ULTRASTRUCTURE OF ANTIBODY-FORMING CELLS FROM NORMAL AND IMMUNOLOGICALLY DEPRESSED ANIMALS

by

Galen H. Neher, B. S.

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[redacted]

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STATEMENT OF THE PROBLEM

The ultrastructure of individual antibody-forming cells has not been extensively studied. Therefore, the present study was undertaken to examine in detail the ultrastructure and development of primary 19S antibody-producing cells. Also, plaque-forming cells from animals immunologically depressed with deuterium oxide, phytohaemagglutinin and antilymphocyte serum were examined with the hope of gaining insight into the mechanisms involved in the immunosuppression produced by these substances.

INTRODUCTION

A. ULTRASTRUCTURE OF IMMUNOLOGICAL PROCESSES

To describe the ultrastructure of antibody formation requires first the identification and description of the cell type or types involved in antibody synthesis, and second the identification of the actual locus of production within the cell or cells.

The plasma cell line is now generally accepted as at least the primary, if not the sole producer of antibodies, and suggestions that other types of cells (macrophages, fibroblasts, etc.) are producers of antibodies generally concern the possibility that these cells are precursors of plasma cells. The first descriptions of the plasma cell were given by Unna (162), Pappenheim (117), and Cajal (25), but Huebschmann (72) was the first to suggest that the plasma cell produces antibodies. Bjorneboe and Barmsen (17) and Fagraeus (46) were the first to show a good relationship between antibody production and plasma cells. Following this, Reisse *et al.* (126), White (166), and Coons *et al.* (31) obtained direct evidence that plasma cells contain and probably manufacture antibody. Other evidence indicates that plasma cells are the primary antibody-producing cell: 1) plasma cells appear after antigenic stimulation and their appearance is followed by detectable elevation of specific antibody in the circulation (46, 141); 2) specific antibody is produced by plasma cell tumors (123); 3) plasma cells have the metabolic machinery necessary for producing protein for export (19); and 4) this synthetic machinery (ribosomes and endoplasmic reticulum) is intimately and causally related to antibody synthesis (47, 85).

During the 1940's several experimental approaches led some investigators to suggest that the lymphocyte is the principle antibody-producing cell (41, 67, 69). This suggestion was not widely accepted, especially because electron microscopic examination of lymphocytes failed to reveal cytoplasmic components related to protein synthesis (92). However, a number of recent reports have shown that lymphocytes or lymphocyte-like cells can produce humoral antibodies (4, 7, 33, 68). Electron microscopic studies of these cells (33, 68, 74) have shown that they are not normal small lymphocytes, but contain in their cytoplasm many free ribosomes, grouped into polyribosomes, not attached to endoplasmic reticulum. These cells are now considered progenators of plasma cells (33, 107) and will be discussed below.

Besides being involved in the production of humoral antibodies, lymphocytes are considered the cell responsible for delayed hypersensitivity and thus the producers of cell bound antibodies (82). A number of studies have shown that lymphocytes can be divided into two populations. About 90% of small lymphocytes have a short life span of 1 to 2 weeks, with the remainder having a long life span of 300 days or more (58). These long-lived lymphocytes are considered the cells involved in immunologic memory. Lymphocytes also fall into two categories in regard to cell volume and electrophoretic mobility (135). Strober (55) found that one group of lymphocytes, fixed in the spleen, respond to certain antigens, whereas another group of lymphocytes, members of the circulating population, respond to other antigens. Recently, it has been reported that two populations of lymphocytes (thymus-derived and bone marrow-derived) interact and are both necessary for normal antibody

production (100, 101, 113). These findings indicate that thymus-derived lymphocytes are the cells which first react with antigen and that bone marrow-derived lymphocytes are the actual progenators of antibody-producing cells. It is obvious that lymphocytes are a heterogeneous population of cells with a number of functions, and although much has been learned about them in recent years, the relationships between the various populations and their exact functions remain to be elucidated.

The problem of the derivation of plasma cells is a long-standing one. Various workers have speculated that plasma cells are derived from an undifferentiated stem cell (46, 153), from reticulum cells (82, 122), and even from perivascular adventitial cells (2). However, the lymphocyte is now generally accepted as the precursor of at least the majority of plasma cells (68, 16, 80, 132, 58, 112). Especially convincing are the studies by Movat and Fernando (107), Harris *et al.* (68), and Cunningham *et al.* (33). Movat and Fernando (107), working with antigenically stimulated lymphoid tissue, demonstrated a decrease in small lymphocytes corresponding to the normal increase in plasma cells. They were able to show transitional forms between small lymphocytes and a cell type they called the immunoblast. This cell was relatively large, had a blast-like nucleus (very little heterochromatin and 1 or 2 large nucleoli), a high nuclear/cytoplasmic ratio, and cytoplasm packed with free ribosomes, with many of the ribosomes being grouped into polyribosomes. They also demonstrated a number of transitional forms leading directly from the immunoblast to the plasma cell. This involved an increase in rough endoplasmic reticulum (RER), enlargement of the Golgi apparatus, a decrease in the number and concentration of free ribosomes,

and a decrease in the nuclear/cytoplasmic ratio and size of the cells. They also showed that immunoblasts and slightly more differentiated plasmablasts correspond to the classical basophilic or pyrininophilic blast cells seen with the light microscope following antigenic stimulation. Harris and co-workers (68), using the agar plaque technique of Jerne *et al.* (80, 81) showed that cells resembling lymphocytes and plasma cells both produce specific antibody to sheep erythrocytes. Cunningham *et al.* (33), using a modification of the plaque technique, showed antibody-producing cells in efferent lymph of sheep resembling lymphocytes except that they contained more free ribosomes. They also found antibody-producing cells covering the range between moderately developed lymphocytes, blast cells and mature plasma cells.

Lymphocytes are usually classed as large or medium, and small (22, 65, 107), with the separation between the two being arbitrary. The main differences are that the medium lymphocyte is slightly larger, has a few more cytoplasmic organelles and a lower nuclear/cytoplasmic ratio than the small lymphocyte. As mentioned above, a number of non-morphologic studies have also indicated that there are two populations of lymphocytes.

Medium and small lymphocytes show similar ultrastructural features: small size, high nuclear/cytoplasmic ratio and lack of formed elements in the cytoplasm (11, 22, 65, 107). Endoplasmic reticulum is quite rare but always present (13, 92), and free ribosomes are fairly numerous but polyribosomes are rare. The Golgi apparatus is small and located close to the nucleus. Mitochondria are relatively large and usually confined to one area of the cell. Azurophil granules and lipid droplets are

present in small numbers. The nucleus of the lymphocyte is round and often shows deep narrow invaginations. The chromatin is quite clumped and electron dense, and one or more prominent nucleoli are always present.

Braunsteiner and co-workers (19, 21) were the first to study plasma cells with the electron microscope. They showed that the most characteristic feature of the plasma cell is its richness in cytoplasmic organelles, especially the extensive RER, which corresponds to the deep basophilia seen in the light microscope. The extensive RER reaches to the periphery of the cytoplasm, and is made up of parallel double membranes irregularly studded with ribosomes. In tangential sections, spirals or coils of ribosomes often appear to be attached to the RER membranes. Free ribosomes occur singly or in small clusters in the cytoplasmic matrix. The cisternae are usually parallel and ordered in a laminar form (164). The appearance of the RER varies greatly depending on the degree of separation of the membranes by the contents of the cisternae. Flat and distended cisternae are often encountered in the same cell, with both being filled with a flocculated material of moderate electron density. This material is sometimes concentrated into fairly dense globules of varying sizes; the larger ones corresponding to Russell bodies (65, 106), which occasionally contain a crystalline material having a periodicity of approximately 120 Å (11, 14, 157).

The Golgi apparatus of the plasma cell is large and usually situated close to the nucleus. It is composed of smooth membrane-bordered saccules, vacuoles, and small vesicles. Distended vacuoles and numerous vesicles are the most prominent features. The vesicles are sometimes

seen in contact with saccules and RER membranes and are usually located in the peripheral regions of the Golgi apparatus. The vesicles are of both the smooth-surfaced and "coated" varieties. Golgi vacuoles are variable and appear either empty or filled with a closely packed flocculated material (164). The centrioles are usually located in the center of the Golgi apparatus, and are similar to those found in other cells (14). Small dense bodies, probably lysosomes, are found in variable locations in the cytoplasm (106, 164).

Round to ovoid mitochondria occur in relatively large numbers in plasma cells and are found between the ergastoplasmic sacs. The cristae are quite numerous and sometimes extend the full width of the organelle. Small dense granules are present in the mitochondrial matrix (14, 164).

The cell membrane of plasma cells has the unit membrane structure. The periphery of plasma cells often show villi or smaller projections, and Movat and Fernando (106) suggest that their presence indicates that plasma cells are concerned with the uptake of substances as well as with secretion.

The nucleus of the plasma cell is enclosed in a typical double membrane, with the outer one being covered with ribosomes. The perinuclear space between the membranes is continuous with the cytoplasmic RER. The nuclear chromatin is fairly dense, with condensation at the periphery of the nucleus, giving the classical "cart-wheel" appearance (47). Movat and Fernando (106) did not find nucleoli in mature plasma cells, but they have been observed by most workers (39, 47, 164).

In addition to producing antibodies under the stimulus of antigen, plasma cells also synthesize γ-globulin in myelomas or plasmacytomas. In the majority of cases, cytologic differences between neoplastic and

normal plasma cells have not been demonstrated (48). Dalton *et al.* (35) studied a number of primary and transplanted plasma cell tumors of the mouse, and were able to find only a few quantitative differences between myeloma cells and normal plasma cells. They found virus-like particles in all the tumors, but felt they were not oncogenic. Cell types other than the plasma cell line have been observed which contain specific antibodies. Using the light microscope, Mellors and Korngold (99) described germinal center and primitive reticular cells which contained immunoglobins in their cytoplasm. These may possibly be immunoblasts (107), which would make them precursors of plasma cells. Schaffner and Popper (173) found a cell in human liver which contained antibody as demonstrated by fluorescence microscopy and showed ultrastructural features of a phagocyte. One side of the cell contained phagosomes and the other moderately well developed RER. The significance of this cell type in antibody formation is unknown and it remains to be shown whether the antibody it contains is made by the cell or has been phagocytized by it.

The problem of identifying the intracellular site of antibody synthesis is a difficult one to resolve because of the question of what can be considered synthesis and what storage. However, there is now sufficient evidence to conclude that the transcription and translation mechanisms responsible for antibody synthesis are similar to those in other systems, i.e. antibodies are synthesized on ribosomes held into polyribosomes by messenger RNA (82, 124, 161). Evidence has also been presented (82, 161) which indicates that the L and H chains of the complete antibody molecule are made on separate polyribosomes of about 190S and 270S respectively. Uhr and Moroz (161) also showed that

assembly of the chains occurs by attachment of free released L chains on nascent H chains and that the carbohydrate moiety of antibody appears to be added primarily to completed and released chains. No evidence has been found that the variable and constant regions of each chain are synthesized separately.

The actual morphologic demonstration of the intracellular site where antibody is found has been achieved (39, 40, 89, 129). Rifkind *et al.* (129) used the ferritin protein conjugate technique of Singer (147) and were able to localize γ-globulin in the cisternae of the RER and in the perinuclear space in myeloma cells of the mouse. Using ferritin as antigen, and after incubating the stimulated cells with ferritin, dePetris *et al.* (39, 40) were able to find antibody specifically localized in similar sites in rabbit lymph node cells. Leduc *et al.* (89) localized antibody in plasma cells during progressive phases of cell differentiation by a method in which peroxidase was localized by anti-peroxidase antibody, and an electron opaque reaction product produced. Their findings indicate that antibody appears first in the perinuclear space and later in the cisternae of the developing RER in cytoplasm. In mature plasma cells, antibody was usually not found in the perinuclear space. Russell bodies and small protein globules found within the endoplasmic reticulum are taken to be manifestations of antibody storage (106, 165). These Russell bodies may be due to stagnation of antibody because of decreased release from the cell or the result of enhanced production. The latter possibility would seem more likely because of the increase in Russell bodies during hyperimmunization (165).

Antibody was found in a few of the Golgi lamellae by Rifkind *et al.* (129) and dePetris *et al.* (39, 40), but Leduc *et al.* (89) noted that lamellar portions of the Golgi apparatus almost always contained antibody. None of these workers found antibody in the large Golgi vacuoles. Thus the function of the Golgi apparatus in the production and storage of antibody remains to be elucidated. These workers also found a small amount of label in the cytoplasm, but the validity and significance of this is unknown. These findings indicate that RER (including the perinuclear space) is the principle organelle involved in the storage of antibody, with some possible storage in the Golgi lamellae, but none or virtually none in the Golgi vacuoles or the cytoplasmic matrix.

Coons *et al.* (31) were able to visualize some antibody in the nuclei of lymphoid cells from immunized mice, but similar observations have not been made with the electron microscope, indicating that this may have been an artifact. Intranuclear inclusions, often resembling Russell bodies, have occasionally been seen in plasma cells from patients with multiple myeloma and macroglobulinemia (48), but at present the site of synthesis, mode of formation, and significance of these bodies are unknown.

The problem of where and how antibody is released from plasma cells is a matter of controversy, and a number of different mechanisms have been proposed. Some investigators (106, 129) have speculated that the material produced in the RER may be segregated in the Golgi apparatus for secretion from the cell, and that this is the purpose of the vacuoles seen in the Golgi area. This suggestion is based on the size of the plasma cell Golgi apparatus and the knowledge that the Golgi

apparatus of other protein secreting cells functions in this way (47, 161). However, this seems unlikely in the case of the plasma cell because antibody has not been demonstrated in Golgi vacuoles (39, 40, 89) and elements of the Golgi apparatus have not been seen in contact with the surface of plasma cells (108). Separation of cytoplasmic fragments (clasmatosis or microclasmatosis) has been observed with light and electron microscopy (66, 129, 150, 158) and considered a possible mode of antibody release. Also, plasma cells are known to have a short life span (7) and lysis of these cells may be another mode of antibody release (48, 89, 158). A considerable amount of evidence now indicates that antibody is secreted from viable and persisting cells by a transient communication between the RER and the plasma membrane. This seems very likely because of the storage function of the RER (39, 40, 89), and because such a communication has been observed by a number of workers (106, 108, 150, 164). Ross and Benditt (133, 134) have made similar observations in fibroblasts, and have suggested this mechanism for the release of collagen from these cells. Another possibility is that antibody may be released by simple diffusion across the membrane (48).

Recent immunologic and histologic evidence indicates that antibody-producing cells fall into at least two divisions, one associated with 19S antibody (IgM), and the other with 7S antibody (IgG). A number of workers (34, 126, 149) have demonstrated 19S antibody in the cytoplasm of "lymphocytoid" or "reticular" plasma cells, and a marked increase of this cell type has been correlated with high levels of circulating 19S antibody (28, 42). Electron microscopic studies of the "lymphocytoid" plasma cell (20, 169) have shown what appears to be an immature plasma

cell with moderately developed RER which is not as abundant as in mature plasma cells. The findings of Moore *et al.* (105) indicate that the immature cells are primarily concerned with 19S antibody production, that these cells do not store antibody, and that plasma cells are primarily associated with the production of 7S antibody. However, Cunningham (32) could not find a consistent difference between 7S and 19S antibody producing cells. The question of whether one cell can produce both types of globulin is still unsettled. Nossal *et al.* (114) found many cells producing both 19S and 7S antibody, especially during the period when the animals were shifting from predominantly 19S to 7S antibody production. However, the work of Schearer (138) does not support the idea that individual cells or clones of cells shift from one type of antibody production to another. A related, and equally unsettled, question is whether a single cell can produce antibodies of 2 different specificities. Although most investigators have not been able to find cells producing more than one antibody (55, 63, 110), some workers have reported that a substantial proportion of cells make more than one antibody (4, 96).

Many unsolved problems regarding antigens and their mode of action remain (48). It has not yet been possible to follow the intracellular course of ingested antigen to determine what becomes of it. Radioisotope labeling, fluorescent tagging, and autoradiographic studies (27, 70, 98, 151) indicate that antigen persists, but its form and distribution are still unknown. Speirs (151) believes that antigen persists as an antigen-antibody complex distributed in macrophages. Nossal (112) has shown that in lymph nodes, antigen appears to be trapped

almost completely on the surface of cell processes of dendritic macrophages, in the form of antigen-antibody complexes. Another major problem is the connection between the uptake of antigen and the production of antibody. At least in the case of particulate antigen, it would seem that phagocytic cells must transfer to antibody producing cells either specific information (RNA or RNA-antigen complex) or molecular fragments of the antigen in order to elicit the synthesis of specific antibody (54). That antigen which goes to the reticuloendothelial system plays a role in the induction of antibody production is also indicated by the fact that only when anatomic relationships are maintained between phagocytic and lymphoid cells, can the induction of a primary response take place *in vitro* (111). Fishman *et al.* (50) have shown that when tritiated RNA from macrophages is incubated with cells from lymph nodes, cells of the lymphocytic series incorporated 3H-RNA into their cytoplasm. Also, structural units in lymphoid tissue consisting of macrophages surrounded by cells of the lymphocytic series have been observed (142, 158). These units were studied with the electron microscope by Schoenberg *et al.* (139) who found areas of direct communication between the cytoplasm of macrophages and some immediately adjacent lymphocytes, with the cytoplasmic membranes of the two cells forming a continuous structure. Deane (36) also found a close association between macrophages and plasma cells and feels that the physical transfer of material is probable.

Using the fluorescent antibody technique, Coons *et al.* (31) reported the finding of soluble antigen in the cytoplasm of lymphocytes. If this is in fact the case with soluble antigens in general, and considering the finding that small lymphocytes are the precursors of at

least most plasma cells (68, 107), it is possible that those lymphocytes which ingest the antigen are stimulated by it to differentiate into plasma cells and produce antibodies (48).

B. PHYTOHEMAGGLUTININ (PHA)

In vitro treatment with phytohemagglutinin (PHA) induces small lymphocytes from peripheral blood of humans and experimental animals to undergo transformation and mitosis (5, 131). This transformation involves rapid ribonucleic acid, deoxyribonucleic acid, and protein synthesis. The transformed cells take on the appearance of blast or reticular cells (156). At least one of the synthesis products of these blast cells has been shown by immunohistochemistry to be γ-globulin (130). Recently, Claman (29) and Claman and Brunstetter (30) demonstrated that thymic lymphocytes will behave in a similar manner when cultured with PHA, and these cells have been studied with the electron microscope (88). The morphologic sequence of events in blast transformation can be summarized as follows. Clumps of lymphocytes are formed rapidly, and is followed in a few hours by cytoplasmic hypertrophy and an increase in free ribosomes. The cells continue to grow until by 90 hours they are approximately four times the size of small lymphocytes and have many free ribosomes grouped into polyribosomes in their cytoplasm. However, most of the cells have only a small amount of rough endoplasmic reticulum (RER) at this time. The nuclei of the transformed cells are "blast-like", with only a small amount of heterochromatin and large nucleoli.

Many investigators have studied the effects of *in vivo* administration

of PHA on immunologic responsiveness, and varying results have been obtained. Some have found that PHA acts as an immunosuppressor (26, 43, 152), while others have found it to be stimulatory (44, 53, 105). This apparent conflict has been resolved by Elves (45) and Petronye *et al.* (121) who have shown that the effect of PHA on the immune response is related to the route of administration of both PHA and antigen. They found that PHA acts as an immunosuppressant only when particulate antigen is used, and only when both antigen and PHA are administered by the intraperitoneal (i.p.) route. If any other route or combination of routes of administration are used, the PHA either has no effect or stimulates the immunological response. In agreement with this observation, it has been shown that PHA either has no effect (44) or accelerates (77) graft rejection. Elves (45) found that the immunosuppressive action of PHA is due to the failure of antigen to gain access to the lymphoid organs because of the effect of PHA on the peritoneal cavity and its cellular population. The i.p. route of administration of PHA causes an increase in the number of macrophages in the peritoneal cavity. It has also been demonstrated that these macrophages contain more heterologous erythrocytes than the macrophages from the peritoneal cavity of control animals after the administration of PHA and antigen (79). Perkins and Makinodan (119) found that these peritoneal macrophages act as scavangers and are probably not involved in the initiation of antibody production. Also, histologic examination has shown that many of the small vessels of the peritoneum appear to be blocked as a result of thrombotic agglutination of host erythrocytes, further inhibiting the release of antigen from the peritoneal cavity (45).

To explain the stimulatory effect of PHA when given by the intravenous (i.v.) route (53, 105), Elves (45) speculated that *in vivo* transformed small lymphocytes, which are morphologically similar to immunoblasts of stimulated lymphoid tissue (45), may be further along the pathway of the immune response than normal lymphoid cells. Also, due to the mitotic stimulation of PHA, more cells should be able to react to antigen. Regardless of the mechanism involved, it now appears that PHA has an enhancing rather than a suppressing effect on immunological reactions (45, 121).

C. ANTILYMPHOCYTE SERUM

The immunosuppressive activity of heterologous antilymphocyte serum (ALS) has become the subject of intensive investigation within recent years. The first work in this area established that specific ALS could be used to inhibit cutaneous delayed hypersensitivity reactions in the guinea pig, as well as to prolong survival of first set skin allographs (163, 167) - it has now been used to prolong the survival of allografts in humans (103). Next, it was demonstrated that ALS could also depress the primary humoral antibody response to Salmonella type II antigen and sheep erythrocytes (61, 102). Further studies have shown that most of the immunosuppressive activity of ALS is associated with the gamma-G immunoglobin fraction, and that this could depress the serologic response to alum-precipitated bovine serum albumin as well as to sheep erythrocytes (75, 76). Some workers have used anti-thymocyte serum (1), while others have used anti-lymphocyte serum (37, 160). Barth *et al.* (8) have shown that both preparations produce the same results.

ALS has been shown to have a considerable mitogenic effect *in vitro* (30) and at least some mitogenic effect *in vivo* (160, 38). Recently, Claman and Brunstetter (30) and La Via *et al.* (88) have studied the *in vitro* effects of ALS on human thymus and peripheral blood lymphoid cells. Addition of ALS to cultures resulted in significant clumping of lymphoid cells and an increased amount of blast cell transformation. In comparison to nonstimulated lymphoid cells, the diameter of these transformed cells was increased 2- to 3-fold, with increased amounts of both cytoplasmic and nuclear material. Considerable RNA and protein synthesis occurred by 27 hours, but DNA synthesis was not apparent until 48 hours. Thymus cells were also stimulated by ALS, but not to as great a degree as peripheral lymphocytes. The transformed cells were examined with the electron microscope (88), and their morphologic features were similar to those of PHA transformed lymphocytes (see above). ALS was cytotoxic when complement was added to the cultures.

One of the most striking effects produced by ALS *in vivo* is the reduction in circulating small lymphocytes. This effect has been described by many workers (1, 62, 102), although exceptions have been reported (78). Agnew (1) suggests that the immunosuppressive effect of ALS is due to both the marked decrease in the number of lymphocytes available to carry out the immune response and a depression of the immunological competence of the remaining small lymphocytes. He found that both the lymph nodes and spleen had fewer lymphocytes after treatment with ALS. Levey and Medawar (90) found very little labeled ALS in lymph nodes and spleen, and it has been suggested that ALS acts primarily on peripheral lymphocytes, affecting the lymphoid organs only to the extent that their

population is recruited from circulating cells (38). Using a different approach, Martin and Miller (95) indicated that the cells destroyed *in vivo* by ALS are almost all thymus-derived small lymphocytes. Most recently, Tyler *et al.* (160) using autoradiographic techniques have shown that the lymphocytes destroyed by ALS are almost exclusively long-lived small lymphocytes, and that some of those which survive are stimulated to enlarge into immature appearing "blast-like" cells. Since long-lived lymphocytes have been shown to be involved in homograph reactions (59), this may account for the effect of ALS in increasing graft survival. Further significance of the reduction in long-lived small lymphocytes produced by ALS is indicated by the observation that these cells may play a role in the initiation of the primary humoral antibody response (60, 75).

That ALS effects cells other than small lymphocytes was shown by DeMeester *et al.* (37) who demonstrated that ALS has a profound effect on the hematopoietic stem cells of bone marrow. Using the spleen colony assay method of Till and McCullock (159), they showed that *in vitro* and *in vivo* treatment with ALS decreased the number of colony-forming units from bone marrow. Single injections of ALS which produced no significant difference in total cell content of the marrow were able to decrease the number of colony-forming units by 50% compared to controls. This ability of ALS to injure hematopoietic stem cells and prevent their proliferating to form a colony lead DeMeester *et al.* (37) to speculate that a similar mechanism may be involved in the immunosuppressive action of ALS. This is compatible with the finding that ALS can, in certain instances, suppress the immune response without causing a detectable

depletion of the general lymphoid population (3).

Other theories on the mode of action of ALS have been proposed by Levey and Medawar (90). The first, labeled "blindfolding of lymphocytes", states that ALS inhibits immunocompetent lymphoid cells in such a way that they do not recognize foreign antigens, and thus are not stimulated to produce antibodies. The second, called "sterile inactivation", states that ALS preempts the ability of immunocompetent cells by nonspecifically activating them so that they are no longer able to respond to antigen; this may be represented by the blast cell transformation produced by ALS. Objections may be raised to all these theories, and much is yet to be learned about the effects and mode of action of ALS. This is exemplified by the fact that Baum *et al.* (9) have reported that ALS can cause an enhancement of antibody production to keyhole limpet hemocyanin, even during periods of marked lymphocytopenia.

D. DEUTERIUM OXIDE

Although the effects of deuterium treatment (in the form of D_2O substitution for drinking water) have not been as extensively studied as the effects of PHA and ALS, it has been shown that the administration of D_2O in drinking water has an effect on immunologic (144, 145) and neoplastic processes (6, 15, 84).

Barbour and Allen (6) were the first to note the effects of D_2O on neoplastic processes when they observed that the growth of transplanted lymphosarcoma and mammary carcinoma was retarded in mice drinking 40% D_2O. Since then, Hughes *et al.* (73) and Finkel and Czajka (49) have reported increased survival times of deuterated mice inoculated with

Ehrlich's and Krebs-2A tumors, and Biggs (15) has observed a significant extension of survival of deuterium-treated animals bearing murine myelogenous leukemia (T49) transplants. Siegel (143) was also able to show that mice given 30% D_2O demonstrated depressed neoplastic processes of known viral etiology (lymphoid leukemia induced by Rauscher virus).

