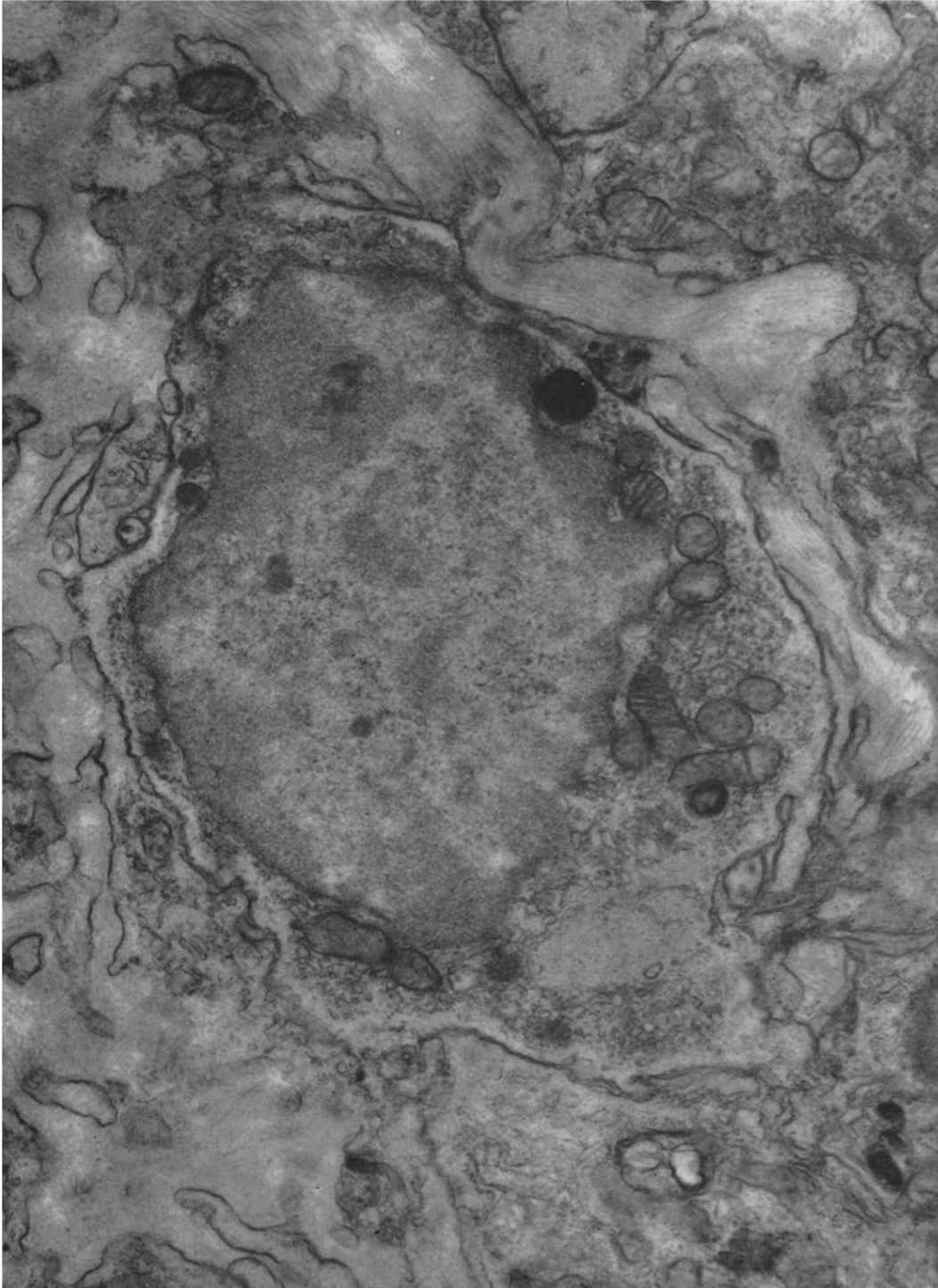
FIGURE 16   Lymph node. This small lymphocyte contains a number of mitochondria and several lysosomes. Many small vesicles and a moderate amount of ribonucleoprotein are present in the cytoplasm. In places the ribosomes are grouped in clusters of four or five, but no polyribosomal pattern is consistently evident. × 40,000.
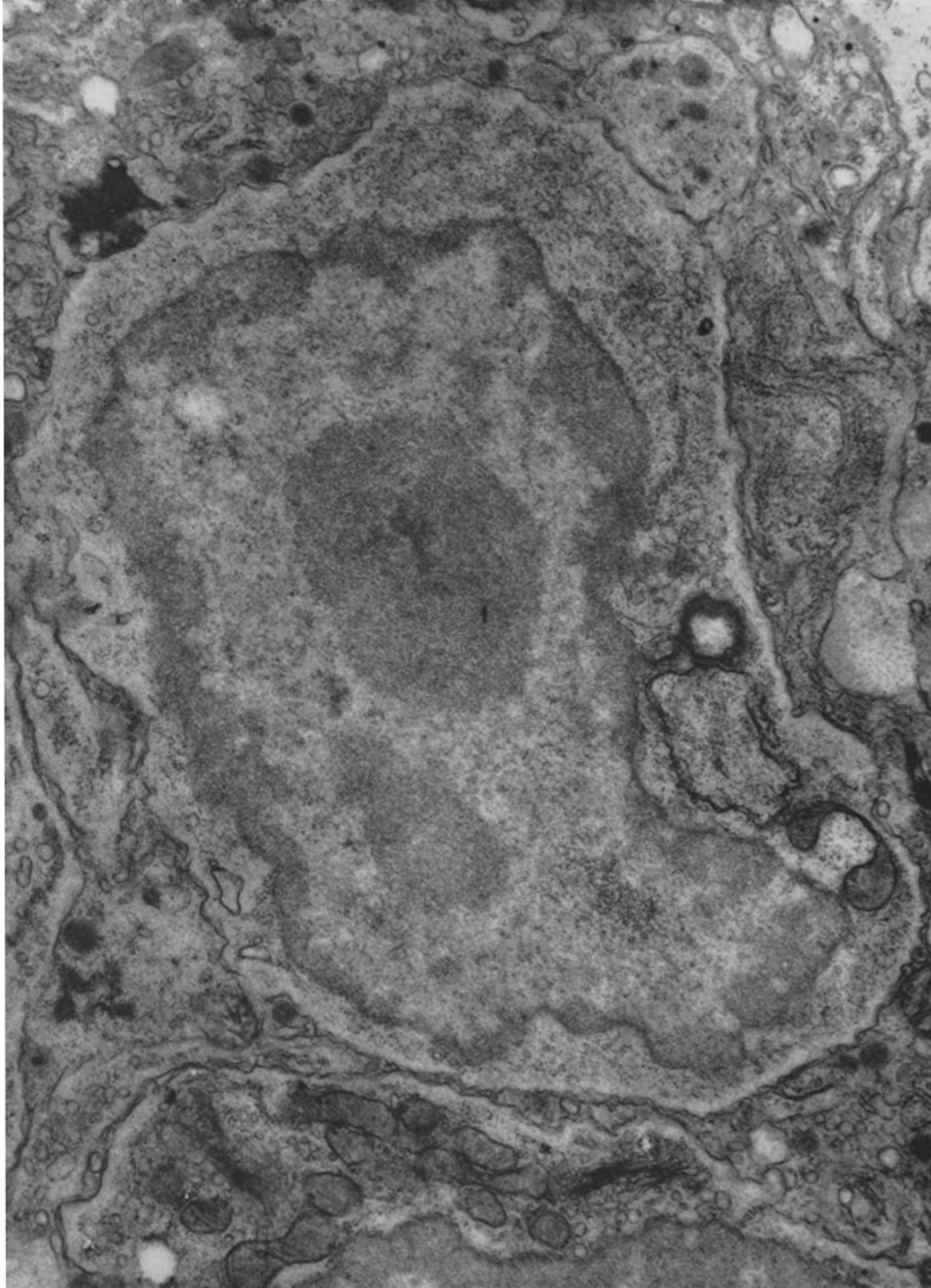
FIGURE 17 Lymph node. This lymphocyte is of medium to large size. It is surrounded by several histiocytes. The cytoplasm at the upper margin is highly characteristic of histiocytes containing a lipid droplet and some lysosomes. See Figs. 18 and 19. × 40,000.