Siegel and Morton found that 30% D_2O administered as drinking water depressed serum antibody levels to bovine serum albumin and sheep red blood cells (145). This depression was shown to be related to the amount of pretreatment (144). Equilibration of body fluids and exchangeable tissue hydrogens with deuterium (10 days pretreatment) did not result in depressed antibody levels. Only after substantial deuterium was incorporated by synthesis into non-exchangeable sites (17 days pretreatment) were circulating antibody titers diminished.

The mode of action of D_2O is probably as a non-specific depressant to cell growth and mitosis (73, 49), inasmuch as it has been shown that organs and tissues with high metabolic rates are especially sensitive to interference by deuteration (83). D_2O has also been demonstrated to decrease the capacity for proliferation and differentiation of mesenchymal leucocytes (120).

E. ELECTRON MICROSCOPY OF ANTIBODY-PRODUCING CELLS

Only a few investigations of the ultrastructure of isolated antibody producing cells have been reported, and none of these have been extensive. Bussard and Binet (24) were the first to study plaque-forming cells with the electron microscope. They examined 12 cells from rabbit lymph nodes taken four days following a secondary stimulus, and

all the cells examined were classed as plasma cells. The next report, by Fitch, Rowley and Coulthard (51), was also quite brief. These investigators studied plaque-forming cells from rat spleens taken on various days following immunization, and described only plasma cells. Harris, Hummeler and Harris (68) reported a more extensive study in which rabbit lymph node cells taken four days after a primary injection of sheep red blood cells were examined. These workers studied a relatively small number of cells which fell into two main categories: cells resembling lymphocytes and cells resembling plasma cells. All the lymphocytes were modified, having some features of blast cells, i.e. large nucleoli and many free ribosomes. The plasma cells, showing considerable pleomorphism, ranged from immature plasma cells with a small amount of RER to mature plasma cells with extensive RER. These authors raised, but did not attempt to answer, the question of whether these represented two different cell lines. Following this, Hummeler *et al.* (74) reported a study on plaque-forming cells from lymph and blood of rabbits four days following a single injection of sheep red blood cells. They found a number of modified lymphocytes and modified plasma cells, which did not fit well into recognized categories. No classical mature plasma cells were seen. Most recently, Cunningham, Smith and Mercer (107) studied cells from lymph nodes and efferent lymph of sheep. These cells had produced clusters of antigen-coated sheep red blood cells in a modification of the Jerne plaque technique. They also found 2 groups of cells, modified small lymphocytes and plasma cells. In addition, cells morphologically intermediate between these two groups were found.

MATERIALS AND METHODS

A. PLAQUE FORMATION

The technique used for obtaining individual antibody producing cells was essentially that of Jerne, Nordin and Henry (81). Young BALB/c mice from Jackson Laboratory, Bar Harbor, Maine, were used. They were injected i.p. with 1.5 x 10^9 sheep red blood cells and sacrificed on days 3 through 7 post-immunization. The spleens were removed and weighed, and a cell suspension was obtained by teasing the spleen through a wire screen into NCTC-109 medium containing phenol red indicator. Petri dishes of 8 cm diameter were used for plating. These were provided with a bottom layer of about 10 ml of 1.4% Difco agar in saline. This layer was not necessary, but it allows hemoglobin from lysed red cells to diffuse out of the top layer and thus clarify the plaques.

The top layer of agar, which was kept fluid in a 45° C water bath, was 0.7% Difco agar dissolved in NCTC-109. Immediately before plating, 0.5 ml of a 1/10 saline dilution of packed, washed sheep red blood cells and the appropriate dilution of spleen cells were added to 10 ml of the agar. Two dilutions, varying five-fold in concentration, were used for each spleen. After thorough mixing, each sample was plated in triplicate, with 2 ml of the mixture being poured onto the bottom layer of each Petri dish. This forms a thin top layer in which the scattered spleen cells become fixed among the red blood cells as the agar solidifies. The plates were incubated at 37° C for 45 minutes, after which 1.5 ml of complement (in the form of 1/10 diluted guinea pig serum) was added to each plate. This was followed by an additional 30 minutes of

incubation at 37° C. By this time, clear zones of hemolysis, or plaques, had developed around those spleen cells which had secreted antibody against the sheep red blood cells. Plaques thus produced are the result of 19S antibody, because 7S antibody required the addition of anti-mouse globulin antibody to produce lysis. Only 19S antibody-producing cells were used in this study.

Most plaques were round and had one lymphoid cell in the precise center. However, some plaques had two lymphoid cells in the center, while others showed none. Only those plaques which displayed one cell in their centers were selected for examination in the electron microscope. Some plaques contained a variable number of unlysed red cells, but most were very clear. The plaques varied from 0.05 to 0.5 mm in diameter, with considerable variation in each plate. This variation was probably due to different rates of antibody production and secretion (81, 71). Agar is known to be anti-complementary (81), and to overcome this, 0.2 mg of DEAE-dextran was added to each 2 ml aliquot of cell containing overlay agar. DEAE dextran is a polycation and is thought to become irreversibly bound to the sulfuric ester groups which are scattered along the strands of agar and are probably responsible for the anti-complementary action of agar (91).

B. D_2O TREATMENT

Two groups of BALB/c mice (taken about 1 year apart) were treated with D_2O. They were given 30% D_2O in place of drinking water when 6-8 weeks old and continued for 3 months, after which they were immunized and then sacrificed. This treatment resulted in the number of plaque-

forming cells being depressed to approximately 20% of H_2O controls.

C. PHA TREATMENT

Young BALB/c mice were injected i.p. with 1 mg of PHA (Riga's preparation) in 0.25 ml of isotonic saline, 2 days prior to the i.p. injection of sheep red blood cells. This treatment depressed the number of plaque-forming cells to about 5% of the control response.

D. ALS TREATMENT

To obtain antilymphocyte serum, rabbits were immunized by subcutaneous injection of 10^8 mouse thymus cells incorporated into complete Freund's adjuvant. After 3 weeks they were given 3 daily booster injections of 10^8 thymocytes each. After one week the rabbits were bled. 0.25 ml of this serum was injected i.p. into young BALB/c mice 2 days prior to immunization with sheep red blood cells. This treatment depressed the number of plaque-forming cells to from 10 to 30% of controls.

E. ELECTRON MICROSCOPY

Plaque-forming cells were fixed at 4° C for at least 2 hours by layering the agar plates in which the plaques had developed with 1.5% glutaraldehyde in 0.067 M cacodylate buffer, pH 7.4, containing 1% sucrose. Employing an inverted microscope, the plaques were then removed from the agar plates with a capillary tube and placed in 0.2% sucrose in 0.1 M cacodylate buffer for from two hours to overnight. Only plaques containing one central spleen cell were selected for study, but they were selected at random with regard to plaque size. They were

post-fixed in osmium tetroxide for from 30 minutes to 2 hours. At this point some plaques were placed in 1% uranyl acetate for 30 minutes to increase contrast and enhance staining properties of the cells.

Plaques were dehydrated in increasing concentrations of ethanols (50, 70, 90 and 100%), passed subsequently into propylene oxide and embedded in Araldite (Ducupan-FLUKA) epoxy resin according to the method of Luft (93). To facilitate subsequent orientation and sectioning of the cells, one drop of azur II-methylene blue stain (128) was added to the 50% ethanol in each vial at the beginning of the dehydration procedure. Subsequent dehydration removed most of the stain from the agar but left the sheep red blood cells and plaque-forming cells clearly stained. The addition of this stain greatly increased the efficiency of the procedure, and as far as could be determined, it had no effect on the ultrastructure of the cells. Before and during sectioning, the distance from the block face to the plaque-forming cell was calculated by viewing the block face from above with a microscope having a calibrated fine adjustment. Stained cells appeared as bright blue dots in the thin sections as they came off the knife, allowing one to determine when the cell was being sectioned.

Sections were cut with an LKB microtome using glass or diamond knives, and were mounted on parlodion coated electron microscopic specimen screens. Sections were treated for contrast enhancement with a saturated solution of uranyl acetate and then with Reynold's basic lead citrate solution (127). Sections were treated for various times with these contrasting agents. The "stained" sections were examined with an RCA EMU-3G electron microscope operated at 50 K.V.

RESULTS

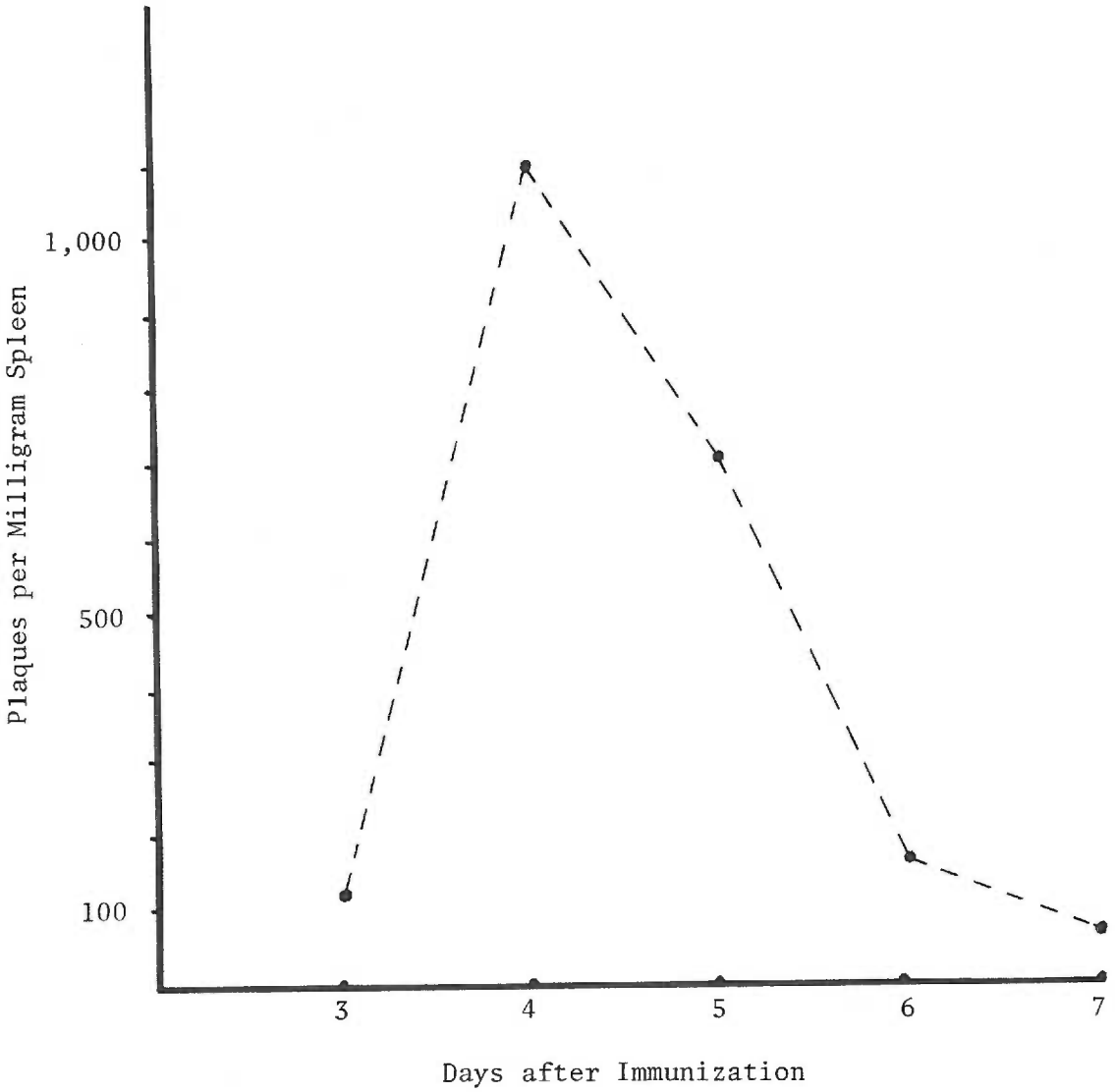
A. PLAQUE-FORMING CELLS FROM UNTREATED ANIMALS

Plaque-forming cells were taken from normal BALB/c mice on days 3, 4, 5, 6 and 7, following a primary injection of sheep red blood cells, and were studied with the electron microscope. This corresponds to the period of rising, maximal, and decreasing numbers of 19S AB-producing cells (graph I). The animals will be referred to as x-day animals, where x corresponds to the number of days after immunization the animal was sacrificed. One hundred cells were examined, with the cells falling into 5 categories - immunoblasts, plasmablasts, proplasmacytes, plasmacytes and abnormal plasmacytes (a new type of antibody-producing cell that has not been previously reported). The nomenclature of the cells involved in the immunologic response is extremely varied (especially concerning the immature or blast-like forms). The system employed by Movat and Fernando (107) is used here because the terms are more descriptive than those used by others. The stages are, of course, arbitrary, and transitions exist.

Immunoblasts.

Only two immunoblasts were seen (figs. 1 and 2), both from the same 3-day animal. These were large cells (at least 13 μ each). A few small blebs were seen at the cell periphery, usually containing a few ribosomes but no polyribosomes. The cytoplasmic matrix of some of these blebs was slightly denser than the rest of the cytoplasm. Mitochondria were round to ovoid, and were located close to the nucleus. The mitochondrial matrix, which usually contained a number of small dark granules, was

GRAPH I


1,000
500
100
Plaques per Milligram Spleen
3
4
5
6
7
Days after Immunization

fairly light in most mitochondria, but was missing in some, giving them a washed out appearance. The cristae were long and narrow and often reached across the diameter of the mitochondria. No Golgi complex was seen in either immunoblast. A few dense lysosome-like bodies were observed in one of the cells. The cytoplasm of these cells was characterized by very numerous free ribosomes, with many being grouped together into spiral- or cluster-shaped polyribosomes. Only a few profiles of rough-surfaced endoplasmic reticulum (RER) were seen. These were all quite short and showed no dilatation.

The nuclei of both immunoblasts showed a thin rim of clumped chromatin along the periphery, and very little clumping in the central areas, giving them the "open" appearance usually associated with blast cells. One nucleus was bilobed and somewhat peripheral in position while the other was ovoid and central with a deep hof similar to those in lymphocyte nuclei (92). Both nuclei contained nucleoli, with one being very large and connected to clumped chromatin at the periphery of the nucleus. In the other cell, only a small portion of the nucleus was seen.

Plasmablasts.

Thirteen of the cells were classed as plasmablasts (figs. 3-6) with 9 of the 13 occurring in 3-day animals. These cells differed from immunoblasts primarily in size (they averaged about 10 μ) and in the amount of RER seen in the cytoplasm. The surfaces of a number of these cells were rippled and cytoplasmic blebing was common. Blebs varied in size, but were generally quite small and did not contain cytoplasmic organelles; being either empty or containing a few free ribosomes. These blebs were often more dense than the rest of the cytoplasm. Three of the plasmablasts

showed pinocytotic processes.

Mitochondria of the plasmablasts were numerous. In a number of instances mitochondria of different appearances were even seen in the same cell. The mitochondria were either grouped close to the nucleus and Golgi apparatus, or located around the periphery of the nucleus. The cristae were long and often extended across the width of the mitochondria, and granules were seen in most of them. The matrix was usually moderately dense, but occasionally appeared electron-lucid, giving the mitochondria a washed out appearance (figs. 4 and 5).

A Golgi complex was noted in about half the plasmablasts (fig. 5). The appearance of this organelle varied considerably from cell to cell. Each of the three major Golgi components (saccules, small vesicles and granules, and vacuoles of different sizes) predominated in one or more of the plasmablasts. The saccules were usually flattened and empty appearing, but a few were filled with a homogeneous, moderately electron-opaque material (fig. 13). In a number of cells they were round, quite distended and appeared empty. The large vacuoles often occurred in groups and were either filled with a moderately dense material or appeared to be empty. It was often difficult to tell the difference between distended saccules and large empty-appearing vacuoles. The Golgi vesicles varied considerably in size and density. They were usually located close to the periphery of the Golgi complex, but were often seen in what appeared to be isolated groups. Many of the vesicles appeared to be empty, but others were coated vesicles, and still others were completely filled with light to very dense material. Connections between the Golgi vesicles and saccules were seen in some of the

plasmablasts. Dense lysosome-like bodies were seen in most of the plasmablasts (fig. 6). These varied in size, shape and location in the cell. They were either round, ovoid or crescent shaped, and were often not located close to the nucleus or Golgi complex. In most instances, however, they were seen at the periphery or toward the inside of the Golgi complex and appeared to be a part of it.

RER in plasmablasts was relatively more abundant than in immunoblasts, but was still present in small amounts. It occurred more or less randomly throughout the cytoplasm and was either slightly or moderately distended (figs. 2 and 3). The RER cisternae were filled with an electron opaque material varying considerably in density from cell to cell. In occasional instances, different RER cisternae in the same cell contained material of varying densities. Such RER appeared randomly mixed in the cells, not separated into different areas. In four of the plasmablasts there was a close association between RER and plasma membrane. Short segments of the RER terminating at or very close to the periphery of the cell were seen. For purposes of recording, this association was considered positive if the RER membrane was so close to the plasma membrane that ribosomes could not fit between the two. As in immunoblasts, the cytoplasm of plasmablasts was filled with ribosomes, many being grouped into spiral- or cluster-shaped polyribosomes.

Microtubules were seen in most of the plasmablasts and, in about 2/3 of them, some or all of the microtubules were closely associated with the periphery of the nucleus (fig. 6). Many were found lying parallel and very close to the surface of the nucleus, and others were grouped in the isthmus between two nuclear lobes. Some microtubules in

the area of an isthmus appeared to fuse with the nuclear envelope (fig. 14). In such areas the space between the two membranes of the nuclear envelope could not be seen and the membranes appeared to be fused. A centriole was observed in one of the plasmablasts (fig. 6), and microtubules and microfibrils were associated with it. This was the only instance in which microtubules appeared to be associated with any cellular organelle other than the nucleus. In two plasmablasts, bundles of microfibrils were seen (fig. 6), but they did not appear to be specifically associated with the nucleus. These structures were free in the cytoplasm and were never limited by a membrane.

The nuclear/cytoplasmic ratio of the plasmablasts was quite high. Nuclei were usually rounded and slightly peripheral in position. Two of them were bilobed. Most of the nuclei had a blast-like appearance, with peripheral chromatin clumping and a large amount of euchromatin in the central areas, but two of the plasmablasts had nuclei with a considerable amount of heterochromatin. These cells were classed as plasmablasts because of their lack of extensive RER. One of the nuclei contained a nuclear body similar to those reported by Brooks and Siegel (23). A nucleolus was present in all plasmablasts which showed a large portion of the nucleus. In most instances nucleoli were very large and connected with the peripheral heterochromatin (fig. 4). This portion of the nucleolus was usually the only place where it was bordered by heterochromatin. In plasmablasts, as in all plaque-forming cells, the outer membrane of the nuclear envelope was studded with ribosomes. In a few of these plasmablasts, the perinuclear space was seen to be continuous with the RER.

Proplasmacytes.

Proplasmacytes were the largest single group, with 37 of the 100 cells being placed in this category (figs. 7-15). They averaged about 8 μ in diameter. The overall appearance of the proplasmacytes varied in several respects, but they always appeared more mature than the plasmablasts, in that their nuclei showed more chromatin clumping; they generally had smaller nucleoli; and their cytoplasm contained a larger amount of RER. The cytoplasmic membrane was intact in all but two of these cells, but the appearance of the cell periphery differed considerably. Plasma membrane rippling was observed in about half the proplasmacytes. Cytoplasmic blebs were also common (fig. 11). These blebs were usually small, and a few contained free ribosomes, but they did not contain RER, vesicles or vacuoles. Micropinocytotic vesicles were found at the surface of several proplasmacytes (figs. 7 and 9). Six of them also had areas along the periphery with a bubbling appearance suggesting the shedding of small pieces of membrane. These membrane fragments were not seen filled with cytoplasm. In a number of instances pieces of cytoplasm and membranes which appeared to be from another spleen cell (non-plaque forming) were seen close to and contacting the periphery of proplasmacytes and the other types of plaque-forming cells (figs. 25-28).

The mitochondria of proplasmacytes were extremely variable in size and appearance. They were either scattered throughout the cytoplasm or grouped close to the nucleus and Golgi complex. In a number of the proplasmacytes the mitochondria were quite small and had very dense matrices (fig. 13). Many of these had longitudinal cristae which were

often slightly dilated. In other mitochondria, the cristae were long and extended almost across their diameter (fig. 7). Small dense granules were seen in most of the normal appearing mitochondria. In a few of these cells, large mitochondria were noted having a "washed-out" appearance.

Golgi apparatus was seen in most proplasmacytes, and was usually very large, but its appearance varied greatly from cell to cell (figs. 8, 10 and 15). The individual components were similar in appearance and distribution to those in the plasmablast. Very dark lysosome-like bodies were seen in many of these proplasmacytes (figs. 7 and 11). Many of them were found within or at the periphery of the Golgi apparatus. Connections between the Golgi apparatus and the RER were noted in many of the proplasmacytes. These connections were in the form of blebs projecting from the RER into a group of similarly sized Golgi vesicles (fig. 8). It was not possible to determine if these blebs were vesicles being formed from RER, or vesicles emptying their contents into RER cisternae. Similar connections were seen between Golgi vesicles and Golgi saccules and vacuoles. Large secretion granules were not seen in proplasmacytes, or any of the plaque-forming cells, and in only a very few instances were any of the components of the Golgi complex close to the periphery of the cell. No connection was observed between any of the components of Golgi apparatus and the plasma membrane. As in the plasmablasts, microtubules were present in many of the proplasmacytes, with many being closely associated with the nucleus (figs. 14 and 15). Others were scattered randomly throughout the cytoplasm or were associated with a centriole - three proplasmacytes showed centrioles (fig. 10).

RER of proplasmacytes varied greatly in amount and appearance. In cells with a moderate amount of RER, some showed little cisternal distention (fig. 7), whereas others showed considerable cisternal distention (fig. 9). Similarly, some cells with abundant RER had considerable cisternal distention (fig. 15), whereas others showed little distention (fig. 8). Some proplasmacytes contained a small amount of ordered or laminated RER as in classical, mature plasma cells (figs. 8 and 11); whereas others had considerable RER having no apparent organization (figs. 7 and 14). Varying degrees of RER distention were commonly seen in the same cell (Figs. 9 and 15). There were also a few instances of differences in density of intra-cisternal material in different segments of RER within the same cell (fig. 9). As in the proplasmacytes, and reported by Neher and Siegel (108), a close association was found between RER and the periphery of many proplasmacytes (fig. 12). In those cells in which there was only a small amount of RER or the RER was not ordered, cisternae appeared to terminate at or very close to the plasma membrane. In those cells in which the RER was more abundant and ordered, small blebs in the outer membrane of the outermost cisternae contacted or came very close to the plasma membrane. Actual contact between the two membranes was seen in many instances. In those cases in which the membranes were well delineated, a single membrane appeared to be present at the point of contact (fig. 12). Approximately 60% of the cells classed as proplasmacytes showed such an association between RER and cell membrane. The portion of RER contacting the cell membrane was usually present for only one section thickness.

In proplasmacytes and all cells showing at least a moderate amount

of RER, the outer leaflet of the nuclear membrane was studded with ribosomes and the perinuclear space filled with an electron opaque substance similar to that found within the cisternae of the RER. Connections between perinuclear space and RER were seen in many proplasmacytes. Numerous free ribosomes filled the cytoplasm of most proplasmacytes. Polyribosomes were seen in a few of these cells (fig. 9), but they were less numerous than in blast cells. A few proplasmacytes showed segments of membrane within RER cisternae. This occurred sporadically in these cells and was considered to be a fixation artifact or due to the *in vitro* manipulation of the cells.

The average nuclear/cytoplasmic ratio of proplasmacytes was lower than that of plasmablasts. The nucleus was slightly peripheral in some proplasmacytes and very peripheral in most (fig. 11). About 40% had more than one nuclear lobe, with some having as many as four lobes. Chromatin clumping was variable. Some had an "open", blast-like appearance and others showed a high degree of chromatin clumping. No correlation was found between the amount of chromatin clumping and the maturity of the cytoplasm as judged by the amount of RER and distention of the cisternae. Such a correlation would be difficult to find, however, because portions of the nucleus were seen in only one or two sections of many of the cells. A single nucleolus was found in about half the proplasmacytes. Nucleoli were usually surrounded by a thin layer of heterochromatin and were close to the periphery of the nucleus. Although smaller than nucleoli in the blast cells, some of them were quite large. Nuclear bodies were seen in two of the proplasmacytes, and in five considerable lengths of nuclear periphery showed no chromatin

clumping. Nuclear pores were common (figs. 8 and 10).

Plasmacytes.

Twenty-seven cells were classed as mature plasmacytes (figs. 16-20). They averaged about 8 1/2 μ in diameter and showed less variation in size and overall appearance than less mature plaque-forming cells. Also, the cell periphery was usually less rippled and had fewer blebs. Blebs, when present, were similar to those described in previous cells in that they were generally small and contained only occasional free ribosomes. Micropinocytotic processes were seen at the surface of two plasmacytes, and a few showed small clear vacuoles close to the plasma membrane, some causing bulges in the cell surface. These vacuoles were not seen in contact with the plasma membrane or opening to the exterior of the cells.

Mitochondria of the plasmacytes were similar to those in proplasmacytes, varying considerably in size, number and appearance from cell to cell. It was not uncommon to find mitochondria of varying size and appearance in the same cell, with structurally different mitochondria lying close to one another. Mitochondria were generally located close to the nucleus and Golgi complex. Some plasmacytes contained small mitochondria having very dense matrices. Many of the small, dense mitochondria had longitudinal, distended cristae, but in some the cristae were similar to those of normal-appearing mitochondria.

The Golgi apparatus of plasmacytes was similar to that seen in the proplasmacytes, varying considerably in size and appearance from cell to cell (figs. 17 and 20). Dense lysosome-like bodies were found in about half of the plasmacytes (fig. 16). These bodies also varied in size and

shape and were either randomly located in the cytoplasm or closely associated with the Golgi complex.