ciated with the immediate type of hypersensitivity (11, 12, 25, 29). Lymphocytes lack a well developed system of cytoplasmic membranes, having a small Golgi apparatus and small but variable amounts of endoplasmic reticulum (3, 4, 5, 18). Lymphocytes, which appear capable of synthesizing protein which is not secreted but remains intracellular, are of the class of cells which includes erythroblasts and cells of the epidermis. Lymphocytes are associated with delayed hypersensitivity. They appear to play their part by moving directly to the allogenic source (35). Thus Weiner *et al.* (36) have described "graft rejection cells," which conform to the structure of lymphocytes, in the bed of a homograft undergoing rejection, and André *et al.* (2) have described hemocytoblasts, which appear to be plasma cells, in the lymph node draining the region of a homograft.

Lymphocytes and plasma cells are among the small free cells of the hematopoietic and connective tissues whose cytoplasm contains ribosomes and which thereby have an affinity for basic dyes such as methylene blue, pyronin, and toluidine blue. The problem of establishing the potentialities for differentiation and functions of such free basophilic cells has occupied morphologists since the time of Ehrlich. Among the criteria upon which a basophilic cell has been identified and its capacities for differentiation judged are cell size, ratio of volumes of nucleus and cytoplasm, nuclear shape, texture of chromatin, number and size of nucleoli, cytoplasmic vacuoles, depth of basophilia, morphologic and tinctorial relationships with known cell types, and association with known cell types (37). Sharply divergent conclusions have been drawn, polarized as the monophyletic and polyphyletic schools of hematopoiesis. The term *hemocytoblast* was introduced and used by monophyletists to indicate a free basophilic cell considered capable of differentiating into any of the blood cells and into some of the connective tissue cells. The term has more recently been used by immunologists to designate a cell capable of differentiating into lymphocytes and plasma cells.