RER was the most characteristic feature of plasmacytes. It was ordered in a laminar form in most of these cells and was very extensive in all of them. In a few plasmacytes RER showed no order. Distention of RER cisternae varied considerably from cell to cell (figs. 16 and 17) and even in different areas of the same cell (fig. 18). Approximately 65% of the plasmacytes showed a close spatial relationship between the RER and the periphery of the cell (fig. 17). In a few plasmacytes, detached membrane segments were seen in the RER cisternae. As in proplasmacytes, a few plasmacytes contained very large, empty-appearing vacuoles. Free ribosomes were present in all plasmacytes, but their concentration in the cytoplasm varied considerably. Even plasmacytes which contained numerous free ribosomes did not contain polyribosomes. Microtubules were associated with centrioles in the two instances where this organelle was seen in plasmacytes (fig. 17). Microtubules were also noted in close association with the nucleus in about one-fourth of the plasmacytes (fig. 16).

Plasmacyte nuclei tended to be slightly smaller and more peripheral in the cell than nuclei of proplasmacytes, and they showed a higher percentage of heterochromatin (figs. 16 and 18). About half of the plasmacytes showed more than one nuclear lobe (figs. 16 and 17) and two of the nuclei contained nuclear bodies (fig. 20). Nucleoli, some of which were quite large, were noted in about one-third of the sections of plasmacyte nuclei. The nucleoli were usually completely surrounded by heterochromatin (fig. 18).

Abnormal Plasma Cells.

The fifth type of cell encountered has not been reported previously and is referred to here as an abnormal plasmacyte (APC) because of the pyknotic appearance of the nucleus and the sparsity of cytoplasmic organelles other than RER (figs. 21-24). Eleven cells were classified as APCs, with only three occurring before day 5. As a group, these were the smallest cells, having an average diameter of about 7 1/2 μ. The plasma membrane of these cells was usually very smooth. A few APCs had membrane blebs at their surfaces which contained no obvious cytoplasm, but cytoplasmic blebs similar to those described previously were only rarely seen. Although APCs varied in overall appearance, the variation was considerably less than in other groups. The two most characteristic features of APCs were the appearance of their nuclei and RER. Most RER in the APCs had a tubular shape and when cut in cross section showed round profiles (figs. 22-24). In those APCs in which RER was cut longitudinally, somewhat orderly, laminar profiles were observed (fig. 21). Connections between different profiles were rarely seen. In a number of APCs, segments of RER contained electron-opaque material of variable density. Such segments were intermixed one with another and not segregated into different areas of the cell (fig. 24). Another feature of APC RER was the even amount of cisternal distention. In many APCs all the round profiles were virtually the same diameter (figs. 22 and 23). In those APCs in which a variation in the amount of distention occurred, the variation was always much less than that seen in most proplasmacytes and plasmacytes. Eight of the 13 APCs had at least one segment of RER in close spatial association with the cell membrane. All the profiles of

RER in APCs were studded with ribosomes and the cytoplasm was filled with numerous, evenly spaced free ribosomes. Polyribosomes were only rarely seen in APCs. The ground cytoplasm of these cells tended to be more dense than that of the other plaque-forming cells (figs. 21 and 23).

Golgi apparatus was seen in only four APCs and these were all quite small, showing either small vesicles or large clear vacuoles; no flattened saccules were seen. Dense lysosome-like bodies were found in only 2 cells. Very clear vacuoles were seen at the periphery of many of the APCs (fig. 21). Mitochondria, reduced in number, were variable in position, size and appearance. In half the APCs where mitochondria were present, they were small and had dense matrices, with longitudinal cristae which were often dilated (figs. 22 and 23). The nuclei of APCs characterized these cells as abnormal. The nuclear/cytoplasmic ratio was the lowest of any of the cell groups. The nucleus, very peripheral in most of APCs and showing more than one lobe in only two, had a pyknotic appearance, with denser and more abundant heterochromatin than seen in the other cell types (figs. 21-24). The pyknotic portions of the APC nuclei were sharply circumscribed and those areas in the normal nucleus usually containing euchromatin were filled with a moderately dense flocculated material, often containing groups of dense granules slightly larger than ribosomes (fig. 22). The two areas of the nuclei were sharply separated and the border between formed either a soft curve (fig. 21) or an almost straight line (fig. 23), extending, in some cases, completely across the nucleus. In many APCs, the nucleus was divided into almost equal parts, one part having no heterochromatin

and the other no euchromatin. No nuclear pores or nucleoli were seen in any of the APCs. In a few APCs, long lengths of nuclear envelope appeared to be absent (fig. 24), exposing the pyknotic portions of the nucleus. In a few instances the nuclear envelope appeared to swing away from the nucleus and into the cytoplasm for a short distance (fig. 24). In other cases, the two membranes appeared to fade away or they came together giving a tube-like appearance. In all such instances, the membranes of the RER and the plasma membrane were well preserved and visible. When both leaflets of the nuclear envelope were present, the outer one was always studded with ribosomes and the space between the two showed very little distention. When portions of the nuclear membrane were not in contact with the nucleus, ribosomes were often attached to both leaflets.

In addition to the cells described above, four plaque forming cells were lysed and could not be classified. Cytoplasm of the lysed cells was almost completely "washed out", showing a few membranes and enlarged, degenerated mitochondria. Other recognizable cytoplasmic organelles such as RER, Golgi, etc., were not seen. Nuclei were recognizable by the remaining heterochromatin. The position of the nuclei and the pattern of chromatin clumping suggested that lysed cells were antibody-producing cells. Such cells showed no pattern in their occurrence in the days after immunization. They were all taken from clear, average to large sized plaques, and no other nucleated cells were within the plaques.

A comparison of the different animals and groups (3, 4, 5, and 6 days post-immunization) is shown in Table 1, and a listing of the

characteristics of the various cell types is found in Table V. Certain totals do not equal 100% because some of the cells were not counted, i.e. lysed cells and those few cells in which only a small portion of the cell or nucleus was seen. Only 5 7-day cells from 1 untreated animal were examined, and thus were not included in Table I. They were, however, included in Table V. The average maturation values were estimated by giving a value of 1 to immunoblasts, 2 to plasmablasts, 3 to proplasmacytes and 4 to plasmacytes. APCs were given a value of 3 or 4 depending on the amount of RER in the cell. The average size of the cells was calculated by measuring the largest diameter observed for each cell. This gives a value somewhat below the actual average diameter of the cells, but the differences noted between the various groups and types of cells should be valid. Plaques were given arbitrary values from 2 to 5 depending on their relative size in the high-dry field of the light microscope used to examine the plastic blocks before sectioning. The approximate corresponding diameters are 2 = 0.05-0.15 mm; 3 = 0.15-0.25 mm; 4 = 0.25-0.35 mm; 5 = 0.35 mm and above.

Virus-like particles (VLPs) were seen in about half the cells studied (figs. 4 and 10). In most instances these were seen within RER cisternae, but some were observed budding from the RER membrane into the cisternae (fig. 20). A few VLPs were in small vesicles, but they were not seen free in the cytoplasm or budding from the plasma membrane. All VLPs resembled "A" particles as described by Bernhard and Granboulin (12). The average diameter of the VLPs was 90 to 100 mμ. They were doughnut shaped, being composed of two concentric dense rings with a less dense ring in between, the whole having a light central core. The core

usually appeared less dense than the area between the 2 dense rings and had a diameter of about half that of the whole particle. In most sections in which VLPs were seen, only 1 or 2 particles were present; however, some sections showed as many as 5 or 6, indicating that these cells may have contained as many as 500 VLPs. No correlation could be found between the presence of VLPs and any morphologic feature of the cells or the size of the plaques produced by these cells. Every animal, except one, had at least one cell which contained a VLP, but the percentage of the cells containing VLPs varied considerably from animal to animal.

A few plaque-forming cells had pieces of cytoplasm close to or touching their peripheries (figs. 25-28). These always had a different appearance from the plaque-forming cells and thus appeared to be from another spleen cell. A few intact red blood cells and numerous red cell ghosts were also seen very close to a number of the plaque-forming cells (fig. 29). Some of these were actually in contact with the plasma membrane (fig. 30). The pieces of cytoplasm, red blood cells and red cell ghosts were associated with all classes of plaque-forming cells.

Plaque-forming cells from three animals in the 3 day group were examined. The cells from these animals had the lowest average maturation and the largest average size of any of the groups. These three animals, however, showed a considerable spread in their values (Table I). Cells from animals 3B and 3C had average maturations of 2.1 and 2.7 respectively and average diameters of 10 μ. Cells from animal 3A, however, were smaller (8 μ) and had an average maturation of 3.4. Both these values fall within the range of the animals in the later groups.

Cells within each group and cells from individual animals varied

greatly with respect to maturation, plaque size and morphologic features. However, the average maturation increased with each day and a similar pattern was noted for the average plaque size.

Table V provides a comparison between the different classes of cells, with immunoblasts and plasmablasts being grouped together because of their morphologic similarity and the low number of immunoblasts. The percentage of cells showing an association between RER and plasma membrane is very similar for the 3 classes which contained significant amounts of RER (proplasmacytes, plasmacytes and APCs). This association was even seen in 4 of the blast cells which had a very small amount of RER. The presence of VLPs was quite consistent throughout the 4 cell types, with only the plasmacytes showing particles in less than 50% of the cells. Dense lysosome-like bodies seem to be more abundant in the blast cells, but no significant difference was found between the number of proplasmacytes and plasmacytes showing these bodies. A considerably lower percentage of APCs contained dense bodies, reflecting the low number of cytoplasmic organelles other than RER seen in their cytoplasm. At least one centriole was seen in each of the classes except the APCs. Pinocytotic processes were also found in all classes except the APCs; however, plasmacytes did not appear to be as active in this respect as the less mature cells.

The largest number of mitochondria in any one section of each cell was recorded and an average value obtained in order to estimate the abundance of mitochondria in each type of cell. Those cells which showed only 5 or less mitochondria were not used to obtain the averages. As the cells became more mature, the number of mitochondria per cell went

down correspondingly. Mitochondria with dense matrices were not seen in the blast cells but significant percentages of the cells in the other groups had such mitochondria, and APCs seemed to have a significantly higher percentage than proplasmacytes and plasmacytes.

In scoring cells as positive for the presence of nucleoli, those cells in which only a small portion of nucleus was seen were not counted. There was a direct correlation between the percentage of cells showing nucleoli and the maturation of the cells in the first three categories. No nucleoli were seen in the APCs. The main difference noted between the average sizes of the cells in the different classes was that blast cells averaged 1 1/2 to 2 1/2 μ larger than the other cells. In going from less mature to more mature cells, an increase was noted in the average size of the plaques produced by these cells. However, the cells in each of the groups produced all sizes of plaques.

B. CELLS FROM D_2O TREATED ANIMALS

Seventeen animals were treated with D_2O, and a total of 119 cells were examined. A summary of the findings from each of the animals is presented in Table II, and a listing of the characteristics of the different types of cells is given in Table VI. As indicated in the methods section, two sets of animals received the same treatment, one being taken about a year previous to the other. The earlier set was composed of animals 3C, 4D, 5D, 6C and 7C. The only apparent morphological differences between the 2 groups were that many nuclei from the second group of cells showed small pieces of extra-dense chromatin (fig. 31) not seen in the first group, and all cells that were lysed occurred in the second set.

Cells from D_2O treated animals fell into the same six categories as cells from untreated animals. Twenty-six cells from D_2O animals were lysed and thus could not be classified, leaving approximately the same number of classifiable cells in both groups (100 control and 93 D_2O). The percentage of immunoblasts, plasmablasts and abnormal plasmacytes (APCs) from the D_2O animals was very similar to those from control animals, but the percentage of proplasmacytes and plasmacytes was reversed in the two groups, with D_2O animals having fewer proplasmacytes and more plasmacytes than controls. Cells from D_2O animals produced plaques averaging approximately the same size as those produced by the cells from untreated animals. Fewer cells from this group showed a close association between RER and the periphery of the cell, as compared to control cells. VLPs were observed in at least one cell from each of the D_2O treated animals, and about 60% of the total cells were observed to contain at least one VLP. As in the cells from control animals, those cells showing only 5 mitochondria or less were not used in calculating the average number of mitochondria per cell. The D_2O cells averaged 13 mitochondria per cell as compared to 10 for control cells. Also, each different cell type from the D_2O animals averaged from 2 to 5 more mitochondria per cell than the corresponding cell type from control animals.

A number of D_2O cells contained myelin figures (fig. 35). These occurred free in the nucleus and cytoplasm, in the perinuclear space, in RER cisternae, and in smooth vesicles in the cytoplasm. Their appearance varied greatly, and they showed no pattern in their occurrence as to cell type or days after immunization. They were more common,

however, in cells from a few animals, suggesting that myelin figures were fixation artifacts. The several cell types from D_2O treated animals (figs. 31-44) showed the same general morphologic features as the control cells and thus will not be described in great detail.

Immunoblasts and Plasmablasts.

Sixteen immunoblasts and plasmablasts were seen (figs. 31-35). These were similar to those from control animals, having a "blast-like" appearance with cytoplasm filled with free ribosomes (many grouped into polyribosomes), and showing small amounts of RER with little or no distention of the cisternae. All nuclei were large and contained a high percentage of euchromatin, with the heterochromatin appearing in a narrow peripheral rim. The nucleolus was usually very large and was often attached to peripheral heterochromatin. D_2O blast cells had a much higher percentage of polyribosomes arranged in spiral form than did blast cells from untreated animals, and the D_2O cell ribosome spirals tended to contain more ribosomes than those observed in control cells (fig. 32). Blast cells from D_2O animals appeared to have significantly more mitochondria per cell than control blast cells. Blast cells from D_2O animals had cytoplasmic blebs similar to those found in untreated cells (figs. 31 and 33). These blebs were often more dense than the remaining cytoplasm.

Proplasmacytes.

Proplasmacytes from D_2O treated animals were very similar to those from control animals, showing similar morphologic characteristics, and

having the same general pattern of occurrence (figs. 36 and 37). The D_2O group had 10% fewer proplasmacytes, but the occurrence and appearance of their nuclei, RER and cytoplasmic organelles was the same as in cells from the control animals. Proplasmacytes from D_2O treated animals averaged three more mitochondria per cell. Many of these cells also showed cytoplasmic blebs, a number of which were more dense than the rest of the cytoplasm (fig. 36).

Plasmacytes.

The D_2O animals had 10% more plasmacytes than the controls, and many showed the same morphologic characteristics as control plasmacytes (figs. 39 and 40). More of the plasmacytes from D_2O treated animals had unordered, non-laminar RER. In some of these cells, the RER was random, very extensive and distended; while in others, it was tubular with round profiles. One D_2O plasmacyte was observed in contact with a red cell ghost (figs. 38 and 39). One portion of the red cell ghost was within a micropinocytotic process at the surface of the plasmacyte.

Abnormal Plasmacytes.

APCs from D_2O treated animals (figs. 41-43) showed more dense bodies and mitochondria per cell and considerably larger Golgi apparatuses than control cells. About half the RER profiles in many APCs from D_2O treated animals were long and ordered. The RER cisternae showed a uniform amount of distention, but this was less marked than in APCs from untreated animals. Nuclei of APCs from D_2O treated animals showed chromatin clumping similar to that seen in control APCs, with heterochromatin being more abundant and usually denser than in other plaque-forming cells, giving

the nuclei a pyknotic appearance. As in the APCs from control animals, a few D_2O APCs contained groups of dense granules slightly larger than ribosomes in the euchromatin portion of their nuclei. However, D_2O APCs differed in that about one-third had a recognizable nucleolus (fig. 41) and some had nuclear pores. A number of cells classified as proplasmacytes or plasmacytes from D_2O treated animals showed characteristics suggesting development toward the APC state.

Twenty-six lysed cells occurred in D_2O treated animals as compared to only 4 from untreated animals. Most of the lysed cells came from 5 animals in the 5 and 6 day groups, and as mentioned previously, all the lysed cells came from the later set of D_2O treated animals. These lysed cells were similar to those from control animals, except that a few appeared to be less degenerated (fig. 44).

C. CELLS FROM PHA TREATED ANIMALS

Four animals were treated with PHA - two 4-day and two 5-day animals. Summaries of the findings from individual animals and the different cell types are found in Tables III and VII respectively. Thirty-five cells were examined as compared to 44 cells from 4 and 5 day untreated animals. Cells from PHA treated mice fell into the same categories as cells from control animals, but the proportion in the several categories was different. The average maturation of PHA cells was 2.9 as compared to 3.3 from the corresponding control cells. In comparing the average maturation of the cells from the individual animals, the lowest value from controls is higher than the highest value from PHA treated animals on the same day. That cells from PHA treated animals secreted normal amounts

of antibody is indicated by the average plaque size being the same as that produced by 4 and 5 cells from control animals.

As in control cells a few cells from PHA treated animals had extraneous pieces of cytoplasm close to or touching their peripheries. These cytoplasm pieces always had a general appearance different from that of the plaque-forming cell, suggesting that they were from another cell. A number of red cell ghosts and a few intact red blood cells were close to or touching some of the PHA cells.

Plaque-forming cells from PHA treated animals (figs. 45-53) showed about the same number of mitochondria per cell, but fewer VLPs than control cells. They also contained more dense bodies, and areas of close association between RER and plasma membrane (figs. 38 and 39). Many cells from PHA treated animals had very clear cytoplasm and considerable amounts of plasma membrane detached from the surface of the cells. A few of these cells also had portions of RER bulging at the surface of the cell and/or distortions in the membranes of the nuclear envelope. These differences were not seen in a large proportion of PHA cells, which were generally very similar to the corresponding types of cells from control animals.

D. CELLS FROM ALS TREATED ANIMALS

Seventeen cells from two 4-day ALS treated animals were studied (figs. 45-49). Summaries of the findings from individual animals and the different cell types are found in Tables IV and VIII respectively. These cells fell into the same categories as the 4 day cells from untreated animals, but the relative numbers of plasmablasts and

proplasmacytes were quite different. This gave the ALS cells a lower average maturation - 2.9 as compared to 3.3 for 4 day control cells. As in PHA treated cells, many ALS cells had very clear cytoplasm, some plasma membrane shedding, and more myelin figures than were seen in control cells. Also, in one plasmablast which had mitochondria with dense matrices, some mitochondria had very distended cristae and others were broken up into small dense globules (fig. 56). In general, however, the morphologic features of ALS treated cells were similar to those noted for untreated cells.

That ALS treated cells secreted at least as much antibody as untreated cells is indicated by the fact that their average plaque size was larger than that of control cells. The same pattern of increasing average plaque size with increasing maturity seen in control cells was observed with the cells from ALS treated animals. As with control, D_2O and PHA treated animals, individual cells from each class showed a considerable variation in plaque size.

TABLE I

PLAQUE-FORMING CELLS FROM UNTREATED ANIMALS

Day and Animal	3A	3B	3C	3 DAY TOTALS	4A	4B	4C	4D	4E	4 DAY TOTALS	5A	5B	5 DAY TOTALS	6A	6B	6C	6 DAY TOTALS	TOTAL CELLS
Number of Cells	10	9	10	29	7	3	4	9	4	27	11	6	17	6	7	9	22	95
Average Maturation	3.4	2.1	2.7	2.7	3.4	3.3	3.3	3.1	3.3	3.3	3.3	3.5	3.4	3.7	3.0	3.8	3.5	3.3
Average Size	8	10	10	9μ	8	8	8	7	8	8μ	8	8	8μ	9	8	9	9μ	9
Average Plaque Size	3.9	3.2	3.2	3.4	3.3	3.3	3.5	3.7	2.8	3.4	4.0	3.0	3.6	3.2	3.7	4.0	3.7	3.5
RER-Periphery Association	5	4	6	15	5	2	1	1	1	10	9	5	14	2	3	7	12	51
Virus-like Particles	8	4	9	21	1	2	2	3	2	10	9	3	12	2	1	1	4	47
Dense Bodies	4	7	6	17	4	2	4	2	3	15	1	1	2	5	1	6	12	46
Nucleoli	0	7	6	13	3	0	2	2	1	8	1	2	3	2	3	2	7	31
Immunoblasts	0	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Plasmablasts	0	4	5	9	0	0	0	2	1	3	0	0	0	0	1	0	1	13
Proplasmacytes	5	3	3	11	5	2	2	4	1	14	5	2	7	2	2	2	6	38
Plasmacytes	4	0	2	6	1	1	1	2	2	7	1	2	3	4	1	6	11	27
Abnormal Plasmacytes	0	0	0	0	1	0	1	1	0	3	5	2	7	0	1	0	1	11
Lysed Cells	1	0	0	1	0	0	0	0	0	0	0	0	0	0	2	1	3	4

TABLE II

PLAQUE-FORMING CELLS FROM D_2O TREATED ANIMALS

Day and Animal	3A	3B	3C	3 DAY TOTALS	4A	4B	4C	4D	4 DAY TOTALS	5A	5B	5C	5D	5 DAY TOTALS	6A	6B	6C	6 DAY TOTALS	7A	7B	7C	7 DAY TOTALS		TOTAL CELLS
Number of Cells	9	8	6	23	9	9	8	5	31	7	9	8	5	29	6	8	6	20	3	7	6	16		119
Average Maturation	2.3	2.9	2.2	2.5	3.1	3.6	3.8	2.6	3.4	4.0	3.7	3.6	3.4	3.6	3.7	4.0	3.7	3.8	3.7	4.0	3.2	3.6		3.5
Average Size	10	9	9	9μ	10	9	8	8	9μ	8	9	8	8	8μ	9	9	8	8μ	8	8	8	8μ		8μ
Average Plaque Size	3.1	2.8	-	2.9	3.1	3.6	4.1	-	3.6	3.3	3.6	3.1	-	3.3	3.3	3.8	-	3.6	3.3	3.3	3.3	3.3		3.4
RER-Periphery Association	1	2	2	5	5	6	3	2	16	1	1	3	2	7	2	1	4	7	2	2	4	8		43
Virus-like Particles	1	7	4	12	3	9	4	3	19	2	3	3	1	9	3	3	1	7	2	3	2	7		54
Dense Bodies	5	2	3	10	6	7	3	0	16	0	2	2	2	6	2	1	3	6	2	2	4	8		46
Nucleoli	5	2	5	12	6	5	3	1	15	1	2	1	2	6	2	3	3	8	0	1	5	6		47
Immunoblasts	1	0	1	2	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0		3
Plasmablasts	5	2	3	10	1	0	0	1	2	0	0	0	1	1	0	0	0	0	0	0	0	0		13
Proplasmacytes	2	5	2	9	3	3	1	2	9	0	1	1	0	2	1	0	2	3	1	0	4	5		28
Plasmacytes	0	1	0	1	3	4	5	0	12	2	3	2	2	9	2	4	2	8	2	5	1	8		37
Abnormal Plasmacytes	0	0	0	0	1	1	0	2	4	0	0	2	2	4	0	0	2	2	0	0	1	1		11
Lysed Cells	1	0	0	1	0	1	2	0	3	5	5	3	0	13	3	4	0	7	0	2	0	2		26

TABLE III

PLAQUE-FORMING CELLS FROM PHA TREATED ANIMALS

Day and Animal	4A	4B	4 DAY TOTALS	5A	5B	5 DAY TOTALS	TOTAL CELLS
Number of Cells	10	5	15	10	10	20	35
Average Maturation	2.8	2.8	2.8	3.2	2.9	3.1	2.9
Average Size	9	10	9μ	9	9	9μ	9μ
Average Plaque Size	3.3	3.6	3.4	3.5	3.6	3.5	3.5
RER-Periphery Association	8	4	12	6	4	10	22
Virus-like Particles	1	1	2	3	4	7	9
Dense Bodies	4	3	7	5	5	10	17
Nucleoli	5	2	7	7	7	14	21
Immunoblasts	0	0	0	0	0	0	0
Plasmablasts	4	2	6	1	2	3	9
Proplasmacytes	4	1	5	6	5	11	16
Plasmacytes	2	1	3	2	0	2	5
Abnormal Plasmacytes	0	0	0	1	1	2	2
Lysed Cells	0	1	1	0	2	2	3

TABLE IV

PLAQUE-FORMING CELLS FROM ALS TREATED ANIMALS

Day and Animal	4A	4B	TOTALS
Number of Cells	9	8	17
Average Maturation	2.6	3.1	2.9
Average Size	10	9	9μ
Average Plaque Size	3.9	4.1	4.0
RER-Periphery Association	6	4	10
Virus-like Particles	8	6	14
Dense Bodies	1	6	7
Nucleoli	2	1	3
Immunoblasts	0	0	0
Plasmablasts	4	2	6
Proplasmacytes	3	2	5
Plasmacytes	1	3	4
Abnormal Plasmacytes	0	1	1
Lysed Cells	1	0	1

TABLE V

CHARACTERISTICS OF PLAQUE-FORMING CELLS
FROM UNTREATED ANIMALS

Cell Type*	1&2	3	4	5	6
Number of Cells	15	39	29	13	4
Average Size	10	8μ	8μ	7μ	-
Average Plaque Size	3.1	3.5	3.7	3.4	3.0
RER-Periphery Association**	27	56	61	61	-
Virus-like Particles**	53	51	38	53	-
Dense Bodies**	73	48	52	15	-
Nucleoli**	100	48	30	0	-
Average Number Mitochondria	13	10	9	7	-

TABLE VI

CHARACTERISTICS OF PLAQUE-FORMING CELLS
FROM D_2O TREATED ANIMALS

Cell Type*	1&2	3	4	5	6
Number of Cells	16	28	38	11	26
Average Size	10	9μ	9μ	8μ	-
Average Plaque Size	2.7	3.3	3.6	3.2	3.6
RER-Periphery Association**	25	36	55	55	-
Virus-like Particles**	31	60	61	45	-
Dense Bodies**	44	53	45	55	-
Nucleoli**	86	45	65	36	-
Average Number Mitochondria	18	13	11	9	-

* 1&2 = Immunoblasts and Plasmablasts
3 = Proplasmacytes
4 = Plasmacytes
5 = Abnormal Plasmacytes
6 = Lysed Cells

** values given are percentages

TABLE VII

CHARACTERISTICS OF PLAQUE-FORMING CELLS
FROM PHA TREATED ANIMALS

Cell Type*	1&2	3	4	5	6
Number of Cells	9	16	5	2	3
Average Size	10	9μ	8μ	8μ	-
Average Plaque Size	3.3	3.6	3.6	3.0	3.3
RER-Periphery Association**	56	63	80	100	-
Virus-like Particles**	33	56	40	0	-
Dense Bodies**	33	75	60	0	-
Nucleoli**	80	69	40	50	-
Average Number Mitochondria	10	11	9	0	-

TABLE VIII

CHARACTERISTICS OF PLAQUE-FORMING CELLS
FROM ALS TREATED ANIMALS

Cell Type*	1&2	3	4	5	6
Number of Cells	6	5	4	1	1
Average Size	10	9μ	8μ	-	-
Average Plaque Size	3.8	4.0	4.2	-	-
RER-Periphery Association**	67	100	25	-	-
Virus-like Particles**	67	80	100	-	-
Dense Bodies**	33	20	75	-	-
Nucleoli**	67	80	75	-	-
Average Number Mitochondria	11	11	8	-	-

* 1&2 = Immunoblasts and Plasmablasts
3 = Proplasmacytes
4 = Plasmacytes
5 = Abnormal Plasmacytes
6 = Lysed Cells

** Values given are percentages

DISCUSSION

Previous reports have dealt with the ultrastructure of individual antibody-producing cells (24, 51, 68, 74), but they did not follow the development and maturation of these cells. The present study follows the development of 19S antibody-producing cells of the primary immune response. Cells were taken from the time 19S plaque-forming cells begin to appear at 3 days post-immunization through 7 days post-immunization, when very few of these cells remain (graph I).

It is generally accepted that at least most plasma cells arise from small or medium lymphocytes (16, 86, 105, 107, 146) which first differentiate into large blast cells. This differentiation (107, 140) involves an increase in size of both the cells and the nuclei, with the nuclear/cytoplasmic ratio remaining quite high. The nucleus develops a large nucleolus and the chromatin becomes very diffuse, giving it an "open" appearance. The cytoplasm becomes packed with ribosomes, many being grouped into polyribosomes, and the mitochondria increase in size and number. These cells correspond to the basophilic blast cells seen in light microscopy (107). As the blast cells develop into proplasmacytes, and plasmacytes (immature and mature plasma cells), there is increased development of the RER, mitochondria decrease in size, the Golgi apparatus enlarges and becomes more complex, the size of the cell and the nuclear/cytoplasmic ratio decreases and the nuclear chromatin becomes more clumped, giving the nucleus the typical "cart-wheel" appearance (164).

This general pattern of development was observed in this study. The least mature cells were large blast cells or immunoblasts (figs. 1

and 2). Although no modified lymphocytes were seen, it has been demonstrated by others (33, 68, 74) that such cells can produce plaques. One of the immunoblasts in this study had a large nuclear hof (fig. 2) similar to those seen in lymphocytes (13), giving at least some support to the lymphocytic origin of these cells. Cunningham (32) has shown a difference in the reaction of lymph nodes and spleen to the same antigen in the same animal. This may account for the presence of modified plaque-producing lymphocytes, which produced plaques, in previous reports (74, 68, 33), because they studied lymph node cells; whereas spleen cells were used in this study. Species differences may also be important in this regard.

Development of plaque-forming cells from the immunoblast through the plasmacyte stages is reflected by the average maturation rating, which increased with each day post-immunization (Table I). This development is asynchronous as indicated by the presence of cells of varying maturation on all days. This asynchrony of differentiation may be due to continuous antigen stimulation for a number of days, and/or because some plaque-forming cells differentiate more rapidly than others. That antibody-forming cells may differentiate at varying rates is indicated by the observation that some plaque-forming cells from the same animal divide much more rapidly than others, and that dividing plaque-forming cells can produce daughter cells of unequal size (87).

Although one blast cell was seen after day 4 in control animals, many proplasmacytes were found on days 5 and 6. Schoenberg *et al.* (140) and Moore *et al.* (105) saw plasma cells releasing segments of cytoplasm and noted a collapse of RER with time, and suggested that at least some

plasma cells regress to lymphocyte-like cells. If this is in fact the case, the proplasmacytes seen on days 4, 5, and 6 in this study may have been developing both toward and away from plasmacytes. This possibility seems unlikely, however, because the cytologic features which they noted (shedding of large pieces of cytoplasm containing distended RER, and collapsed RER in the later stages of the antibody response) were not seen in these cells. Although some 6 day cells had narrow RER (fig. 17), the same feature was seen in 3, 4, and 5 day cells (fig. 19). Proplasmacytes and plasmacytes were observed which had considerable RER that was well ordered and laminated (figs. 11, 17, 19), while others had an equal amount which showed no organization (figs. 9, 13, 40). These may be functionally different, i.e. one may develop into a memory cell (140) or a 7S antibody-producing cell (115); or they may be varying morphologic expressions of the same developmental pattern. Mature plasma cells are generally considered to be end cells with a life span of only a few days (7, 111), but the eventual fate of these cells remains to be elucidated.

The significance of abnormal plasmacytes (APCs) in the life cycle of antibody-producing cells is difficult to determine from these data, but it does appear that they are a distinct cell type. They were observed in all groups (control, ALS, PHA, and D_2O). Harris *et al.* (43) demonstrated a plaque-forming cell similar in appearance to the APCs, but they did not discuss the significance of the cell. APCs may be normal cells that have undergone changes caused by being subjected to the artificial environment of the plaque technique, but a number of points argue against this: 1) pyknosis, and especially a decrease in cytoplasmic organelles (dense bodies, mitochondria, Golgi apparatus), are changes

which probably do not take place during the 60 to 90 minutes they were incubated before fixation; 2) no APCs or cells resembling APCs were seen from 3-day animals; and 3) all APCs had moderate to extensive RER, i.e. they were fairly mature cells. That these cells are in fact abnormal is indicated by their pyknotic nuclei, very few of which contained nucleoli; clumps of dense granules in many of the nuclei, which appear to be disrupted nucleoli; the decreased number of cytoplasmic organelles, especially the lack of extensive Golgi apparatus; and the occurrence of nuclear envelope breakdown. APCs also lacked the cytoplasmic processes seen at the periphery of other plaque-forming cells. The arrangement of much of the RER into evenly distended tubules is atypical, if not abnormal. Thus, because of their morphology and distribution, it is suggested that APCs are degenerated plasmacytes or proplasmacytes, and that they are probably the end stage of the normal life cycle of antibody-producing cells.

The ultrastructure of developing and mature plasma cells has been studied by many investigators (35, 40, 57, 64, 164). The organelles and ultrastructural features of the cells in this study are similar to those reported by others, except for the micropinocytotic processes and the microtubules seen in close association with the nuclei of many of the cells. The micropinocytotic invaginations were usually of the "coated" variety (figs. 7 and 9), which are considered to be localized specializations of the cell membrane for the uptake for specific substances (47). The possibility that at least some of this uptake is related to the immunological functions of these cells is indicated by the finding of a portion of a lysed red blood cell within one of these processes (figs.

38 and 39). This suggests that well differentiated antibody-producing cells interact with antigen in a more complex manner than simple immuno-cyto-adherence (107). These processes may, however, result from the *in vitro* conditions of plaque formation, which would explain why they have not been reported previously. Nevertheless, they are an indication that, as Movat and Fernando (106) suggested, antibody-producing cells are concerned with the uptake as well as the secretion of substrates.

The second unique feature of the plaque-forming cells in this study was the presence of many microtubules in close association with the nucleus. The visualization of microtubules in antibody-producing cells is due to the use of glutaraldehyde for fixation (10, 136). Microtubules observed close to the nuclear envelope of many plaque-forming cells may be related to their rapid rate of mitosis (18), inasmuch as microtubules form the spindle apparatus of dividing cells (47). Microtubules are also considered to be cytoskeletal elements and in plaque-forming cells may be involved in maintaining the shape of nuclei, especially multilobed nuclei (which often show bundles of microtubules at the isthmus between lobes). Microtubules are made up of numerous filamentous subunits (47), suggesting that the bundles of microfibrils seen in some plaque-forming cells may be related to the microtubules seen in these cells. The finding of cytoplasmic filaments close to the nucleus of plasma cells by Weinstock (164) may be related to the microtubules seen in the present study, especially since he used osmium tetroxide as the only fixative. In a recent study, Moore *et al.* (104) examined cultured hematopoietic cells fixed in glutaraldehyde, and found many microtubules, a number of which were closely associated with the nucleus. Hence, the lack of microtubules in previously reported studies of plasma cells is

almost certainly due to the use of osmium tetroxide as the sole fixative.

Examination of Table V indicates further characteristics of the maturation process of 19S antibody forming cells. As the cells mature, mitochondria decrease in both size (not shown in Table V) and number. The appearance of small mitochondria with dense matrices in mature cells is of unknown significance. They suggest mitochondrial degeneration because none were seen in blast cells. Also, cells with few mitochondria tended to have mitochondria with dense matrices. A number of cells showed mitochondria of varying morphology (fig. 7), but the significance of this finding is unknown. Numerous cells with otherwise well preserved structures showed mitochondria with a "washed-out" appearance (figs. 7 and 15). Such mitochondria were also observed by Moore *et al.* in cultured hematopoietic cells fixed with glutaraldehyde (104), suggesting that they resulted from *in vitro* manipulations and/or glutaraldehyde fixation.

Blast cells are significantly larger than more mature cells, but have less RER. Inasmuch as plaque size increases with maturity, the large amount of RER found in proplasmacytes and plasmacytes indicates that RER is necessary for efficient antibody synthesis.

Although the Golgi apparatus of the average antibody-producing cell is very large and complex (figs. 10, 16, and 17), its exact function or functions are still unknown. Rambourg *et al.* (125) found that the Golgi apparatus of virtually every cell in the rat contains glycoproteins, and Uhr and Moroz (161) have shown that the addition of the carbohydrate moiety to antibody occurs on released and completed protein chains. Thus, it appears that at least a major function of the Golgi apparatus in

these cells is the addition of carbohydrate to form the completed antibody molecule. The finding of connections between RER and Golgi vesicles (fig. 8) and between these vesicles and Golgi lamellae (fig. 37) suggests the passage of Golgi-produced carbohydrate (125) to the RER. This suggestion is strengthened by the fact that the Golgi apparatus apparently plays no part in antibody storage and release (see below). However, the demonstration of antibody molecules in Golgi lamellae (40, 89, 129) suggests that the exchange is probably in the opposite direction (from RER to Golgi). This is the direction observed in other protein secreting cells (47).

Many cells were seen with dense lysosome-like bodies within or at the periphery of the Golgi apparatus (figs. 7 and 20), which agrees with the findings of others that the saccules, small vesicles and dense bodies of the Golgi apparatus are part of the lysosomal system (52, 116). These two major functions, addition of carbohydrate to antibody and lysosome formation, may account for at least some of the variability which the Golgi apparatus displays. Extensive development of the Golgi apparatus is apparently not necessary for production and secretion of functional antibody molecules inasmuch as blast cells, which produce some very large plaques, showed relatively small Golgi apparatuses. The Golgi apparatuses of APCs were also small, but, in this case, plaque formation by these cells may represent release of preformed molecules stored in the RER.

A number of mechanisms have been proposed for the release of immunoglobulin from antibody-producing cells. The Golgi complex has been implicated (129, 106), but this seems highly unlikely because in this

and numerous other studies (40, 68, 89, 106, 108) large secretion granules and components of the Golgi apparatus were not seen in contact with the cell periphery of antibody-producing cells as is the case with other protein secreting cells (47). Another proposed mechanism for the release of antibody is by clasmatosis or microclasmatosis, i.e. by the shedding of pieces of cytoplasm containing RER and thus antibody. Evidence for such a mechanism has been seen both in microcinematographic (158) and electron microscope studies (66, 105, 129, 164), indicating that such a mechanism probably exists. Large pieces of cytoplasm seen close to some antibody-producing cells in the present study appeared to be from other spleen cells (figs. 25-28), but none of them were observed containing segments of RER. Thus, although microclasmatosis may be one method of antibody release, it appears that antibodies are not released by this method under the conditions of this study.

Because plasmacytes are end cells with a short life span (111), lysis and destruction of these cells have been suggested as methods of antibody release (158). Lysed cells were observed in the present study, but the significance of these cells is unclear. One possibility is that they are due to damage produced in the preparation of cell suspensions. In both control and D_2O animals, more lysed cells in 5 and 6 day animals were noted, suggesting that some cells on these days may be more fragile than others. Another possibility is that the cells may have been partly disrupted when the animal was sacrificed. That some, if not all, lysed cells were artifacts was indicated by the observation that most were from only a few animals, and that every plaque-forming cell examined

from one animal (not included in this study) was lysed. A similarly treated animal sacrificed at the same time showed no lysed cells.

Many investigators have observed connections between RER and the cell membrane of plasma cells (106, 108, 150, 164). Such an association has also been seen in cultured hematopoietic cells (104), and in fibroblasts (24, 68) where it was considered to be related to the release of collagen. In the present study, an association between RER and cell membrane was observed in about 60% of the cells (figs. 12 and 17), including over one-fourth the blast cells which have only a small amount of RER. In those cases where actual contact was observed, a single membrane was always present, suggesting that if antibody is released in this manner, diffusion or pumping across this membrane is involved. The connections observed between RER and cell membrane are considered to be the principal mode of antibody release from plaque-forming cells observed in this study.

Some antibody-producing cells contain very little RER, as shown in this and other studies (33, 68, 74). Leduc *et al.* (89) demonstrated that the first antibody which appears in such cells is in the perinuclear space. Thus, it would seem that at least some of the antibody secreted by these cells must have been produced on the free polyribosomes from which completed chains are released into the cytoplasmic matrix. These completed antibody molecules are probably released by direct diffusion across the plasma membrane. Such a mechanism either does not operate, or secretes only a small amount of antibody in more mature antibody-producing cells, because such cells have relatively few free ribosomes, and almost no polyribosomes (figs. 16, 17 and 20).

It is concluded from these data that antibody is released from plaque-forming cells in 3 possible ways: 1) diffusion or pumping from RER cisternae to the extracellular space across a single membrane produced by fusion of small segments of RER and cell membrane; 2) by diffusion or pumping across the cell membrane directly from cytoplasmic matrix to extracellular space (primarily in immature cells); and 3) by lysis of antibody-containing cells (due at least in part to disruption of viable cells).

Macrophages are thought to take part in the induction of the 1° immune response (111). They probably act by transferring either specific information (RNA or RNA-antigen complex) or molecular fragments of antigen to potential antibody-producing cells (54). Macrophages surrounded by lymphocytic cells have been observed in lymphoid tissue (142, 158). Schoenberg *et al.* (139) studied such structural units with the electron microscope and found areas of direct communication between the cytoplasm of macrophages and lymphocytic cells. In a number of instances in the present study, pieces of cytoplasm were seen adjacent to plaque-forming cells. These pieces of cytoplasm were considered to be from non-plaque-forming spleen cells because of their general appearance and because they did not contain RER similar to that of the adjacent plaque-forming cells. In most instances they were not large enough to allow identification as to the cell of origin, but the cytoplasm adjacent to the cell in Figures 25 and 26 appears as though it could have been from a macrophage. A considerable amount of the periphery of this piece of cytoplasm and cell membrane of the plaque-forming cell are closely opposed or in contact. The fact that this and other pieces of cytoplasm

adhered to plaque-forming cells even after the preparation of cell suspensions and plating of the cells, indicates that there must have been strong bonds between the plaque-forming cells and the cells from which the pieces of cytoplasm came. Thus, it appears that some type of intimate relationship probably exists between at least some mature antibody-producing cells and other cells of the spleen.

Cunningham *et al.* (33) found that all cells capable of producing plaques also demonstrated immuno-cyto-adherence and cluster formation when complement was not present. In the present study many plaque-forming cells had red cell ghosts very close, or attached to their peripheries (fig. 36), suggesting that these cells were also capable of cluster formation. In a few instances, intact red blood cells were seen in the area of plaque-forming cells. Some of these were probably from the spleens used to obtain plaque-forming cells, but others demonstrated small blebs or projections, approaching or contacting plaque-forming cells (figs. 29 and 52), indicating that they were sheep red blood cells attracted to antibody attached to the surface of the plaque-forming cell. It is not known why these sheep red blood cells were not lysed, but it probably resulted from variable surface antigens. The finding of such projections suggests that at least some antibody is bound to the surface of 19S antibody secreting cells, and that the attraction between this antibody and the corresponding antigen (sheep red cell membrane) is very strong. Blebs were probably produced by this attractive force after the cells became fixed in the agar.

Myelin figures, varying considerably in size and appearance, were seen in many cells (figs. 13, 35, and 45). They were generally found in

cells processed at the same time (for example, cells from control animal 5A and the ALS treated animals were fixed on the same day and showed similar myelin figures), and thus are considered fixation artifacts.

Virus-like particles (VLPs) were observed in approximately half the cells examined in this study (figs. 10, 19, and 44). Inasmuch as only 1 to 3 sections of many of the cells (and never more than 5) were photographed, the actual percentage of cells which contained VLPs must be much higher. All VLPs observed were type A particles (12) which lack the central nucleoid thought to contain the reproductive nucleic acid. Thus these particles would appear to be incomplete and non-infectious. Although many VLPs were observed budding into RER cisternae and smooth-surfaced vesicles, none were seen budding from the peripheral cell membrane, further supporting the idea that these were non-infectious particles. That the presence of VLPs did not decrease the ability of these cells to produce and secrete antibody is suggested by the fact that no correlation could be found between the presence or number of VLPs in a cell and the size of the plaque produced by the cell. Also, animals which showed both high and low percentages of cells with VLPs produced similar numbers of plaques. VLPs observed in these cells may have been present in the animals before immunization, or they could have been introduced at the time of immunization. The latter possibility is raised because previous studies in this lab have indicated that washed sheep red blood cells can introduce virus into BALB/c mice.

Cells from D_2O treated animals showed some differences from control cells, but these were all quantitative (i.e. no characteristic or typically abnormal structures were seen in these cells). On an individual

basis D_2O cells were indistinguishable from control cells. The increased numbers of lysed cells from D_2O treated animals appears to have been due to mechanical trauma experienced during the preparation of cell suspensions for 2 reasons: 1) almost 80% of the lysed cells came from only 5 animals; and 2) none of the lysed cells were from the first set of D_2O treated animals (28 cells). It may be that D_2O treatment increased the fragility of plaque-forming cells, accounting for some of the increase in their incidence, but this cannot be determined from these data. In this series, 26 cells were lysed, but only 1 was from a 3 day animal, further suggesting that plaque-forming cells are less fragile during early stages of antibody production.

D_2O treated animals showed an increase in the average number of mitochondria per cell. This was noted in all cell types, but the biggest increase was noted in blast cells which averaged 5 more mitochondria per cell than untreated blast cells. Mitochondria from D_2O treated animals were indistinguishable from mitochondria from control animals in regard to structure and variability. Thus, it appears that treatment with 30% D_2O decreases the efficiency of mitochondria, resulting in increased numbers being found in these cells. However, the average size of the plaques produced by D_2O treated cells was not lower than that produced by control cells, indicating that D_2O treated cells receive the energy necessary for maximal antibody production. This, together with the fact that the average maturation of D_2O cells on each day was similar to controls, indicates that D_2O treatment, in that it depresses antibody production, does so by decreasing the rate of proliferation of antigen-stimulated cells. Alternatively, D_2O could inhibit the afferent side of

the antibody response, i.e. the handling of antigen and the stimulation of potential antibody-producing cells. D_2O has been shown to decrease the ability of leucocytes to proliferate and differentiate (120), and this appears also to be true of antibody-forming cells.

Elves (45) and Petronye *et al.* (121) have shown that the immunosuppressive effect of PHA occurs only when PHA and antigen are administered i.p., and that it is due to the effect of PHA on the peritoneal cavity and not on the immunologic apparatus, and results from the failure of antigen to reach lymphoid organs. Thus, as was observed in the present study, one would not expect to find morphologic or even quantitative differences between plaque-forming cells from PHA and untreated animals. Any differences noted were within the range of normal variation, or due to fixation artifacts (cells with very clear cytoplasm and detached pieces of cell membrane). The lower average maturation of the cells from PHA treated animals (2.9 compared to 3.3 for corresponding control cells) is probably related to decreased antigen reaching lymphoid organs, because both PHA and sheep red blood cells were given intraperitoneally. Koros *et al.* (87) have shown that the rate of cellular proliferation during early stages of a primary antibody response is dependent on the dose of antigen. The lower average maturation of the cells from PHA treated animals suggests that lower doses of antigen also decrease the rate of maturation of these cells. The depressed numbers of cells is thus probably due both to fewer lymphocytes being stimulated, and to fewer progeny being produced from those cells which are stimulated. Inasmuch as more mature cells were found to produce larger plaques than less mature cells, the decreased rate of maturation must also add to decreased

circulating 19S antibody in PHA treated animals.

The general morphology of cells from ALS treated animals was similar to that of control cells. Despite the fact that many factors (besides the amount of antibody secreted) can effect plaque size (71), the average size of plaques produced by the ALS treated cells (4.0 as compared to 3.4 for control cells) is a good indication that at least for short intervals, ALS treated cells are able to secrete as much antibody as control cells. That these cells can produce equal amounts of antibody over longer periods of time is indicated by the fact that ALS treated cells contained approximately the same amount of RER as control cells and thus had about the same amount of stored antibody. The principal difference noted between cells from ALS treated animals and 4 day cells from control animals was the average maturation of the two groups (2.9 for ALS cells compared to 3.3 for control cells). This indicates that those cells in ALS treated animals which are stimulated to produce antibody and develop into plasma cells probably do not mature as rapidly as similar cells in untreated animals. It seems likely that such cells may also proliferate at a reduced rate, giving fewer progeny cells, and resulting in less antibody production. It has been suggested that ALS acts primarily on early events of the antibody response (102, 121). The reduced average maturation of ALS treated cells indicates that there is at least some effect on the stimulated cells during the peak period of 19S antibody production which probably contributes to the decreased numbers of antibody-producing cells seen in these animals. Agnew has demonstrated that the immunosuppressive effect of ALS is due not only to a

decrease in number of small lymphocytes available to carry out an immune response, but also to decreased immunological competence of the remaining small lymphocytes. The decreased average maturation of the cells from ALS treated animals noted in the present study indicates that one manifestation of this decreased immunological competence may be slower maturation and proliferation of these cells. This might account for decreased immunologic responses in ALS treated animals which showed no decrease in lymphocytes (78).

SUMMARY AND CONCLUSIONS

Ultrastructural examination of primary 19S antibody-producing cells obtained by the Jerne plaque technique on days 3, 4, 5, 6, and 7 post-immunization, revealed a developmental pattern similar to that described by others working with lymph node or spleen sections from immunized animals. The cells observed were classifiable as immunoblasts, plasmablasts, proplasmacytes, plasmacytes, abnormal plasmacytes, and lysed cells that could not be classified. The proportion of mature cells increased with each day post-immunization, but the response was asynchronous, with a few plasmacytes appearing on day 3 and plasmablasts appearing as late as day 6 post-immunization. Abnormal plasmacytes, as described in the present study, have not been reported previously. Because many ultrastructural features of the abnormal plasmacytes suggested degeneration, these cells are considered to be dying, and represent the end state in the life cycle of antibody-producing cells. They were seen in controls and all three experimental groups. Lysed cells were judged to be the result of physical trauma produced during the preparation of cell suspensions. Micropinocytotic processes and numerous microtubules in close association with the nucleus were observed in many cells in the present study. These have not been previously reported in antibody-producing cells. Other ultrastructural features of these cells were similar to those of previously reported immunologically competent cells.

Ultrastructural evidence suggested 3 modes of antibody release from plaque-forming cells: 1) by a transient communication between rough endoplasmic reticulum and cell membrane, whereby antibody diffuses or is

pumped across the resulting single membrane - close association or contact was observed in over 50% of the cells, 2) by direct passage from cytoplasm to the exterior of the cell in immature cells, and 3) by cell lysis resulting from physical trauma. Microclasmatosis or shedding pieces of cytoplasm containing RER, and release via the Golgi apparatus (possible modes of antibody release suggested by others) were not observed in these cells.

The immunosuppressants used (deuterium oxide, phytohaemagglutinin, and antilymphocyte serum) did not produce characteristic changes in the morphology of plaque-forming cells. However, cells from D_2O treated animals did show 3 to 5 more mitochondria per cell than similar control cells, suggesting that D_2O treatment decreases the efficiency of individual mitochondria. Plaque-forming cells from D_2O treated animals produced plaques averaging approximately the same size as those produced by control cells, indicating that D_2O cells receive the energy necessary for maximal antibody production and release.

In the presence of PHA and ALS fewer mature cells were observed as compared with untreated animals taken on the same day post-immunization. Inasmuch as both PHA and antigen were administered intraperitoneally, the effect produced by PHA was probably caused by reduction of the amount of antigen reaching the lymphoid organs. The occurrence of fewer mature cells in ALS treated animals than in controls suggests that one effect of ALS may be to decrease the rate of maturation (and probably proliferation) of antibody-forming cells.

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ABBREVIATIONS

B - cytoplasmic bleb

C - centriole

DB - dense body

DC - extra-dense chromatin

F - microfibrils

G - Golgi apparatus

GS - Golgi saccule

GV - Golgi vesicle

GVa - Golgi vacuole

Gr - granules

M - mitochondria

MP - micropinocytotic process

MT - microtubule

MY - myelin figure

N - nucleus

NB - nuclear body

NE - nuclear envelope

NH - nuclear hof

No - nucleolus

NP - nuclear pore

P - polyribosome

RBC - red blood cell

RER - rough endoplasmic reticulum

RCG - red cell ghost

Va - vacuole

Vil - villus-like process

VLP - virus-like process

scale marker on each picture = one micron (1 μ)

Figure 1. 3 day immunoblast - control. The cytoplasm is packed with free ribosomes, with many being grouped into polyribosomes. A few mitochondria can be seen close to the nucleus. Very little RER is present, and the cisternae are not dilated. The nucleus is "blast-like", with most of the heterochromatin in a thin rim along the periphery. The large nucleolus is attached to heterochromatin at the periphery of the nucleus.