Now if a free cell contains a definitive structure which may be recognized by an appropriate technique (specific cytoplasmic granulation or hemoglobin stained by acid or basic dye, antibody coupled with antigen-fluorescent antibody, etc.), confidence in defining that cell is warranted. In the present study, the electron microscope, by resolving the rough-surfaced endoplasmic reticu-

lum, has permitted the identification as plasma cells of cells which by light microscopy are simply basophilic. Unless it contains such a definitive structure, the identity of a cell or group of cells may not be possible. A basophilic cell without any morphological evidence of differentiation, moreover, may not be a hemocytoblast, but be fixed and limited in its potentialities for differentiation. The association of such basophilic cells with cells whose type is definite may suggest that the former differentiates into the latter type. But such evidence is not definitive because free hematopoietic and connective tissue cells, unlike the fixed cells of the epidermis, for example, which bear a constant histological relationship to one another, have shifting topological relationships. The techniques of tagging cells by radioactive isotopes or by chromosomal marker, and following their life cycle in their histological context by autoradiography in the former case, and by mitotic analysis in the latter, appear to offer the only satisfactory approaches to establishing the nature, fate, and capacities for differentiation of cells which are not separable by other morphological criteria. The use of the term *hemocytoblast* or *immunoblast*, which implies a known capacity for differentiation, would appear unwarranted if based simply on cell structure and not on the techniques of tagging. It is of interest that lymphocytes, separable primarily by size in preparations stained by acid, basic, and neutral dyes, may be recognized as a variate group of cells by other techniques. By electron microscopy lymphocytes may differ morphologically in possessing varying amounts of endoplasmic reticulum (22), in the development of nucleoli, and in having varying concentrations and aggregations of ribosomes (41). They differ in their immunologic competence, as determined by the capacity to induce homologous disease in F_1 hybrids (17), and in their longevity, as determined by autoradiography after tagging with tritiated thymidine (15).

The widespread death of cells in runt disease is a remarkable matter. Plasma cells and lymphocytes in spleen and lymph nodes and lymphocytes in the thymic cortex are damaged, dead, and often phagocytized. It is of interest that the cellular damage in runt disease is largely confined to plasma cells and lymphocytes. These cells therefore not only mediate many of the effects of hyper-
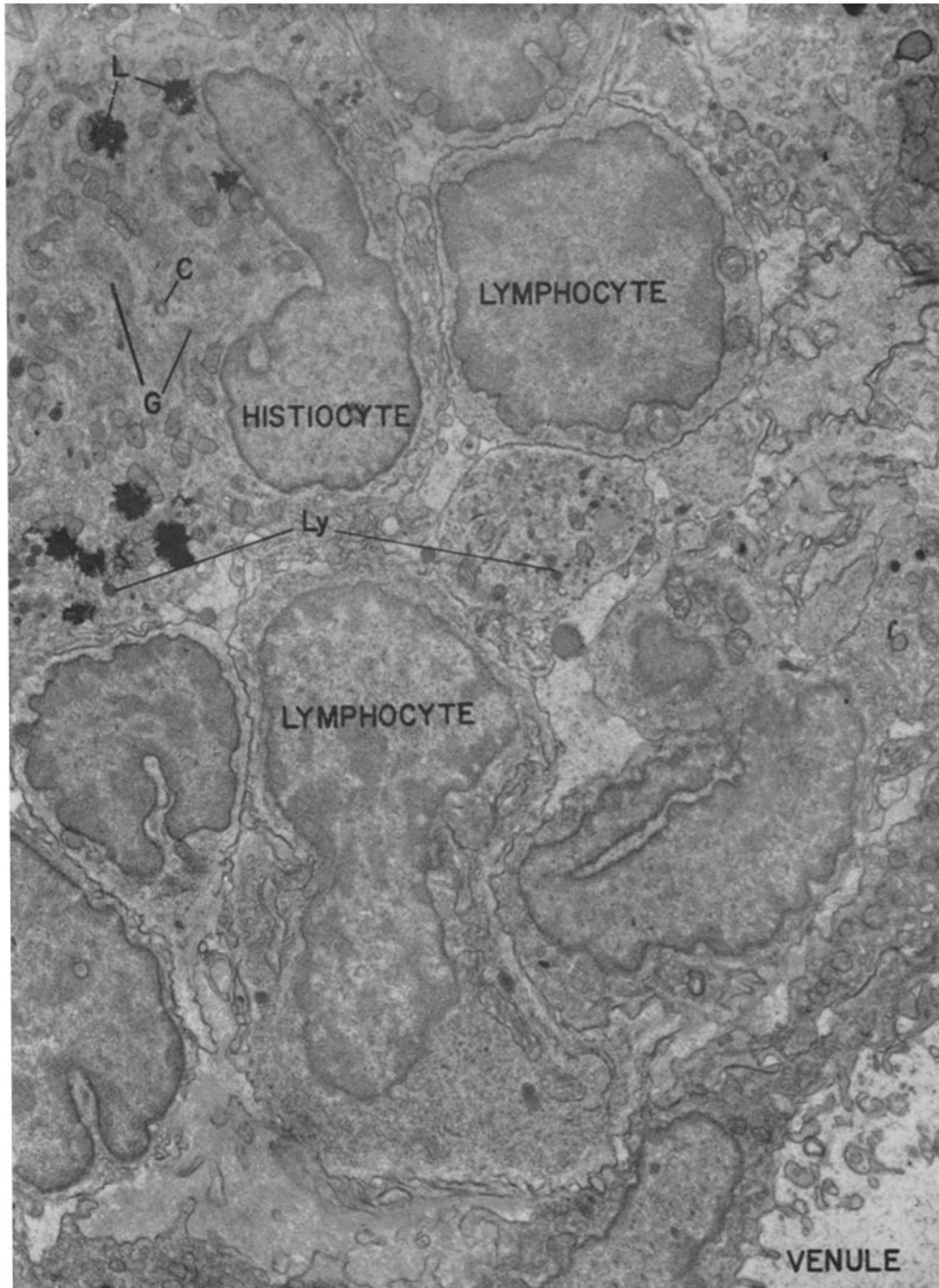
FIGURE 18 Lymph node. Several cell types are grouped about a venule in the cortex of this node. A histiocyte in the left upper corner contains lipid droplets (L), centriole (C), Golgi membranes (G), and some lysosomes (Ly). The nucleus of this cell is characteristically folded. Parts of the cytoplasm of other histiocytes are present in the upper half of the plate. Several lymphocytes are also present here. A venule, its endothelium bearing microvillous processes, is at the right lower corner. X 17,000.

sensitivity but appear also to be selectively damaged.

The damage to plasma cells and lymphocytes may be immunologic, due to antibody produced by plasma cells or to the still undefined influence of lymphocytes. Damage to lymphocytes, particularly thymic lymphocytes, may be due to heightened adrenal cortical activity. Indeed, cortisol may induce a runt syndrome (31). The life span of plasma cells is probably short, measured in hours, days, or weeks (16, 26, 27), and in tissues involved with graft-*versus*-host and host-*versus*-graft reactions their life span may be shorter. The great proliferative activity which produced them, moreover, may result in defective cells having a short life. For example, erythrocytes produced in the hematopoietic burst following an acute massive phlebotomy are shorter-lived than their normally produced cohorts (24). Similarly, heightened mitotic activity in the thymus, coupled with egress of sound lymphocytes to spleen and lymph nodes in response to the incursion of homologous spleen cells, may leave a relatively large number of defective lymphocytes uncovered in the thymic cortex. Thus damaged cells may result from immunologic or hormonal action or may represent the end of a normal or shorter life span.

Runt disease and the wasting syndrome which follows neonatal thymectomy are sufficiently similar so that they have been considered related and the picture of runt disease has been attributed, at least in part, to thymic depletion (23). The cellular responses of the wasting syndrome and of runt disease have, however, not been fully compared. Indeed, the epithelial elements of the thymus, which appear sufficient to induce lymphopoiesis and compensate for neonatal thymectomy, are not destroyed in runt disease. Significant clinical

differences, moreover, exist between runt disease and the wasting syndrome, notably the much more accelerated pace of runt disease. Finally, the finding that runt disease can be induced in germ-free or pathogen-poor animals, whereas the wasting syndrome cannot, indicates that these processes are essentially unalike (23).

Several lines of evidence indicate that the initial proliferation of splenic cells in runt disease is of donor origin and that, within several days, this is followed by a decrease in viable donor cells and a steady proliferation of host cells. The methods used to establish this pattern have depended upon chromosomal markers discernible in metaphase (14, 9, 7), or the capacity of viable cells to condition a second set reaction (20). An important difference between lymph nodes and spleen in runt disease has been disclosed by chromosomal markers. In an experimental model similar to ours, Nowell and Defendi (28) found that large numbers of donor cells proliferate in lymph nodes and not in spleen. This proliferation occurred throughout the period of observation (4 to 21 days) and was correlated with the severity of the disease. It may be noted that the spleen displays a propensity to remove foreign or altered hemal elements (38, 39). Damaged erythrocytes, let pass by other reticuloendothelial organs, are sequestered from the circulation by the spleen and destroyed. Foà-Kurloff cells and leucocytes in shock are taken out of the circulation by the spleen. Antibody to hemal antigen is produced primarily in the spleen, presumably after the hemal antigen is fixed by the spleen. Haller reports that runt disease is prevented or aborted by splenectomy shortly after the homologous cells are injected, suggesting that at least a large number of the