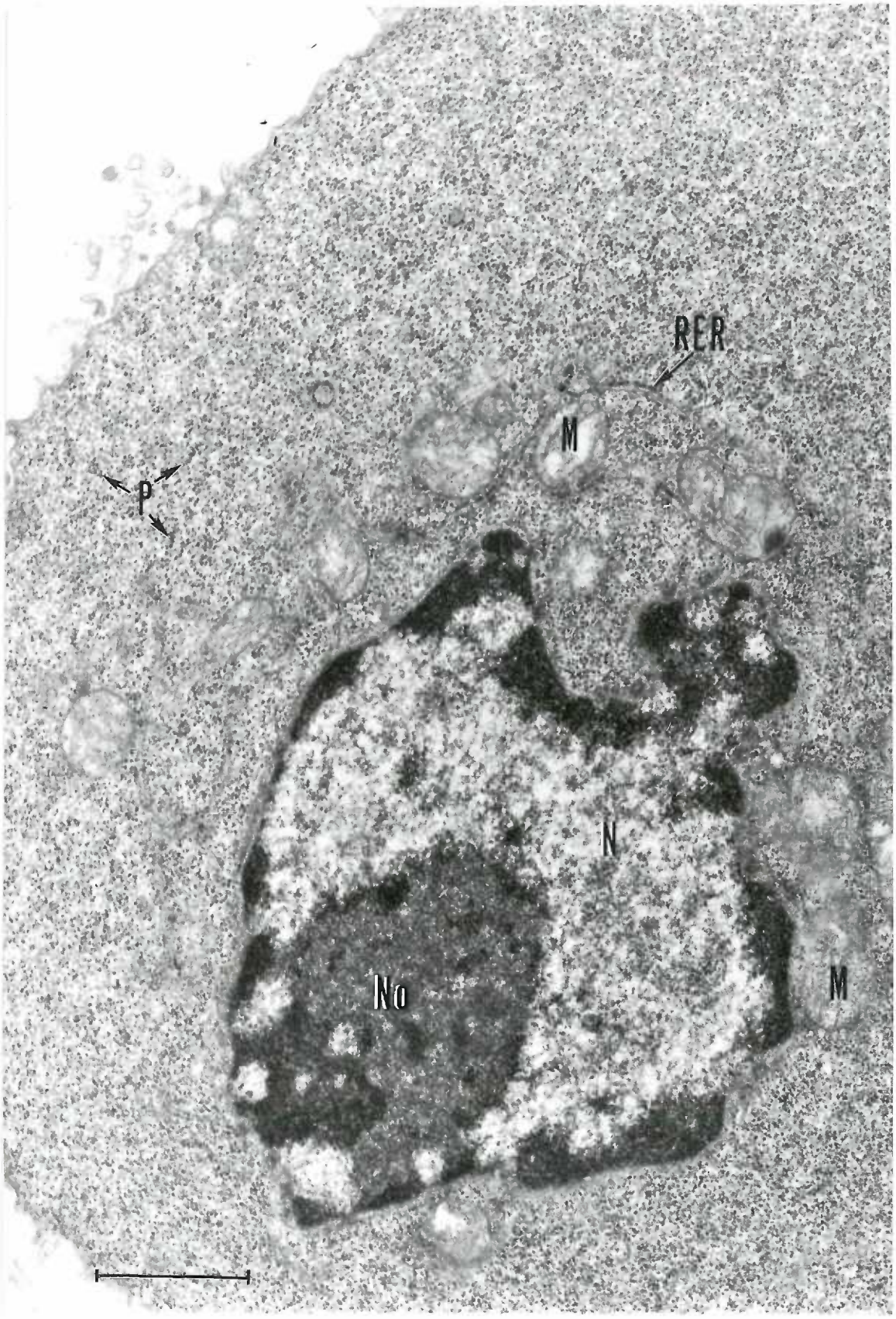

RER
M
P
N
No
M

Figure 2. 3 day immunoblast - control. The cytoplasm is packed with free ribosomes, and although there is slightly more RER than in the previous cell, the cisternae are still not distended. The nucleus is very large and "blast-like". The large nucleolus is attached to heterochromatin at the periphery of the nucleus. A few mitochondria are present.

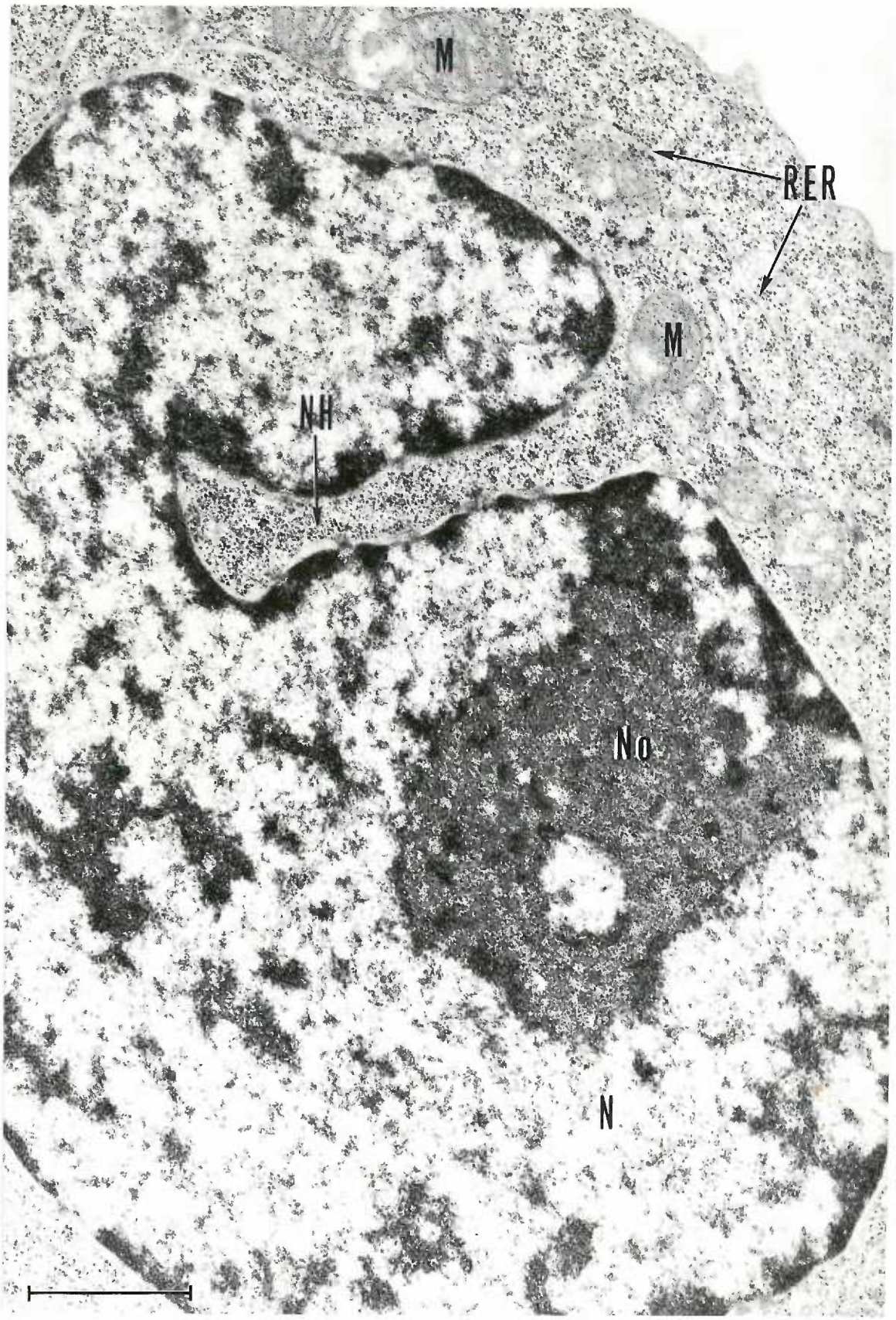

M
RER
M
NH
No
N

Figure 3. 3 day plasmablast - control. This cell shows 2 nuclear lobes and a small amount of nucleolus. Cytoplasm is packed with ribosomes and a number of spiral polyribosomes can be seen. A number of mitochondria are grouped in the center of the cell. This cell contains more RER than the previous cells, and a few profiles show some distention.

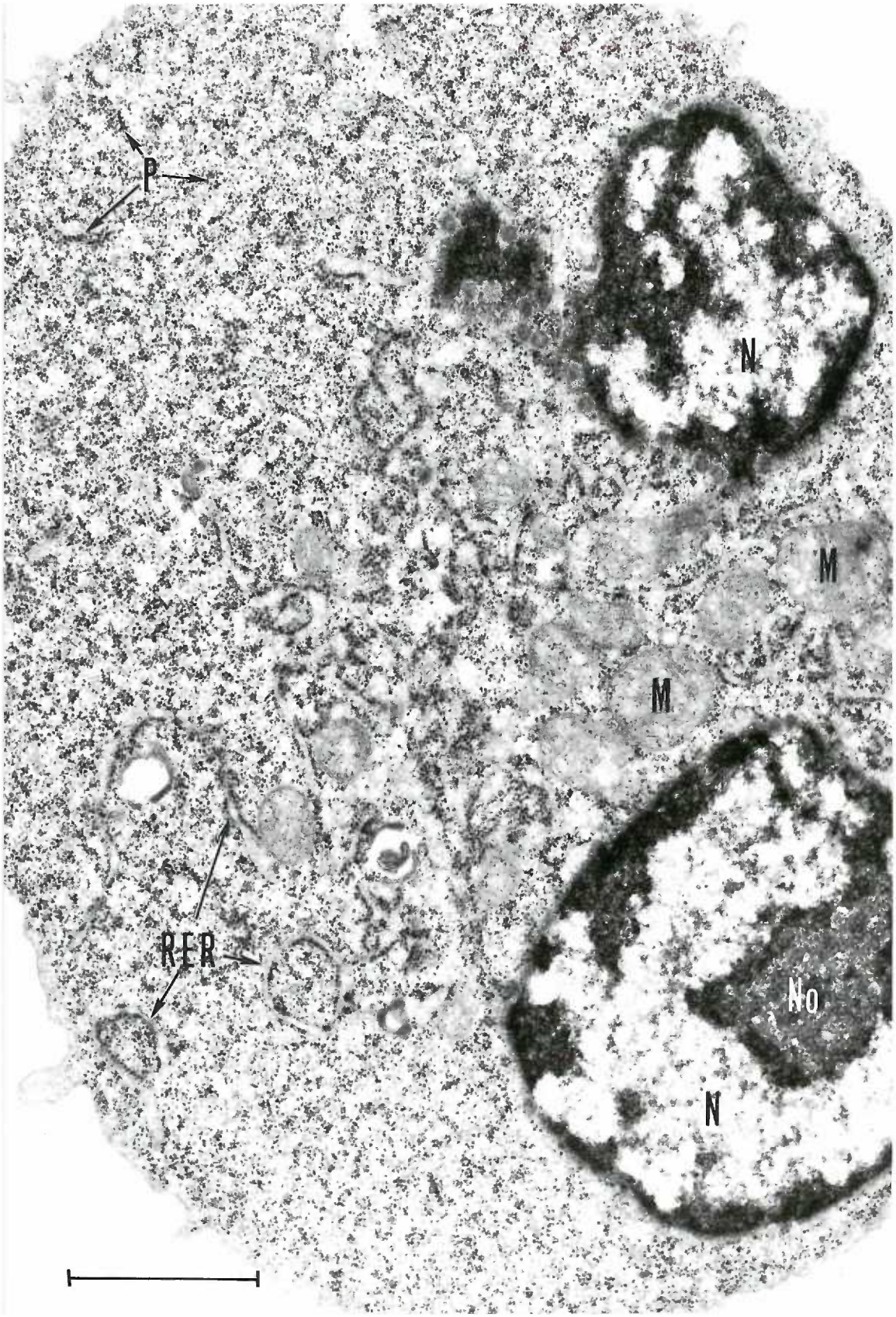

P
N
M
M
RER
No
N

Figure 4. 3 day plasmablast - control. This cell contains a moderate amount of RER, with some portions showing considerable distention. Mitochondria are large and have a "washed out" appearance. The nucleus is slightly peripheral but very "blast-like" in appearance. The large nucleolus is attached to heterochromatin only at the periphery of the nucleus. Numerous nuclear pores can be seen. Two virus-like particles are present.

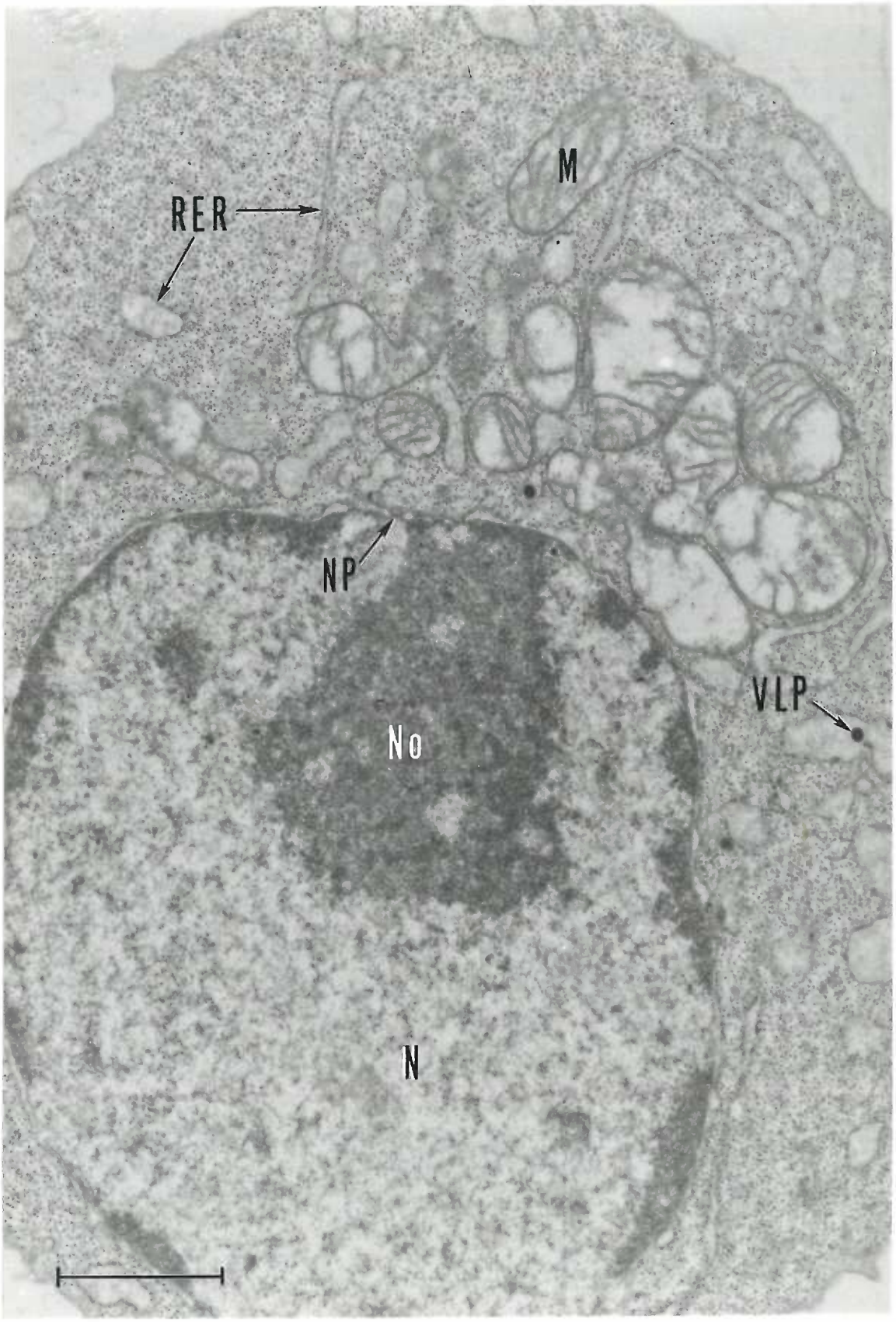

M
RER
NP
VLP
No
N

Figure 5. 3 day plasmablast - control. This cell has a "blast-like" nucleus, but more RER than previous cells. Many profiles are long and parallel to the cell membrane. The Golgi apparatus is quite large, with vesicles and distended saccules being prominent. There is a bleb in the outer nuclear membrane into the area of the Golgi apparatus (double arrow). Many mitochondria are present close to and within the Golgi apparatus.

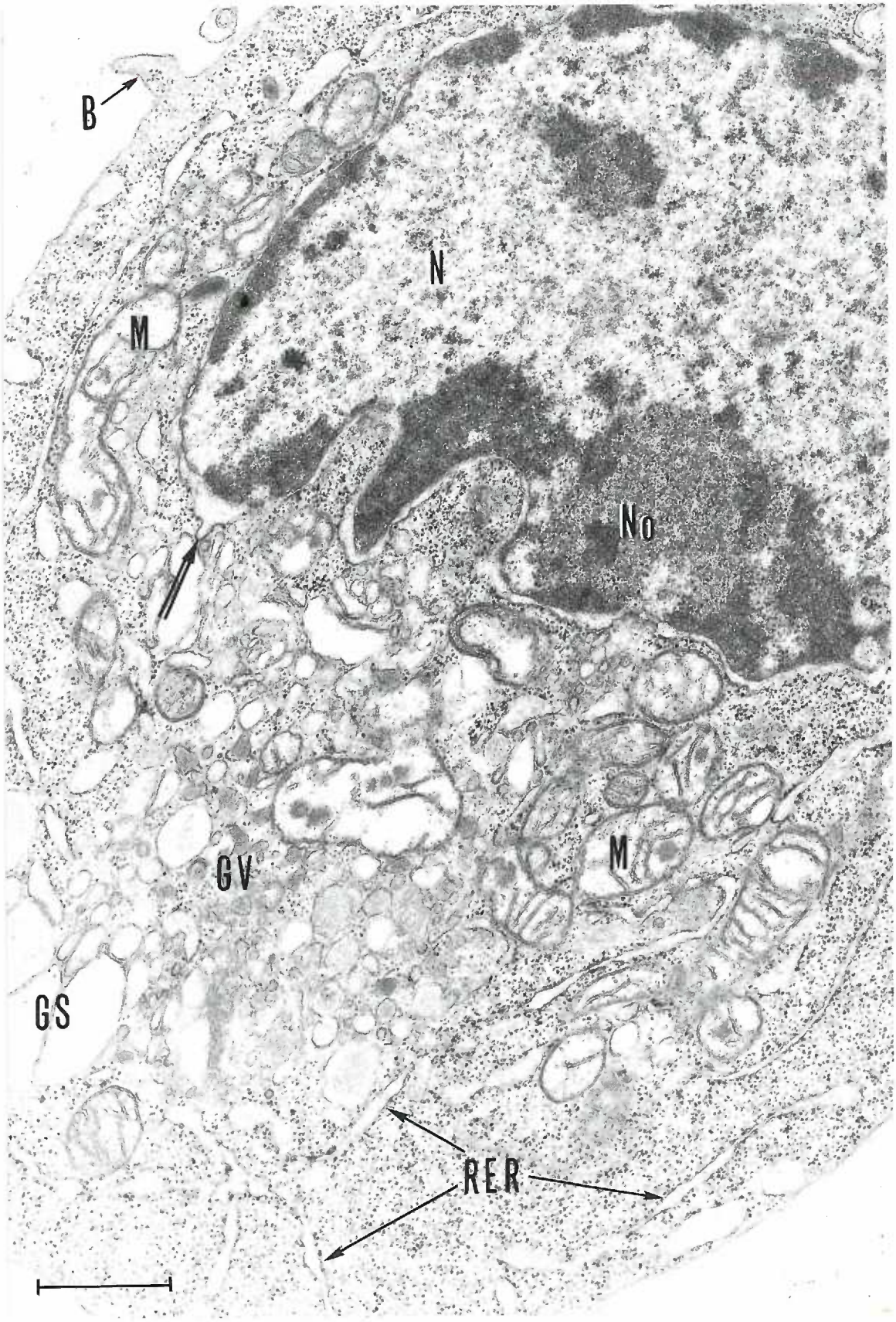

B
N
M
No
M
GV
GS
RER

Figure 6. 3 day plasmablast - control. This cell contains Golgi apparatus, a centriole, numerous microfibrils, microtubules associated with the nucleus and the centriole, a dense body, mitochondria, some RER and many free ribosomes. Some of the microfibrils are associated with the centriole, while others are free in the cytoplasm.

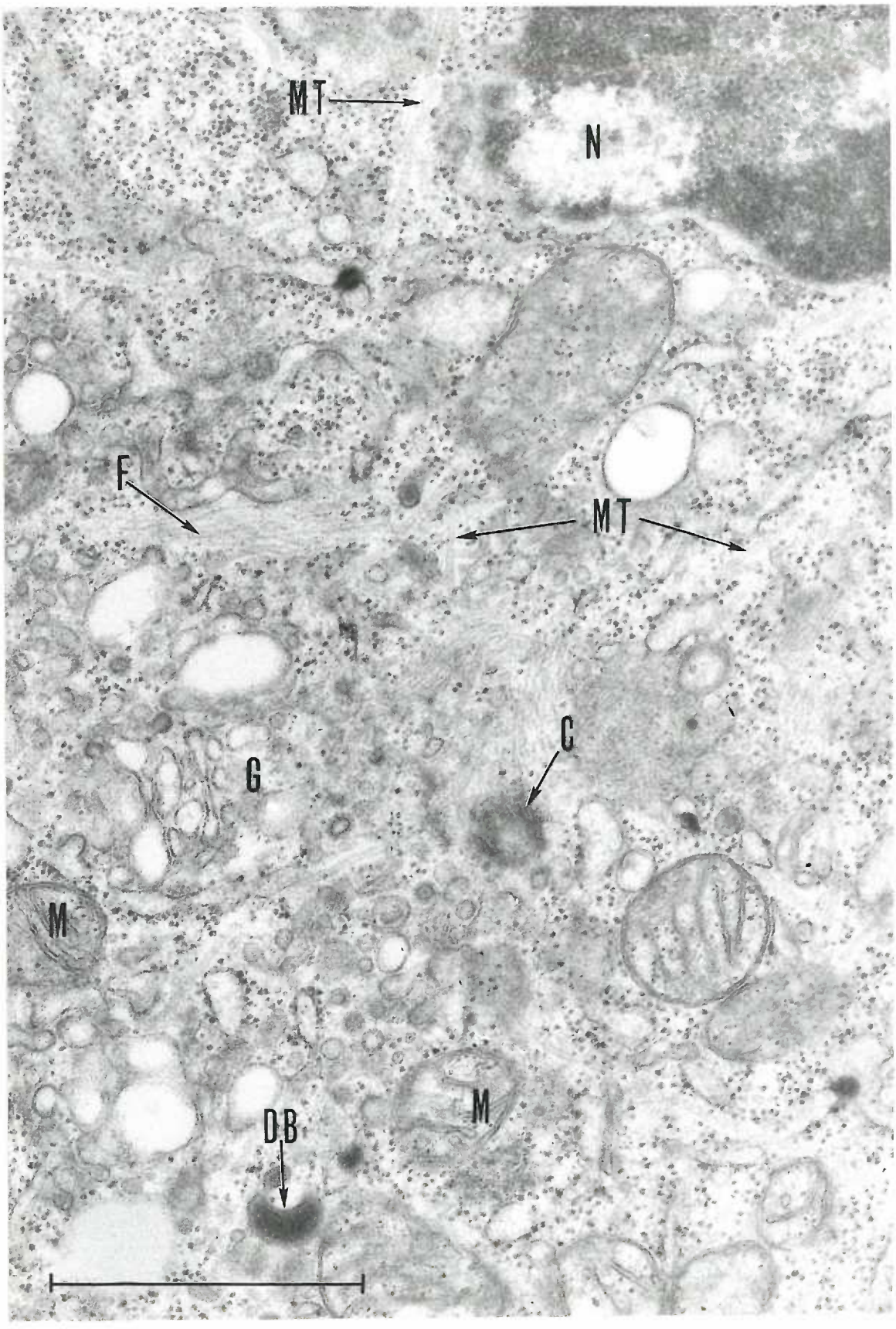

MT
N
F
MT
C
G
M
M
DB

Figure 7. 3 day proplasmacyte - control. This cell has more RER and fewer polyribosomes than less mature cells (figs. 1-6). Most of the RER is seen as long profiles which are slightly distended. Some of the mitochondria have a "washed-out" appearance. A few dense bodies with limiting membranes are located close to the nucleus. A micropinocytotic process is also present (double arrow).

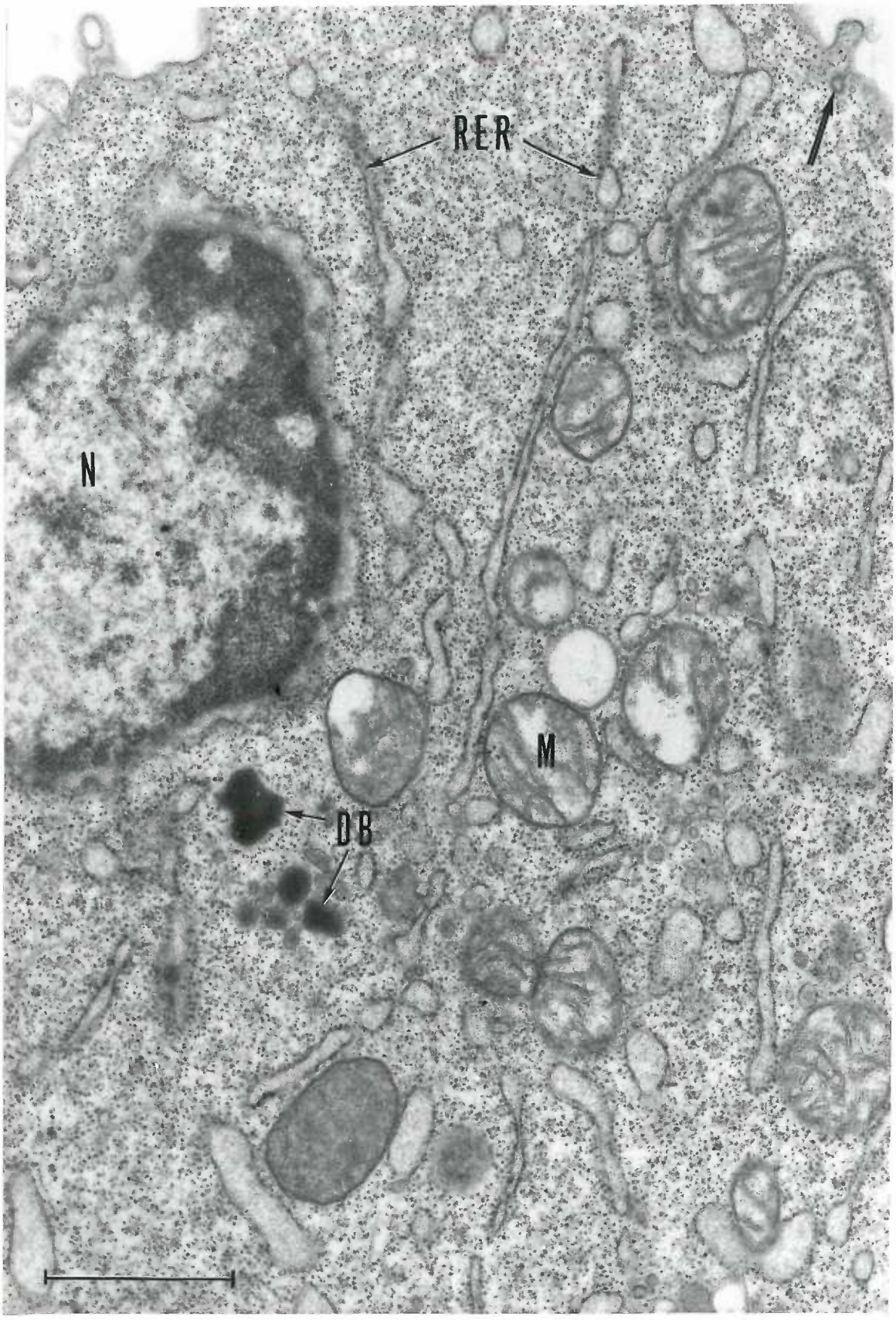

RER
N
M
DB

Figure 8. 3 day proplasmacyte - control. The RER in this cell is ordered in a laminar form and has an even, moderate amount of distention. One portion of RER is in contact with the cell membrane (double arrow). Two areas of Golgi apparatus are present, both close to the nucleus. Golgi vesicles can be seen emptying into or forming from RER. The elongated nucleus contains clumps of extra-dense chromatin, and numerous nuclear pores are present. Mitochondria are grouped around the Golgi apparatus.