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FIGURE 19   Lymph node. A large phagocyte of the histiocyte-macrophage type occupies most of this field. Indeed, the many sections through nuclei (*Nu*) suggest that the cell may, in fact, be a multinucleated giant cell, a cell type formed by fusion of histiocytes. In a portion of the nucleus a grazing section exposes nuclear pores (*Nu P*). The cell contains many lipid droplets (*L*). Many of them, including the two droplets which are labeled, are in intimate association with mitochondria. Packets of Golgi membranes (*G*) are present (see also Fig. 14), the granular endoplasmic reticulum (*ER*) is scattered through the cytoplasm, and a few lysosomes (*Ly*) are present about the periphery of the cell. Two large phagocytic inclusions (*P*) are apparent. The cell touches upon extracellular connective tissue, some of it rich in collagenous fibers (*Co*). × 22,000.
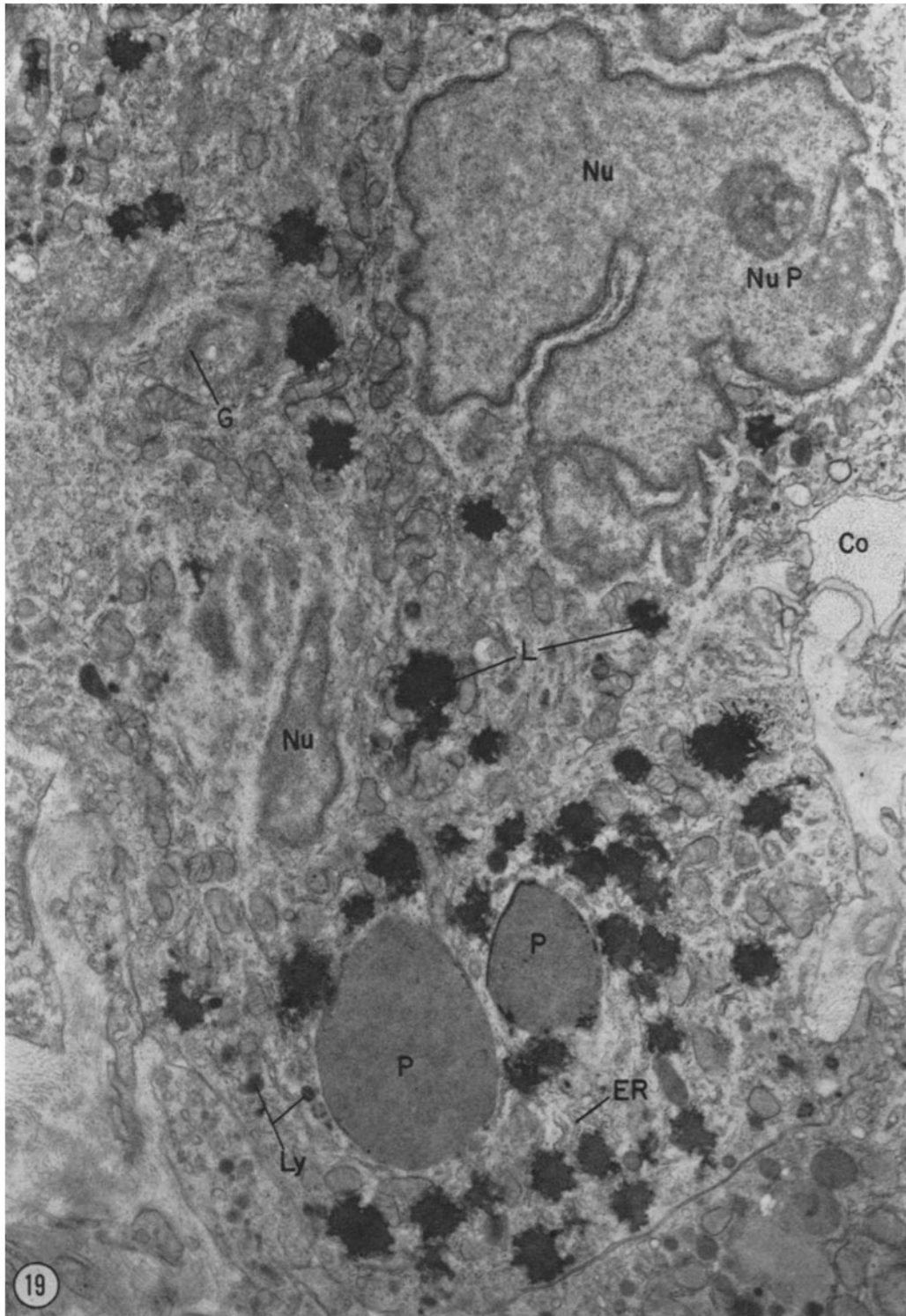
homologous cells locate in the spleen initially and are destroyed or move on (19).

These experiments are not applicable in determining host or donor origin of the large population of damaged and dead lymphocytes and plasma cells. In their electron microscope study of homologous disease in F_1 hybrids in which an immunologic reaction of host against donor is excluded on genetic grounds, Binet and Mathé (8) explicitly state that no plasma cells are present. But Howard (21) has found plasma cells as a concomitant of this homologous disease, and McIntyre et al. (23) has found plasma cell proliferation following thymectomy in newborn mice. The very abundance of apparently vital plasma cells on the 7th to 9th day of the process in our material, and in particular their presence in the spleen, which contains few donor cells, suggests that at least a large proportion of plasma cells are of host origin. It is now clear, moreover, that neonatal and fetal animals may produce plasma cells and have certain immunologic powers (32, 33). With regard to lymphocytes, Nowell and Defendi's work (28) and the finding of large numbers of apparently vital lymphocytes in lymph nodes of our animals (which are rapidly succumbing to runt disease) suggest that significant numbers of these lymph node lymphocytes are of donor origin. We consider it likely that the histiocytes and macrophages

are of host origin. These cells proliferate, accumulate in great number, and are viable more than a week after the administration of homologous spleen cells. This finding, moreover, is in accord with Howard's work on F_1 animals, in which an enhanced capacity of the reticuloendothelial system to phagocytize carbon is demonstrated. That this change is immunologically determined is shown by its earlier appearance when F_1 hybrids are injected with parental cells of one strain iso-immunized against the cells of other parental strains (21). Conceivably, with time, the only cell type persistent in the surviving runted tissues may be the histiocyte, and on this basis our findings may reach accord with those of Binet and Mathé (8). Clearly the proportion and viability of the reactive cell types in runt disease varies with the stage and tempo of the process.

The participation of lymphocytes, plasma cells, and histiocytes in runt disease indicates a complex process wherein occur the processes both of delayed hypersensitivity, presumably mediated by lymphocytes (35), and of immediate hypersensitivity, associated with the plasma cells and the production of antibody.