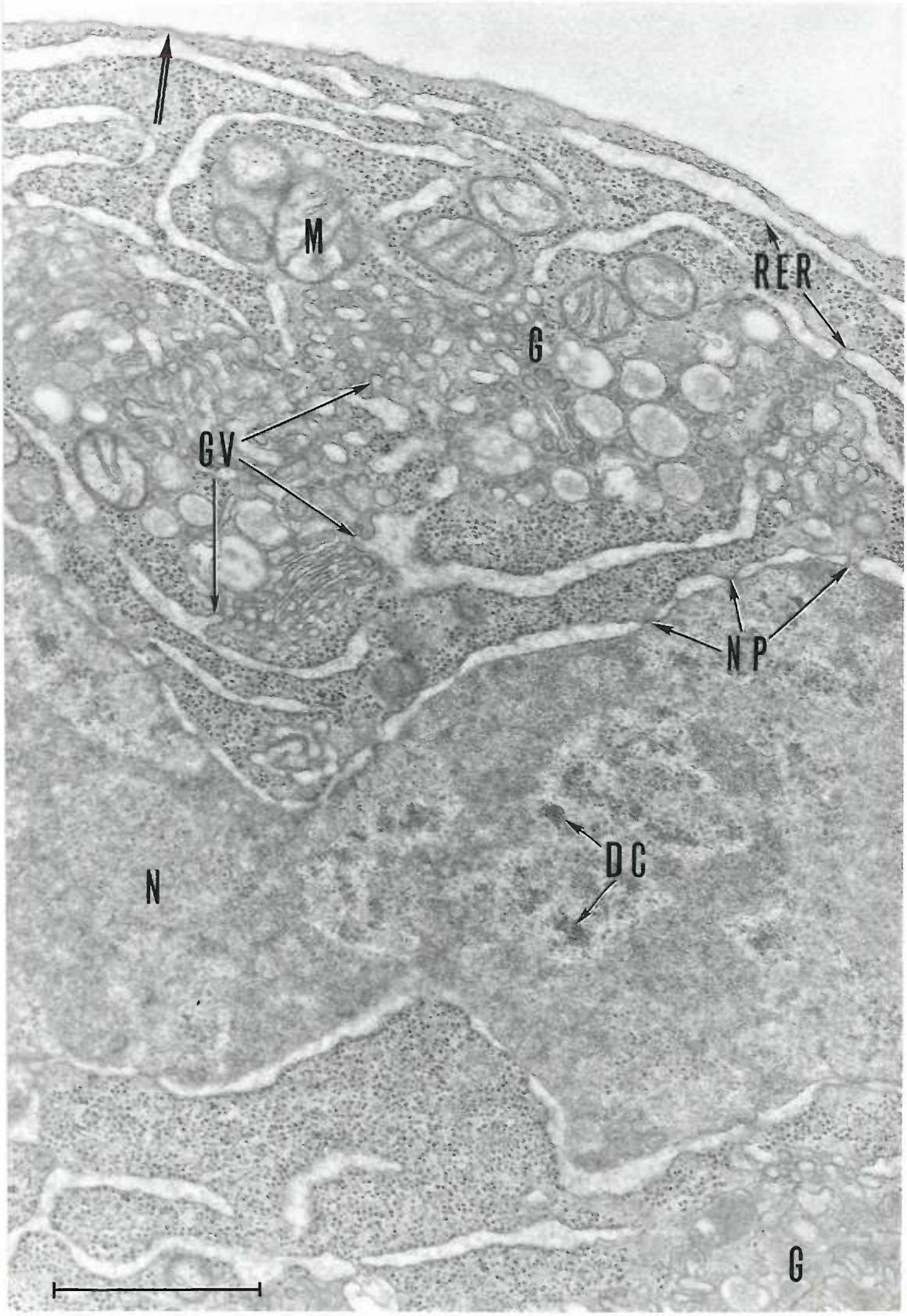

M
RER
G
GV
NP
DC
N
G

Figure 9. 5 day proplasmacyte - control. The RER shows no order and varies greatly in amount of distention and density of the intracisternal material. A micropinocytotic process can be seen at the periphery of the cell. The mitochondria have dense matrices and some cristae are slightly dilated. There are a few polyribosomes in the cytoplasm. The nucleus shows moderate chromatin clumping, and one nuclear pore is present.

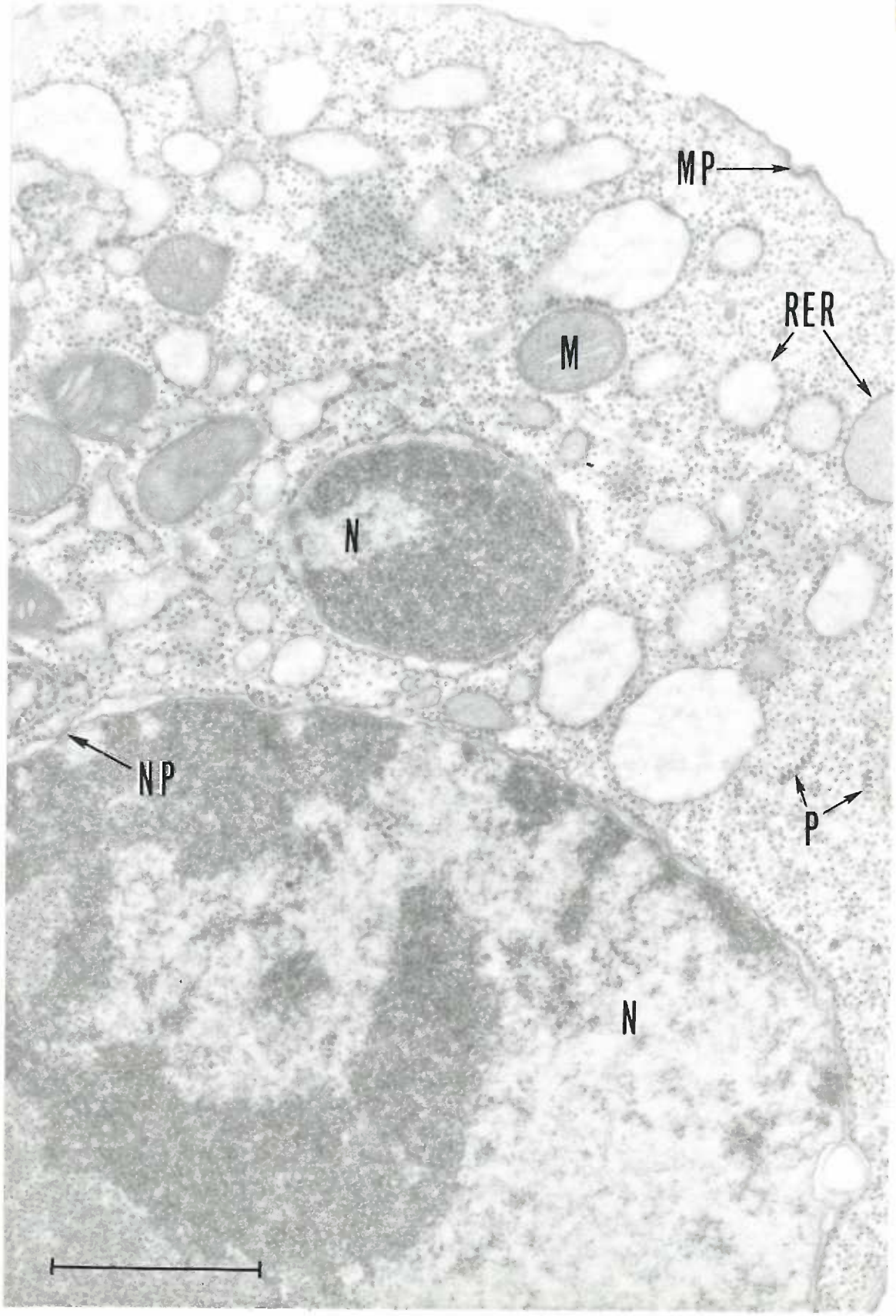
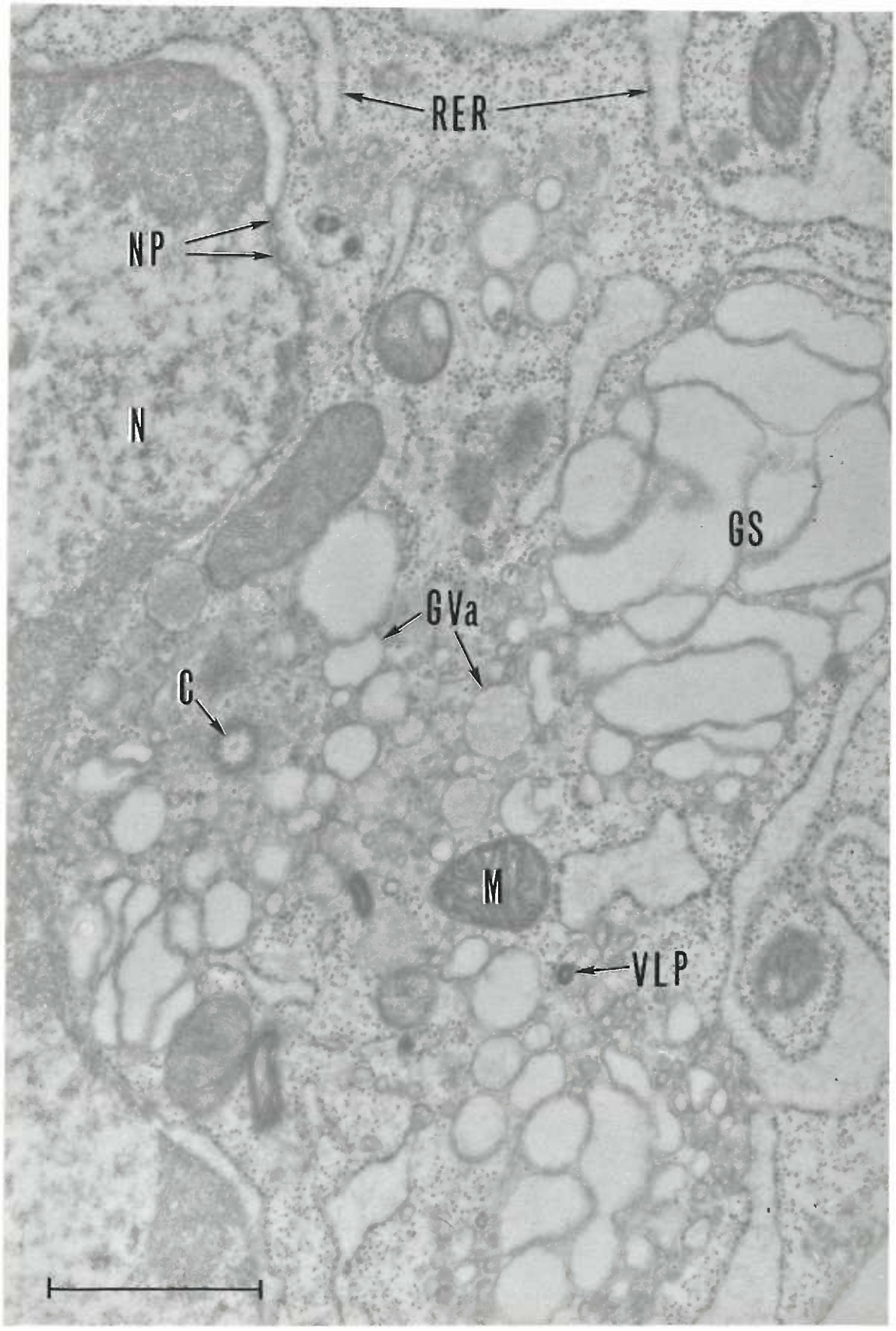

MP
RER
M
N
NP
P
N

Figure 10. 3 day proplasmacyte - control. The large Golgi apparatus is composed mostly of vacuoles and distended saccules. A few Golgi vacuoles containing a slightly electron-opaque material are present. One centriole and a virus-like particle are within the Golgi apparatus. Mitochondria are within or close to the Golgi apparatus. RER is quite distended and there are only a few free ribosomes in the cytoplasm. Numerous nuclear pores are present.


RER
NP
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GS
GVa
C
M
VLP

Figure 11. 7 day proplasmacyte - control. This cell has well ordered, slightly distended RER. A fairly large Golgi apparatus is located next to the nucleus. One dense body and a few mitochondria are present. Cytoplasmic blebs can be seen at the surface of the cell. The nucleus has thick chromatin clumping and is peripheral in position.

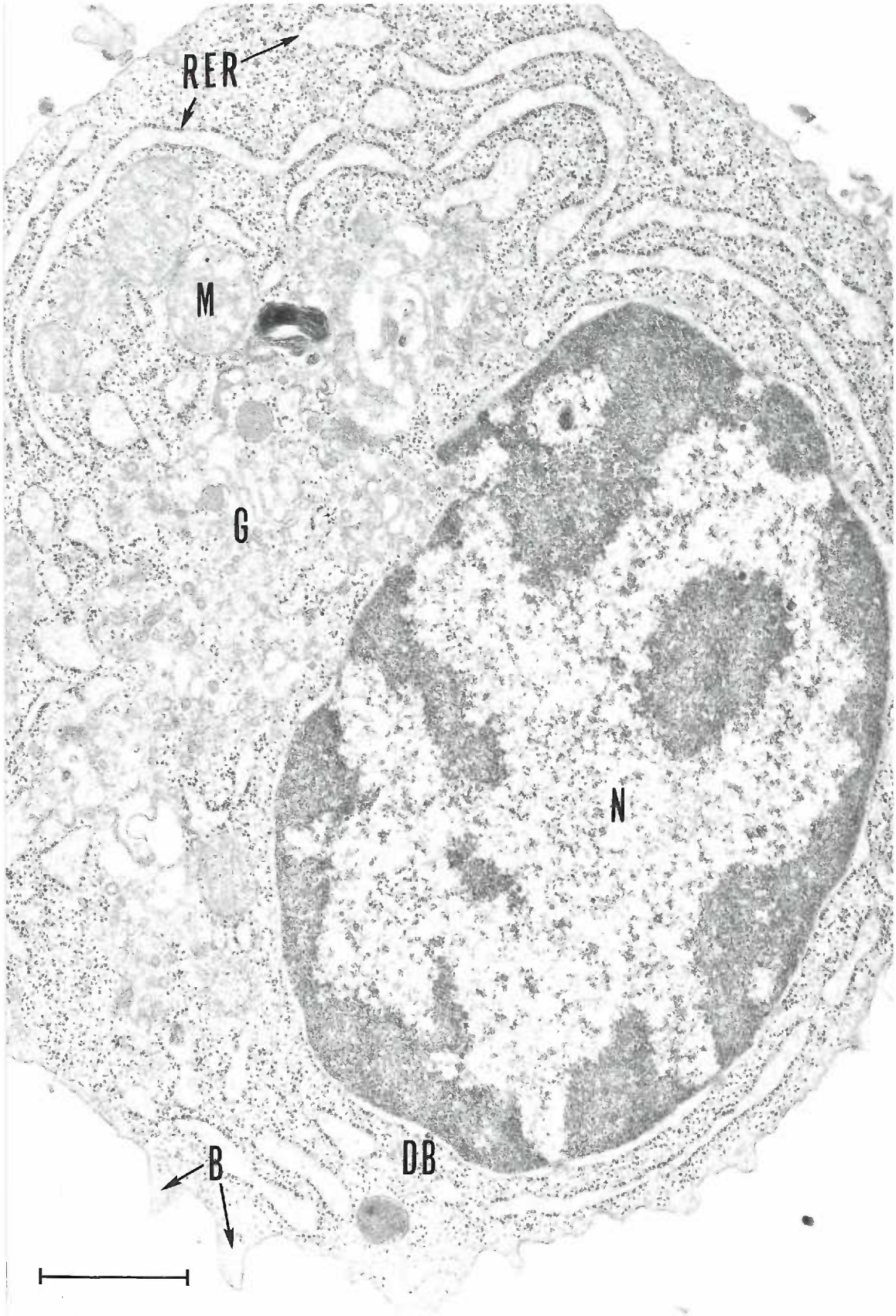

RER
M
G
N
B
DB

Figure 12. 4 day proplasmacyte - control. A portion of RER and peripheral cell membrane appear to be fused (double arrow). The Golgi apparatus contains distended saccules and filled vacuoles. Three lobes of the nucleus are present. Two virus-like particles and one mitochondria are present. The RER is quite distended and contains moderately electron-opaque material.

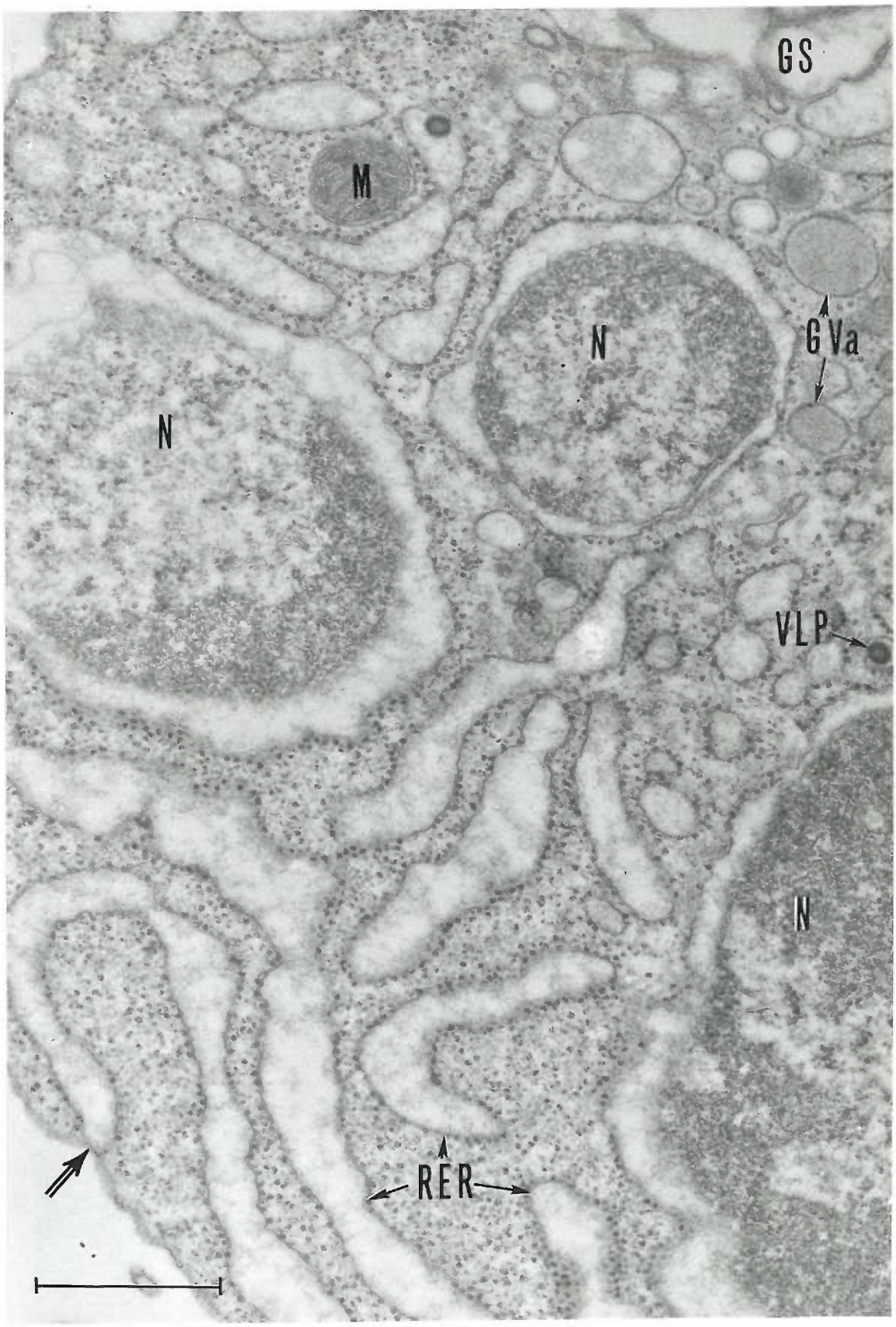

GS
M
N
GVa
N
VLP
N
RER

Figure 13. 5 day proplasmacyte - control. RER is moderately distended, and is in short or rounded profiles. There is one myelin figure and two pieces of membrane bound cytoplasm (double arrows), one containing RER. The mitochondria have distended cristae and dense matrices. Narrow Golgi saccules containing electron-opaque material are present. A portion of the nucleus is present.

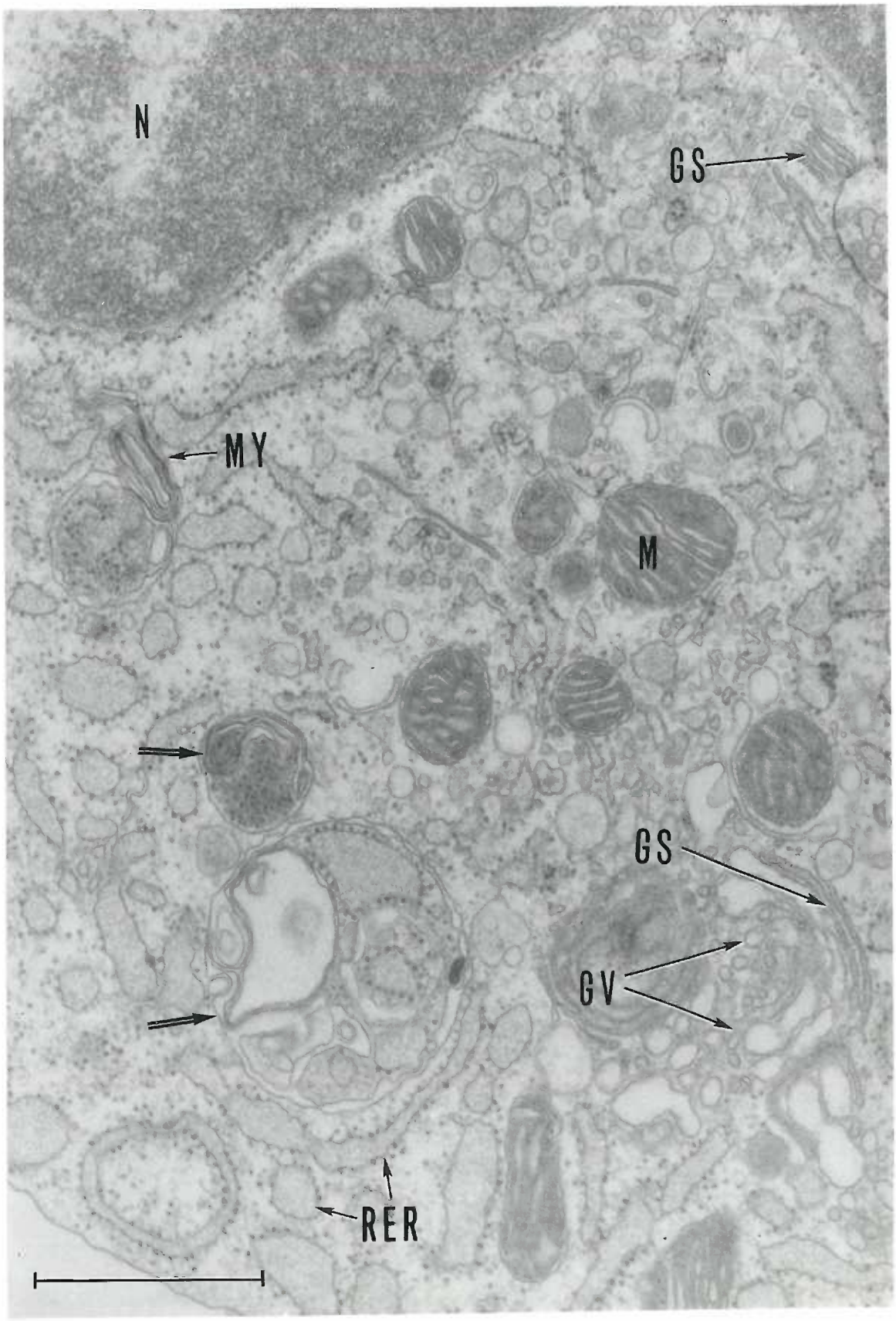

N
GS
MY
M
GS
GV
RER

Figure 14. 5 day proplasmacyte - control. This cell contains microtubules which appear to join 2 portions of the nucleus across a nuclear hof. The upper lobe of the nucleus contains pieces of membrane in the euchromatin (double arrow). RER varies considerably in the amount of distention. Three virus-like particles and a small amount of Golgi apparatus are present. Mitochondria are close to Golgi apparatus and nucleus.