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FIGURE 20 Spleen—periarterial lymphatic sheath. A small arterial vessel (*Art V*) in the upper third of the field is surrounded by strands of extracellular reticulum (*Ex R*) and many free cells. These include phagocytes (*P*) with ingested whole cells and particles. Very many plasma cells are also present. They are small to large in size, and often contain an eccentric nucleus and a perinuclear clear zone present in negative image. Eleven representative plasma cells are indicated by arrows. See Fig. 21. Toluidine blue. × 950.
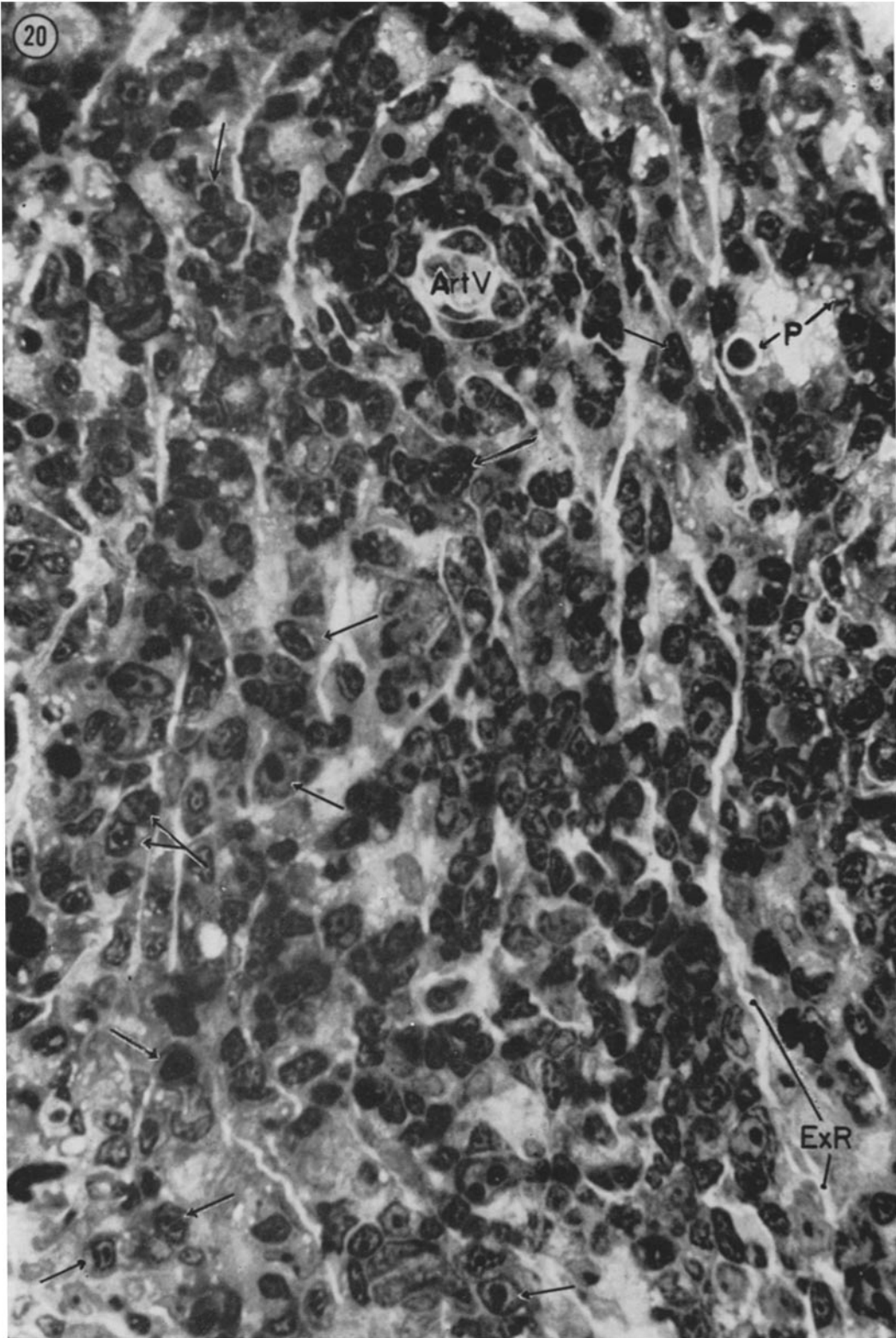
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Figure 21 Spleen—periarterial lymphatic sheath. This portion of white pulp is bounded on the upper left and lower right by small branches of the central artery (*Art V*). Most of the remaining cells are plasma cells (*PC*), their endoplasmic reticulum showing varying degrees of dilatation. Often large zones of the white pulp consist primarily of plasma cells, as in this field. $\times$ 30,000.
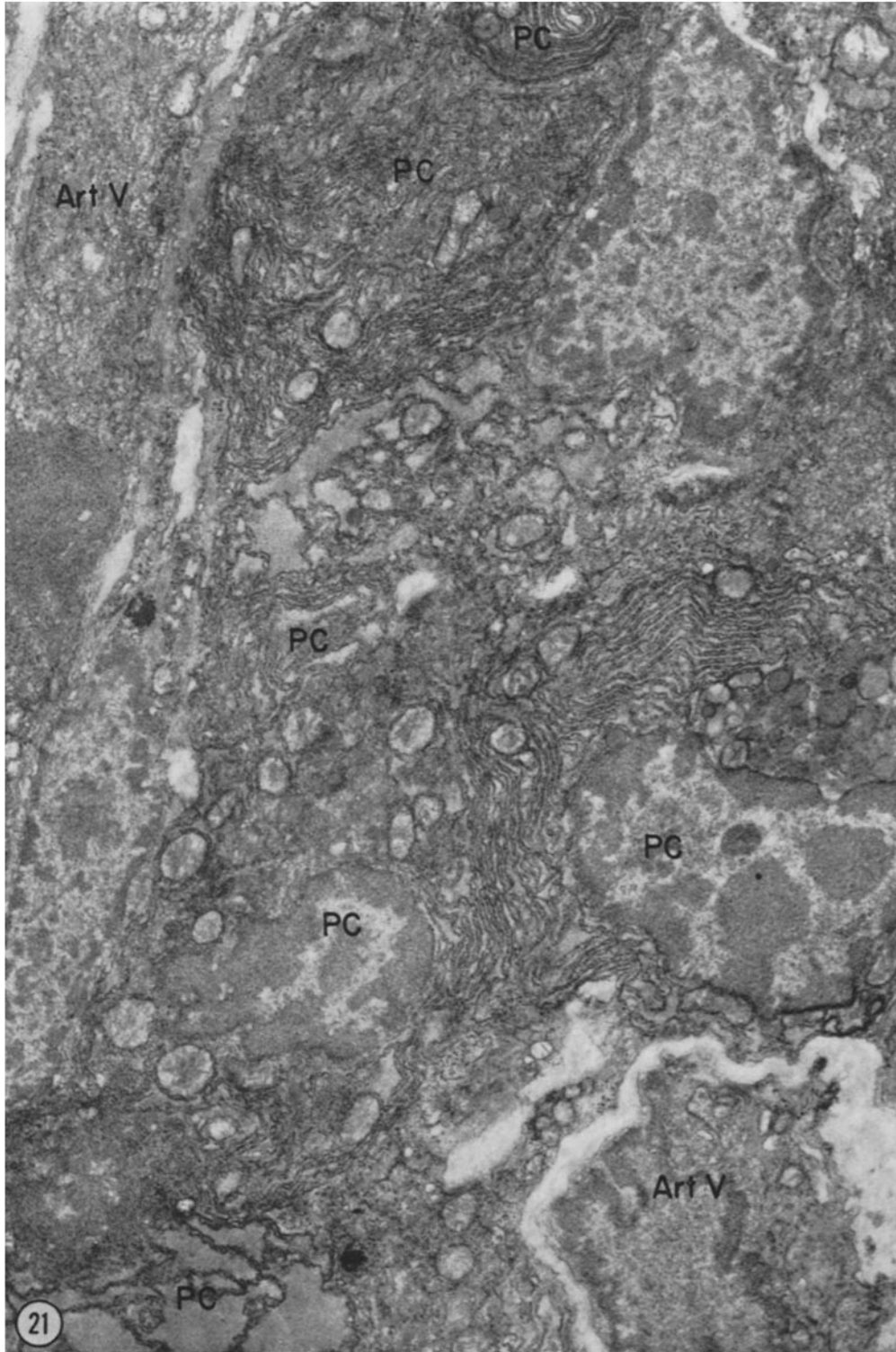
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Figure 22 Spleen—marginal zone. Several strands of extracellular reticulum (*Ex R*) course in this field. A reticular cell (*RC*) is closely applied to the extracellular reticulum at the left upper corner. Two erythroblasts (*E*) are present, as is a plasma cell (*PC*). × 40,000.
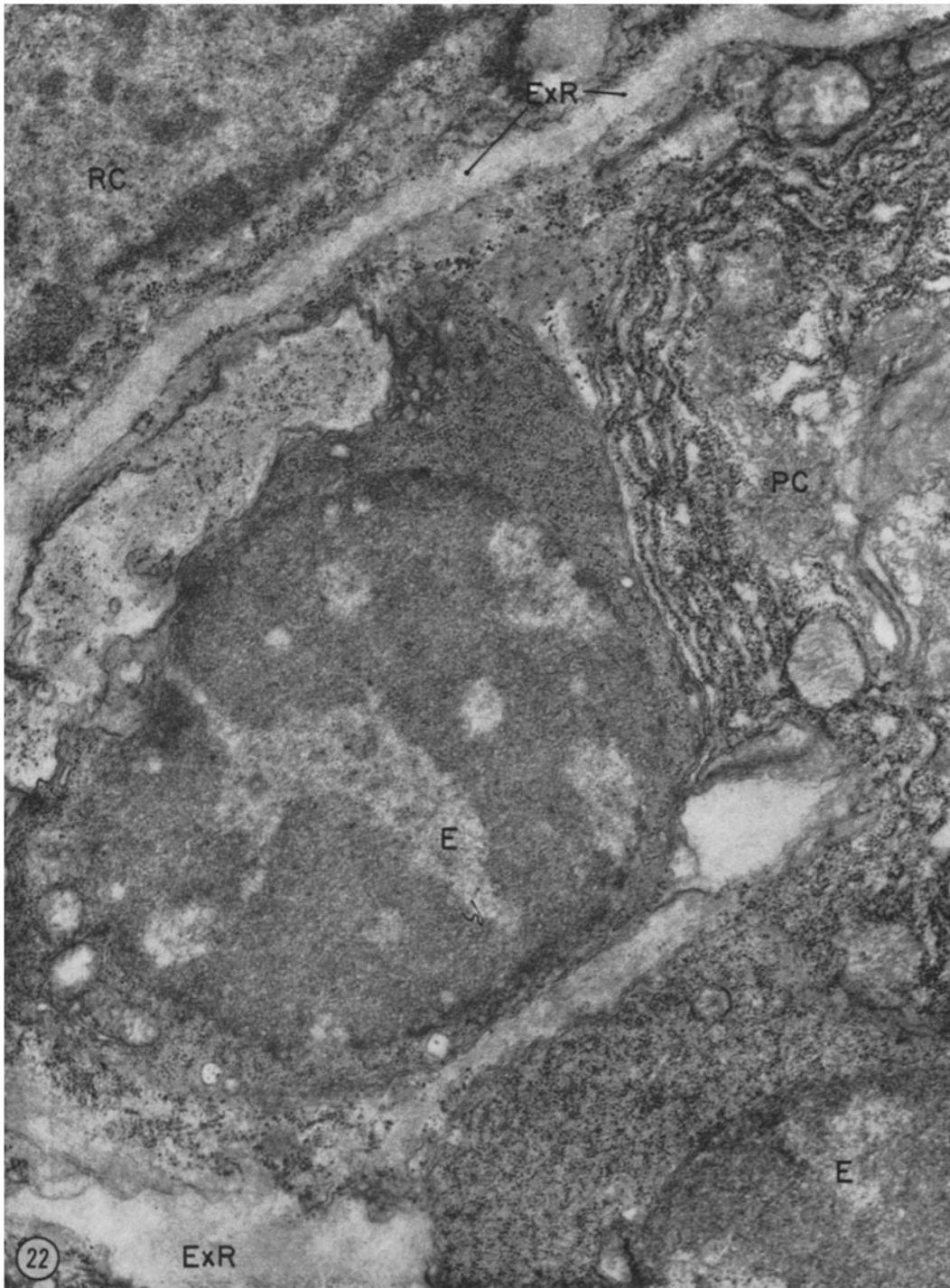
FIGURE 23 Spleen—red pulp. A sinus, not well defined in infant rats, is at the upper left corner. The remainder of the field consists of a splenic cord. It contains erythroblasts (E), plasma cells (PC), a macrophage (P) containing several phagocytic inclusions among which are the remnants of an ingested cell (IC), and reticular cells (RC). This field is representative of the varied cell types present in red pulp. In other areas, however, some lymphocytes may be present. × 19,000.