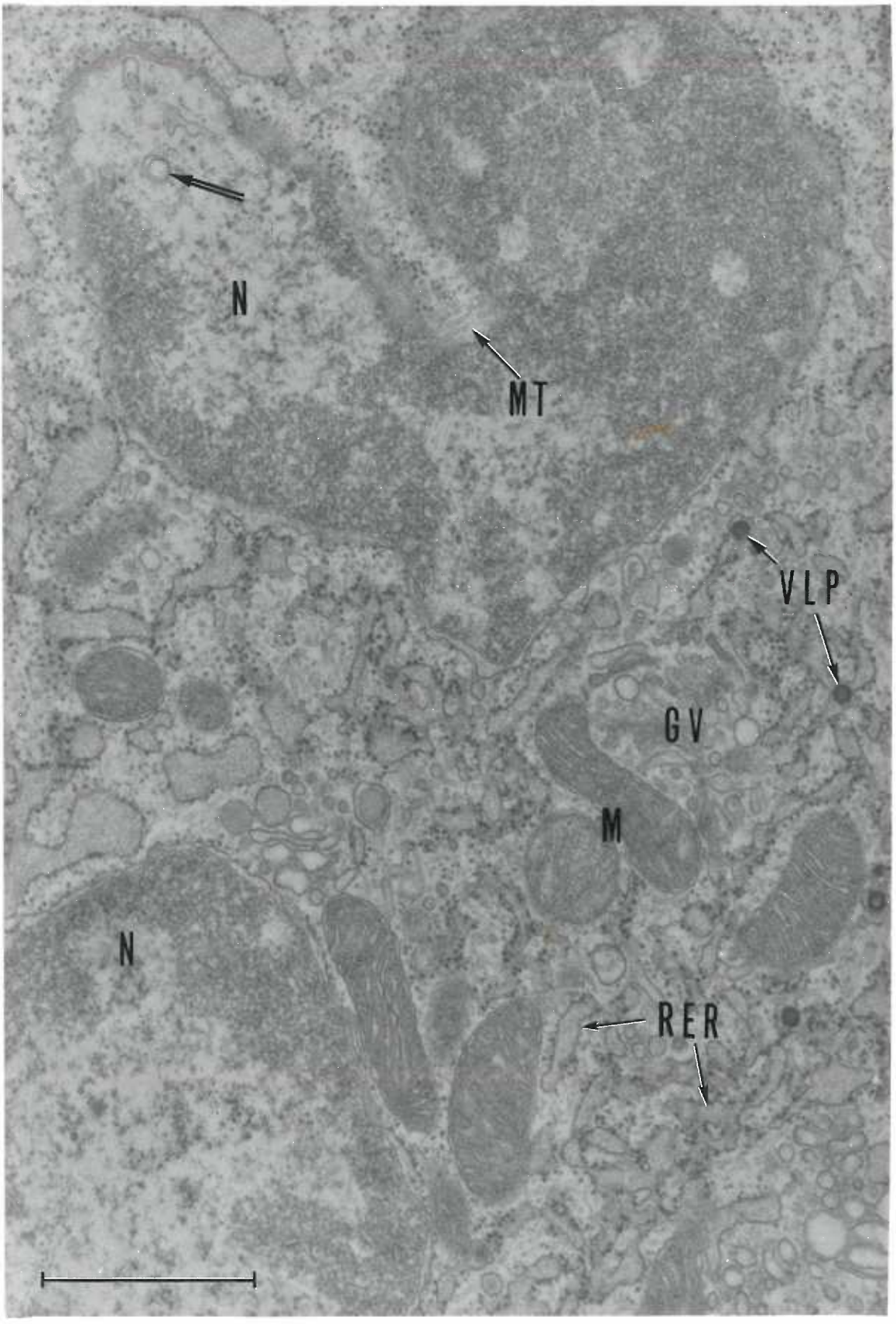

N
MT
VLP
GV
M
N
RER

Figure 15. 3 day proplasmacyte - control. Long microtubules can be seen lying parallel and close to the nucleus. A few microtubules not associated with the nucleus are also present. Some portions of RER are very distended while others show almost no distention. Free ribosomes are numerous. Distended, almost empty-appearing Golgi saccules are also present.

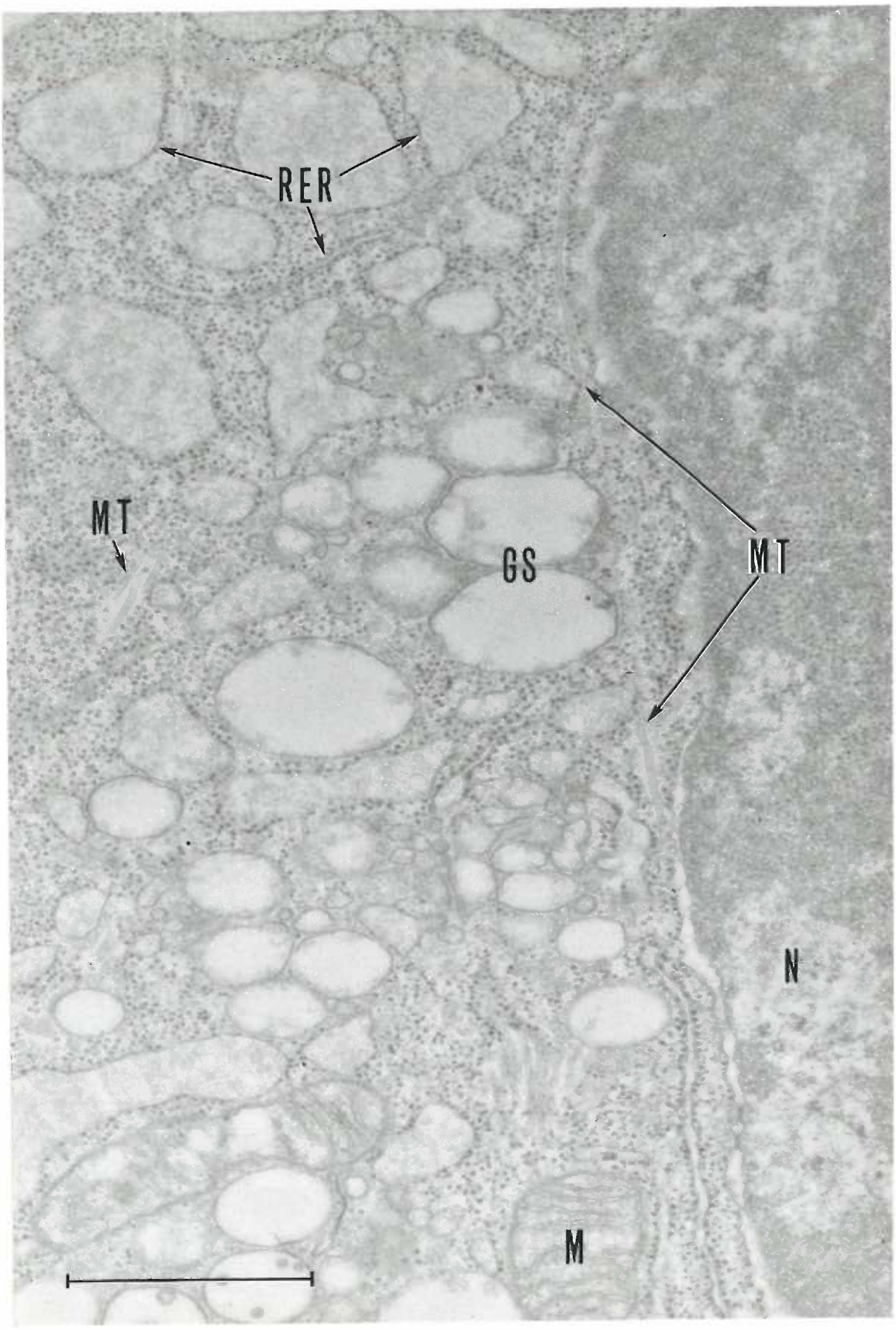

RER
MT
GS
MT
N
M

Figure 16. 6 day plasmacyte - control. This cell has very distended, ordered RER. Mitochondria are located close to the nucleus and the large Golgi apparatus. Dense bodies are both within and at the periphery of the Golgi apparatus. There are 3 lobes of the nucleus with many microtubules in the areas between the lobes.

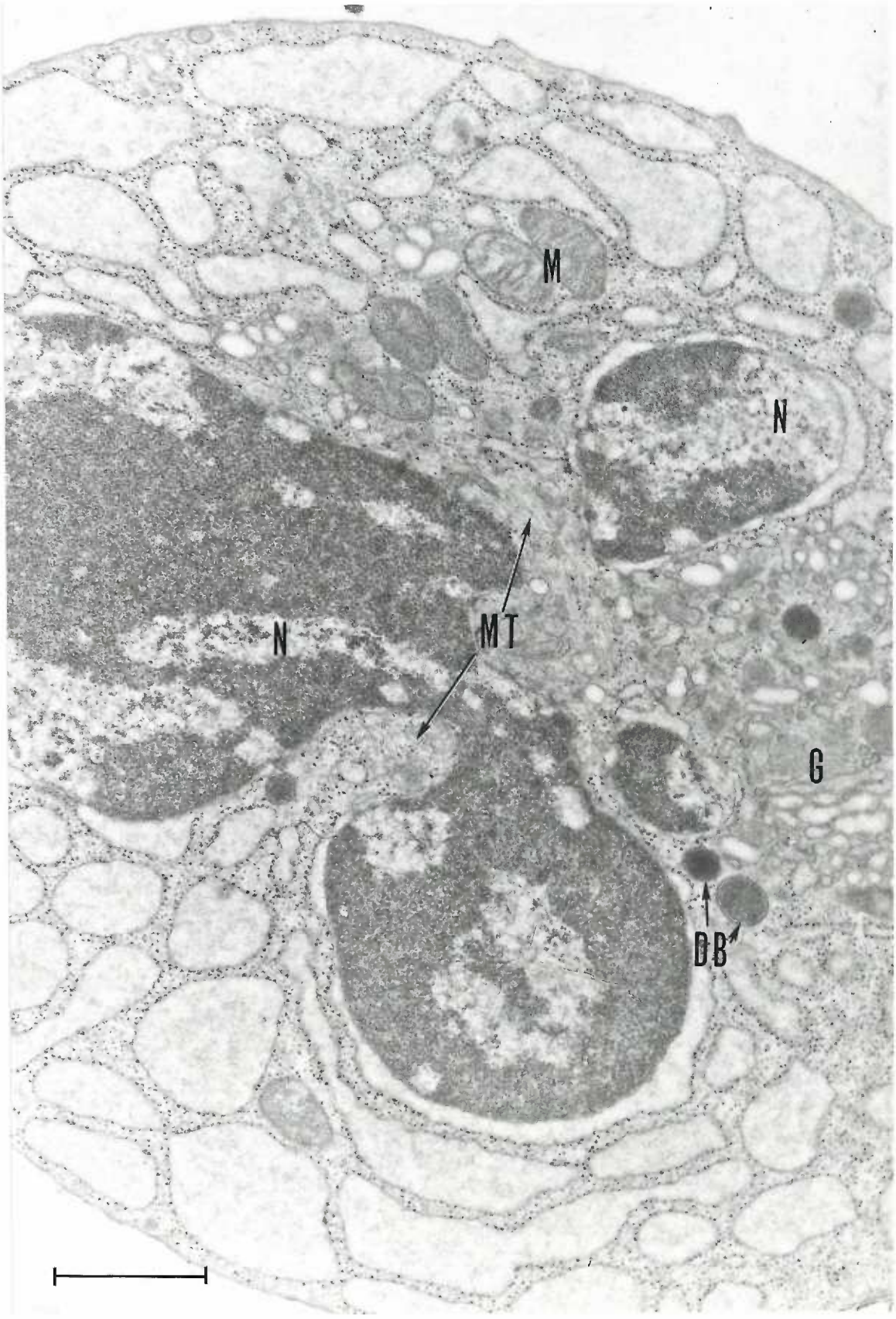

M
N
N
MT
G
DB

Figure 17. 6 day plasmacyte - control. The cytoplasm of this cell is dominated by the well ordered, moderately distended RER and the large Golgi apparatus. Two areas of RER-peripheral cell membrane contact or close association can be seen (double arrows). The nucleus has 3 lobes and a high degree of clumping. A centriole is within the Golgi apparatus.

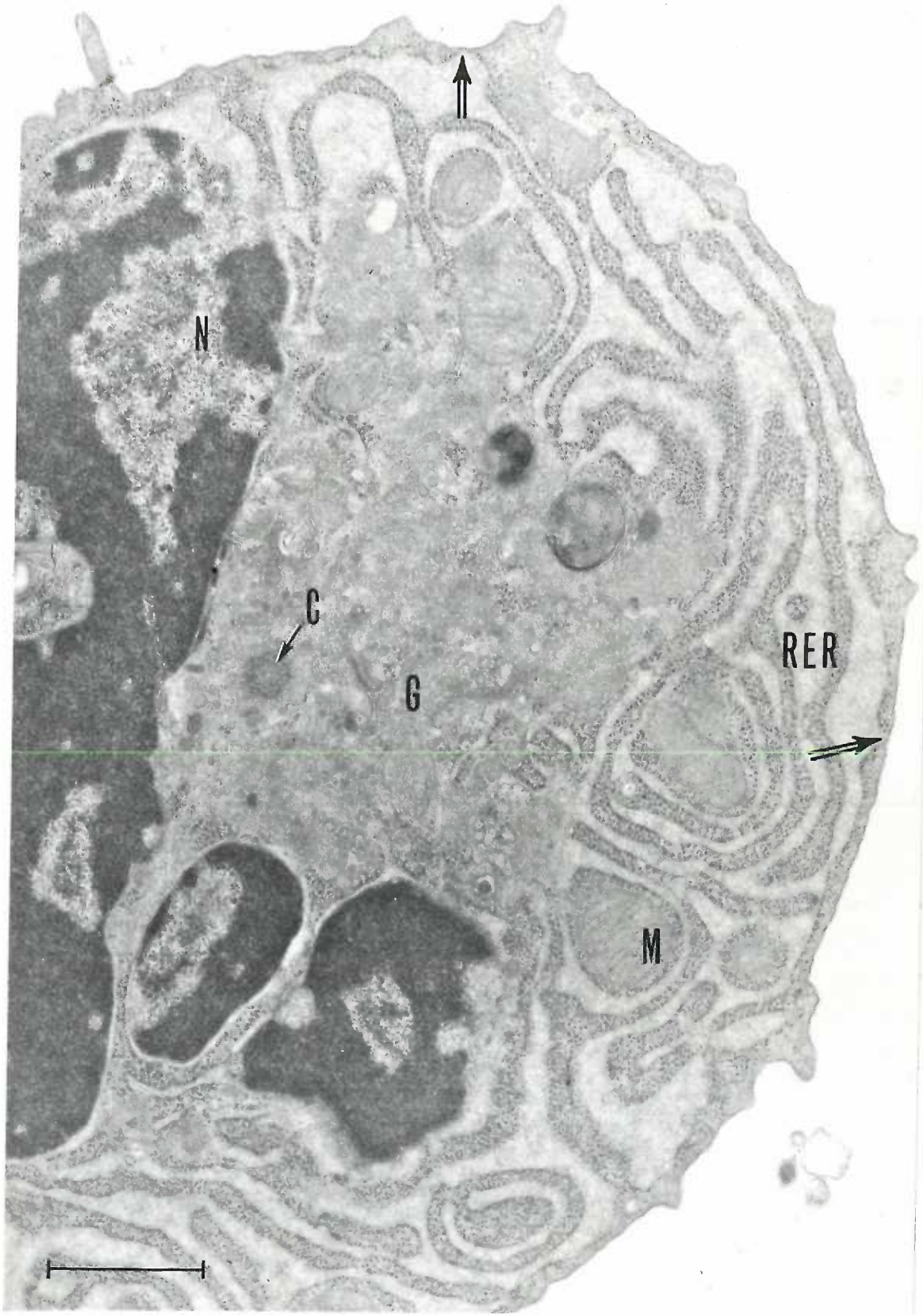

N
C
G
RER
M

Figure 18. 6 day plasmacyte - control. This cell contains 4 nuclear lobes and a small nucleolus which is completely surrounded by heterochromatin. A few microtubules are close to one of the nuclear lobes. Mitochondria showing long narrow cristae and mitochondrial granules are present. The amount of distention of the extensive RER varies considerably. A small amount of Golgi apparatus is present.

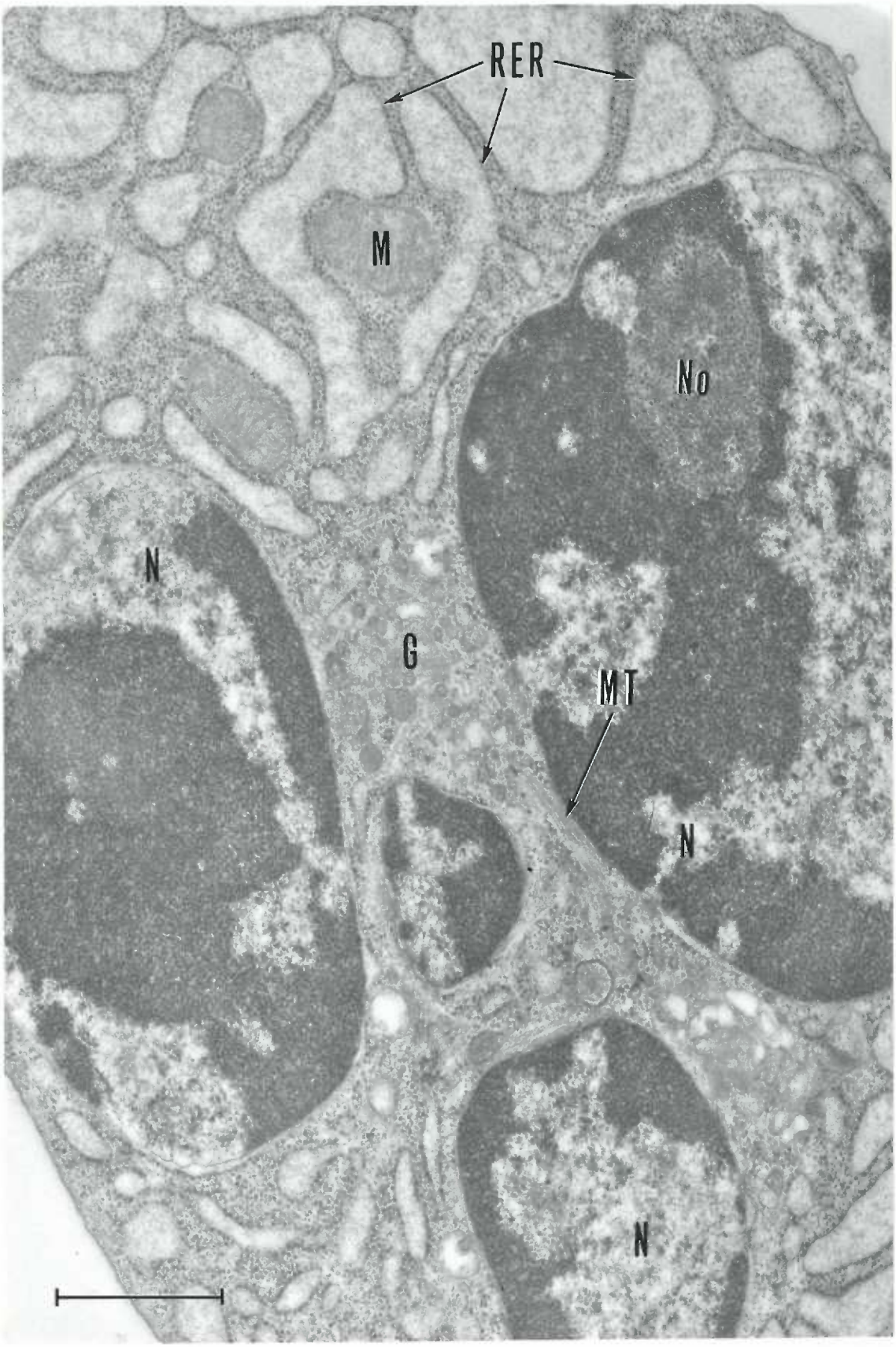

RER
M
No
N
G
MT
N
N
N

Figure 19. 4 day plasmacyte - control. This cell has long narrow profiles of well ordered RER and many free ribosomes. The large Golgi apparatus is located close to the nucleus and is surrounded by mitochondria. The nucleus is elongated and peripheral in position.

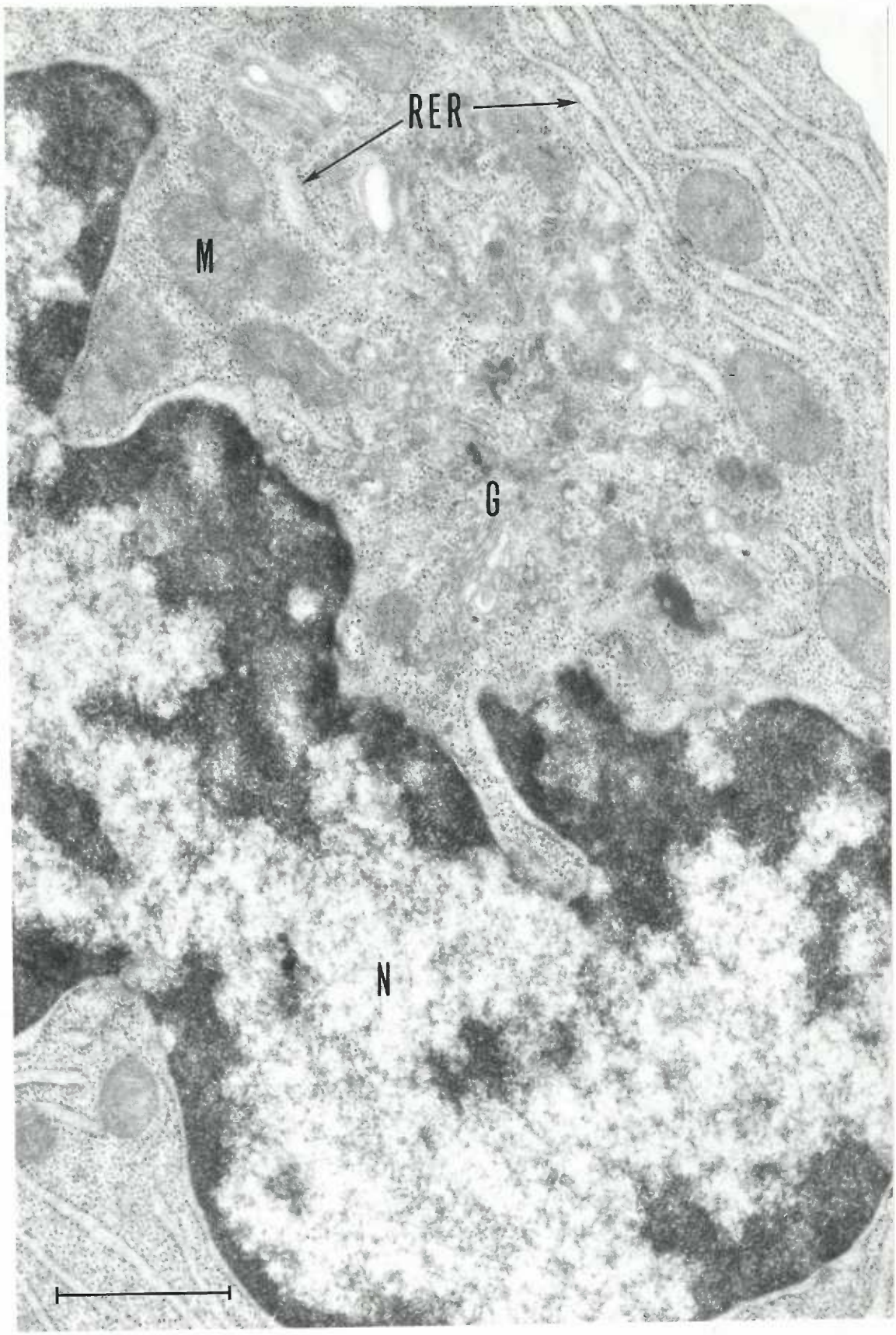

RER
M
G
N

Figure 20. 3 day plasmacyte - control. The distention of the RER of this cell varies considerably. Only a few free ribosomes are present. A considerable amount of Golgi apparatus is seen on both sides of the centrally located nucleus. Mitochondria are close to the Golgi apparatus and the nucleus. Two nuclear bodies are present in the euchromatin. Virus-like particles are seen in the process of budding into RER.

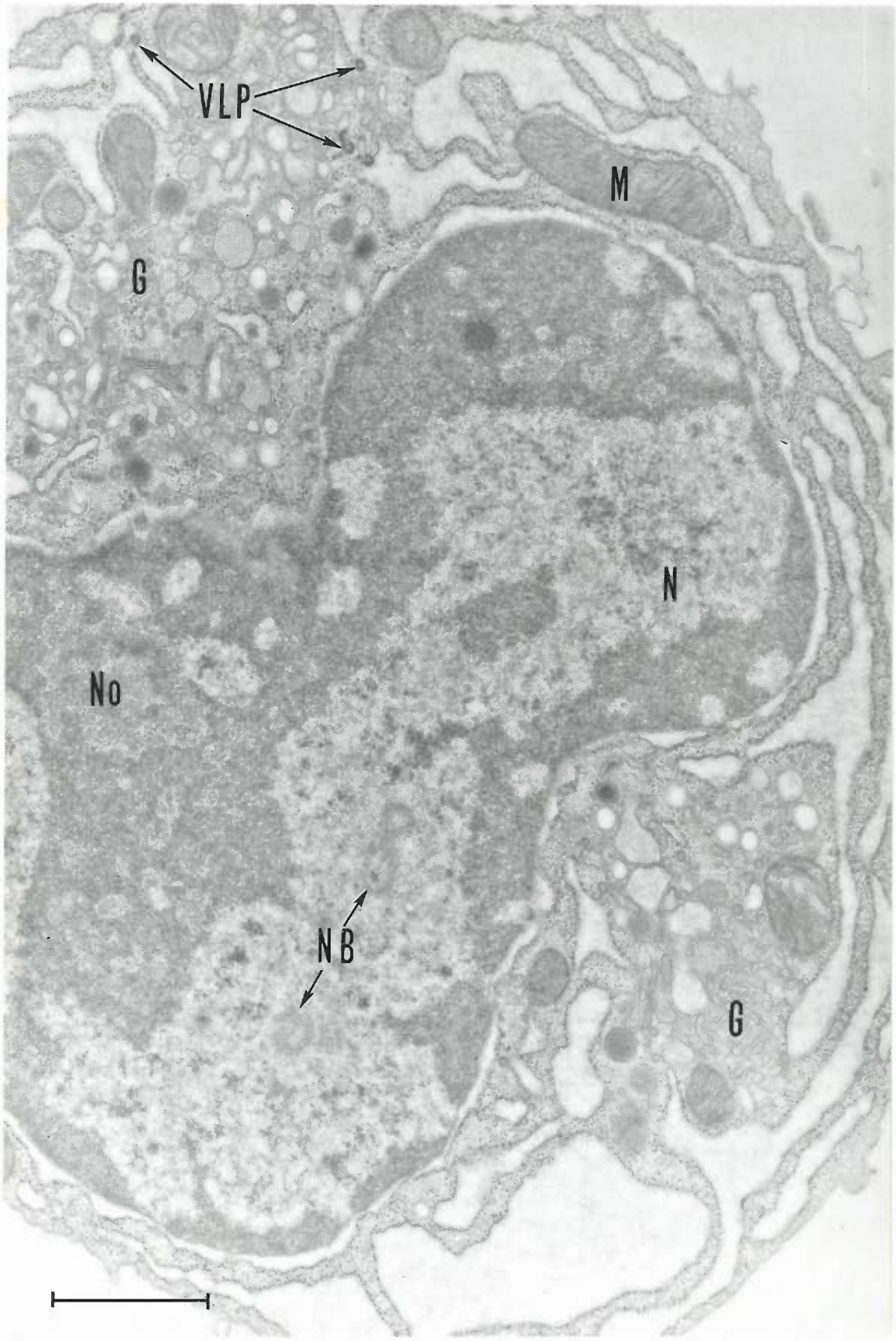
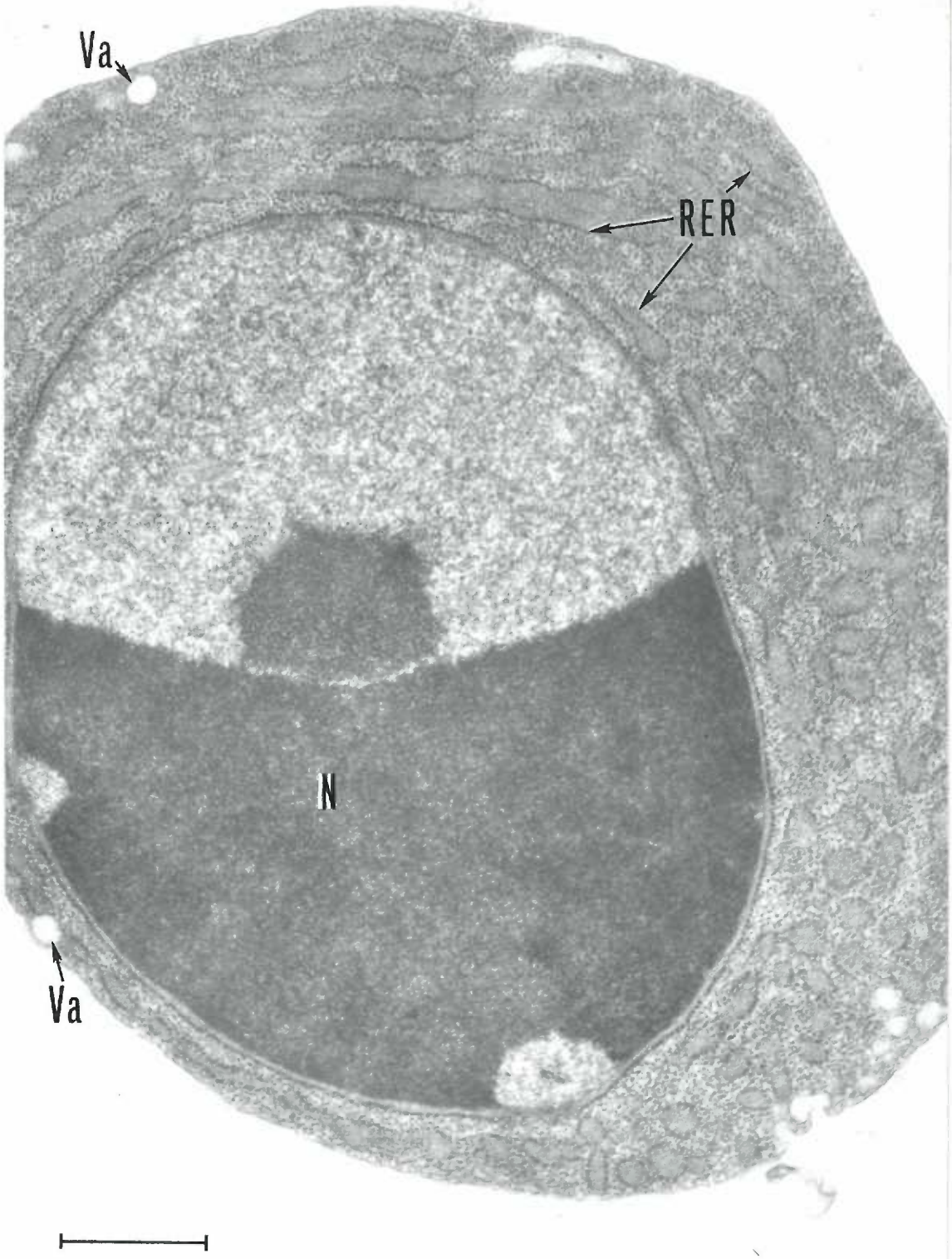
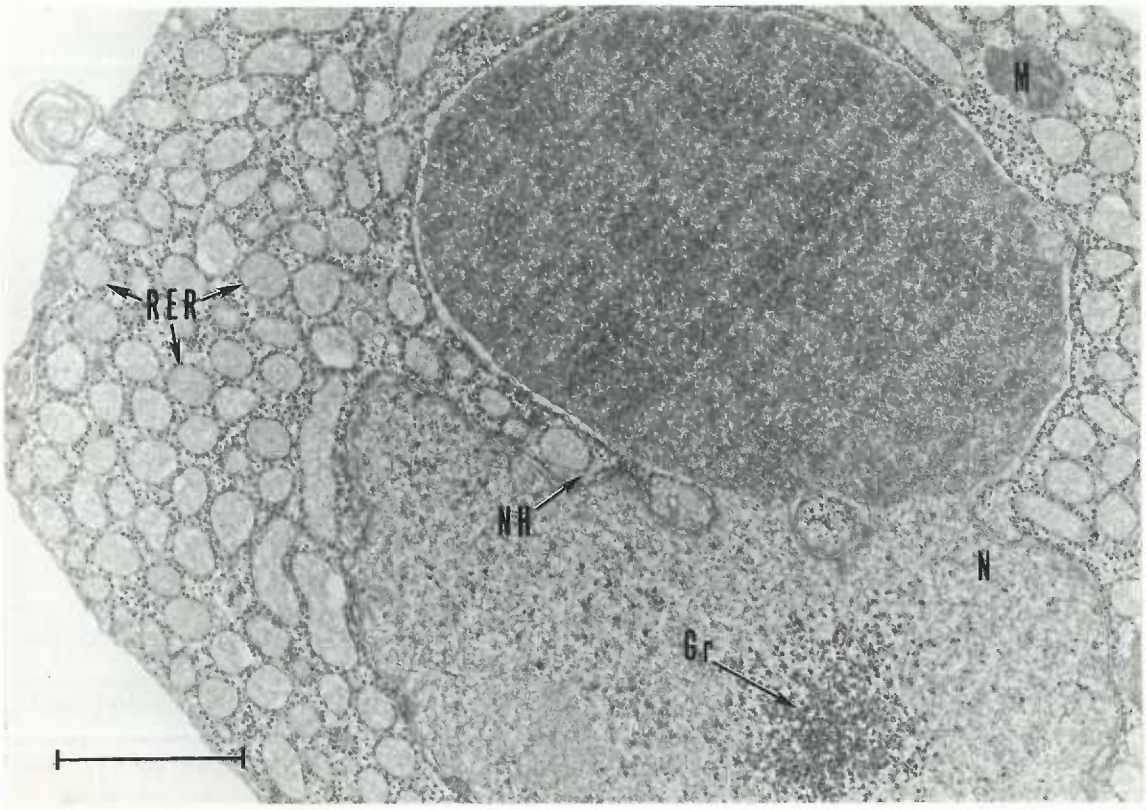

VLP
M
G
N
No
NB
G

Figure 21. 5 day APC - control. The periphery of this cell is very smooth with a few clear vacuoles just under the surface. The cytoplasm is packed with free ribosomes and the RER shows a fairly even amount of distention. The nucleus is slightly peripheral with the heterochromatin being very dense and pyknotic-appearing. There is a very distinct line dividing heterochromatin and euchromatin.


Va
RER
N
Va

Figures 22 and 23. 5 day APC - control. These two sections of the same cell show RER that is very tubular, with most profiles being round and very evenly distended. Only a few small mitochondria are present. Heterochromatin is less dense than in fig. 21, but the line between heterochromatin and euchromatin is just as sharp. A group of dense granules can be seen in the euchromatin. A few VLPs are located within RER cisternae.


M
RER
NR
N
Gr

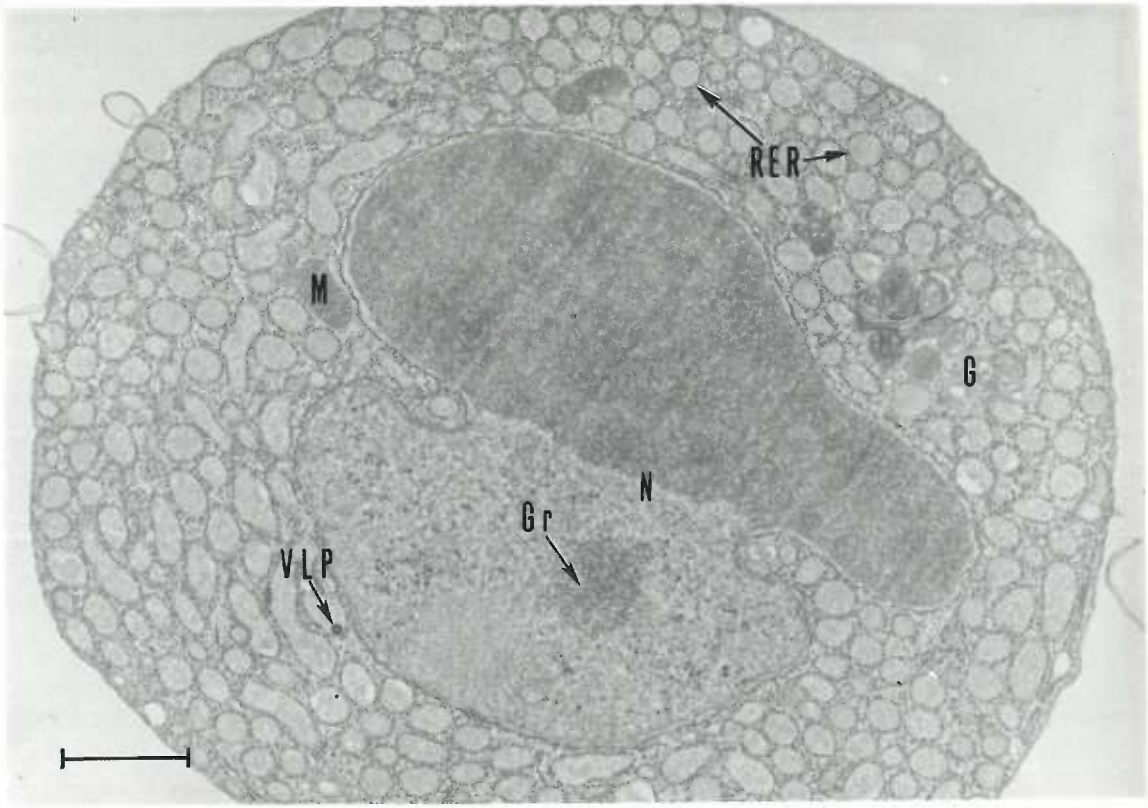
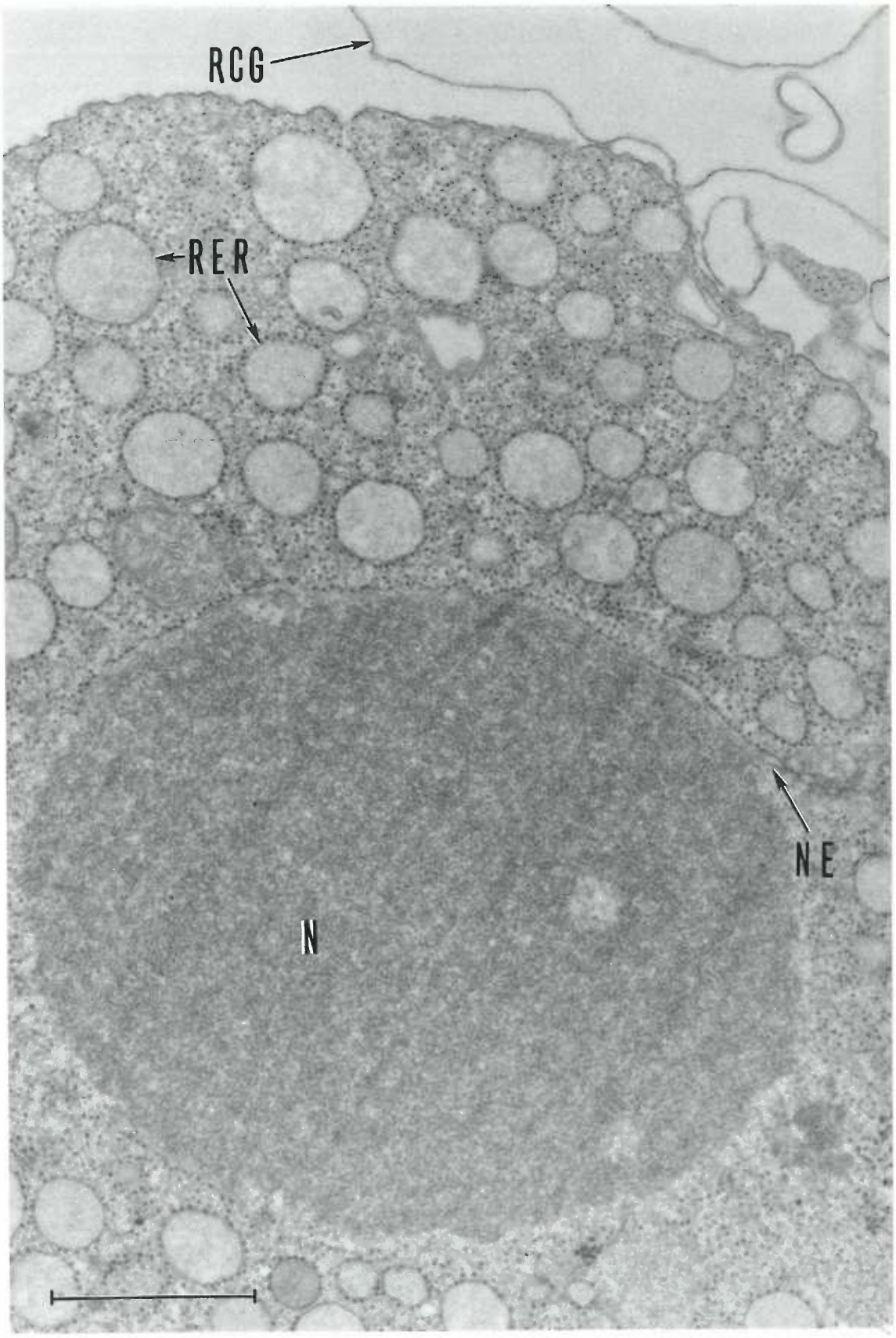
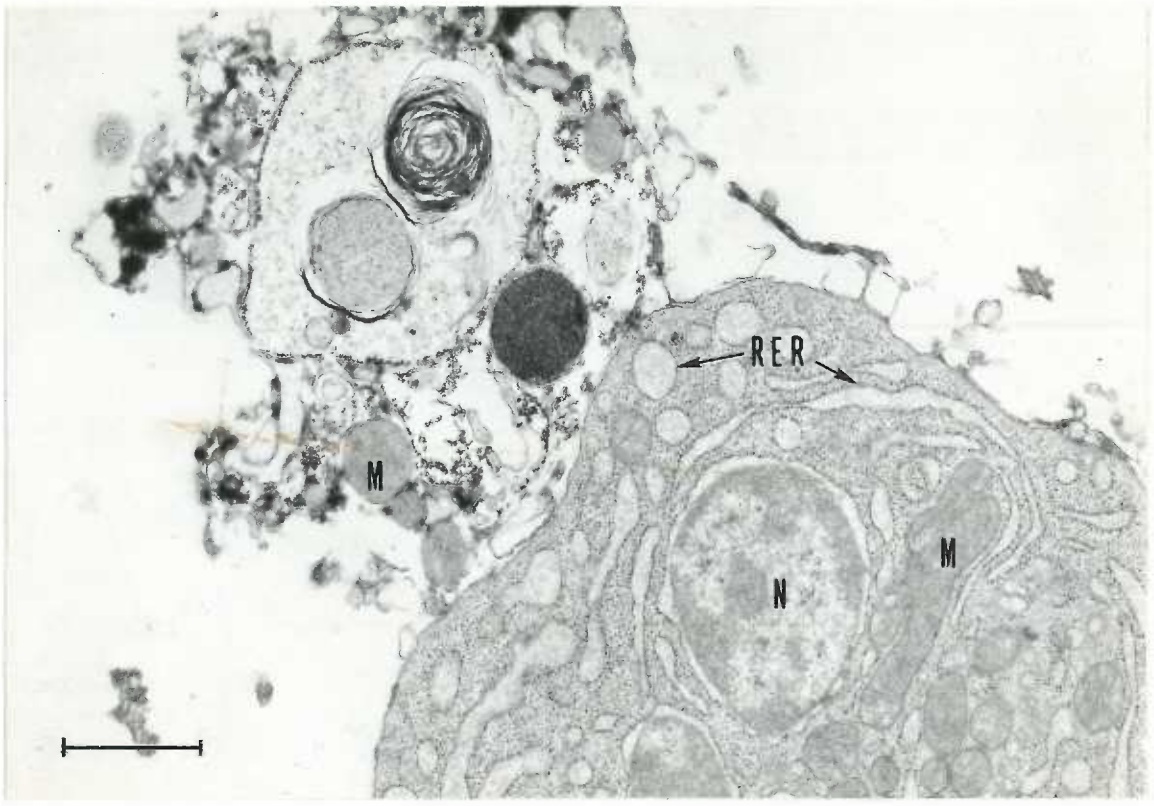
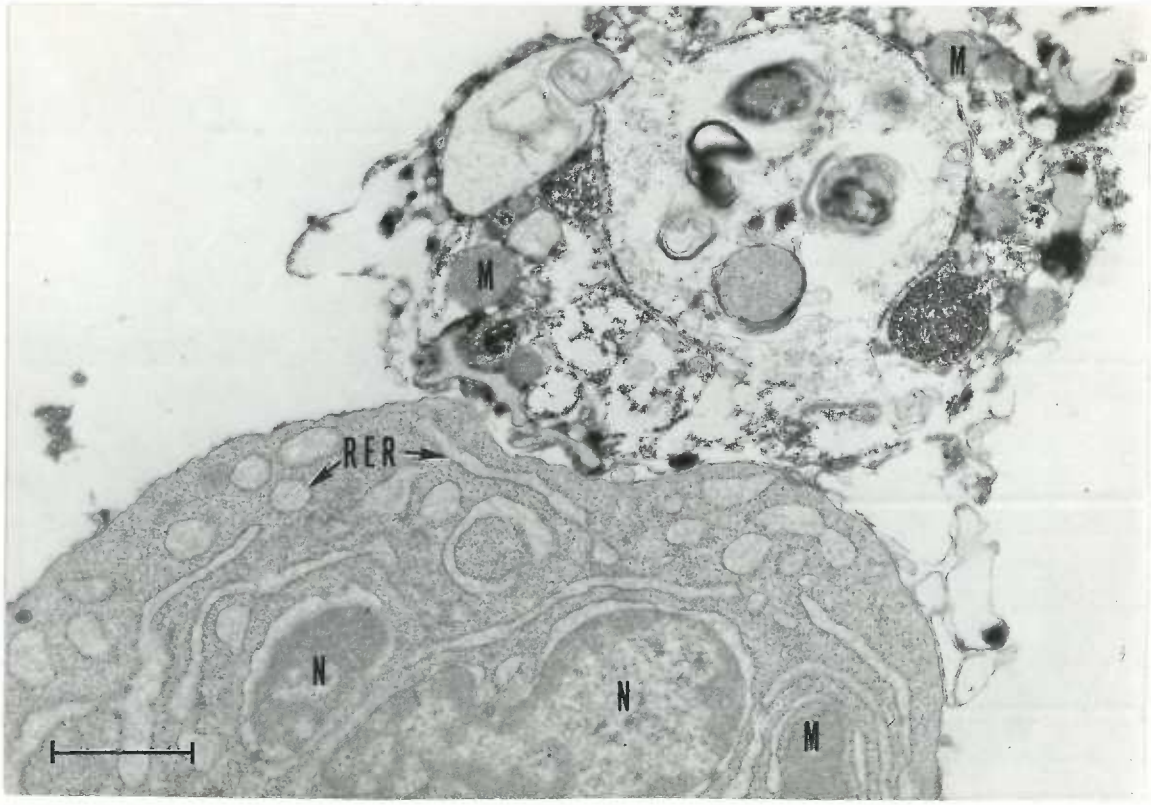

RER
M
G
N
Gr
VLP

Figure 24. 5 day APC - control. This cell has only rounded profiles of RER. The electron-opaque, intra-cisternal material varies in density. A red cell ghost has 2 projections which almost contact the cell membrane. All of the nucleus in this cell appears to be pyknotic, and most of its surface shows no nuclear envelope. A portion of the nuclear envelope can be seen projecting into the cytoplasm.

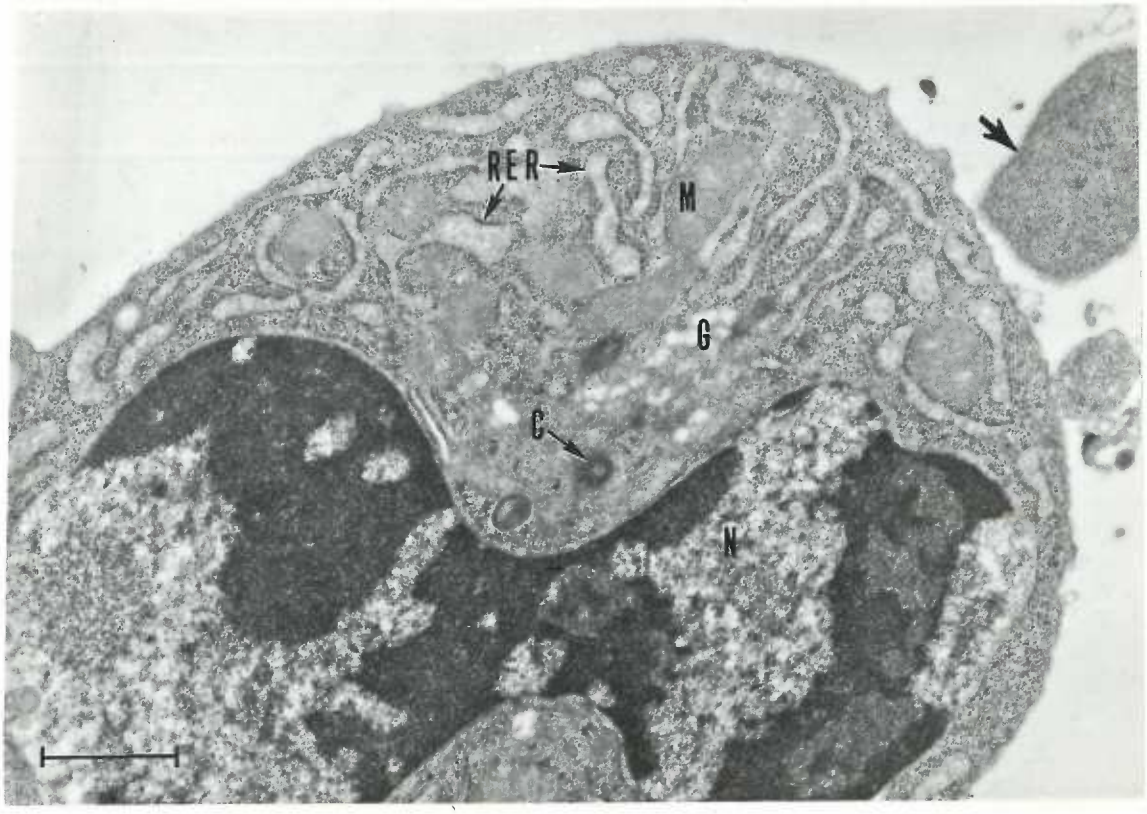

RCG
RER
NE
N

Figures 25 and 26. 3 day proplasmacyte - control. Different sections of the same cell showing a large piece of lysed cytoplasm which is adhered to the surface of the plaque-forming cell. This disrupted cytoplasm contains myelin figures, intact mitochondria and cellular debris. The proplasmacyte has normal-appearing nucleus, RER and mitochondria.


RER
M
N
M


M
M
RER
N
N
M

Figures 27 and 28. 4 day plasmacyte - control. These 2 sections of the same cell show a piece of cytoplasm close to (fig. 27) and adhering to (fig. 28) the surface. The piece of cytoplasm is denser than the cytoplasm of the plaque-forming cell and contains no RER. The plasmacyte has extensive RER, mitochondria, a centriole (fig. 27) and 2 nuclear lobes.


RER
M
G
C
N

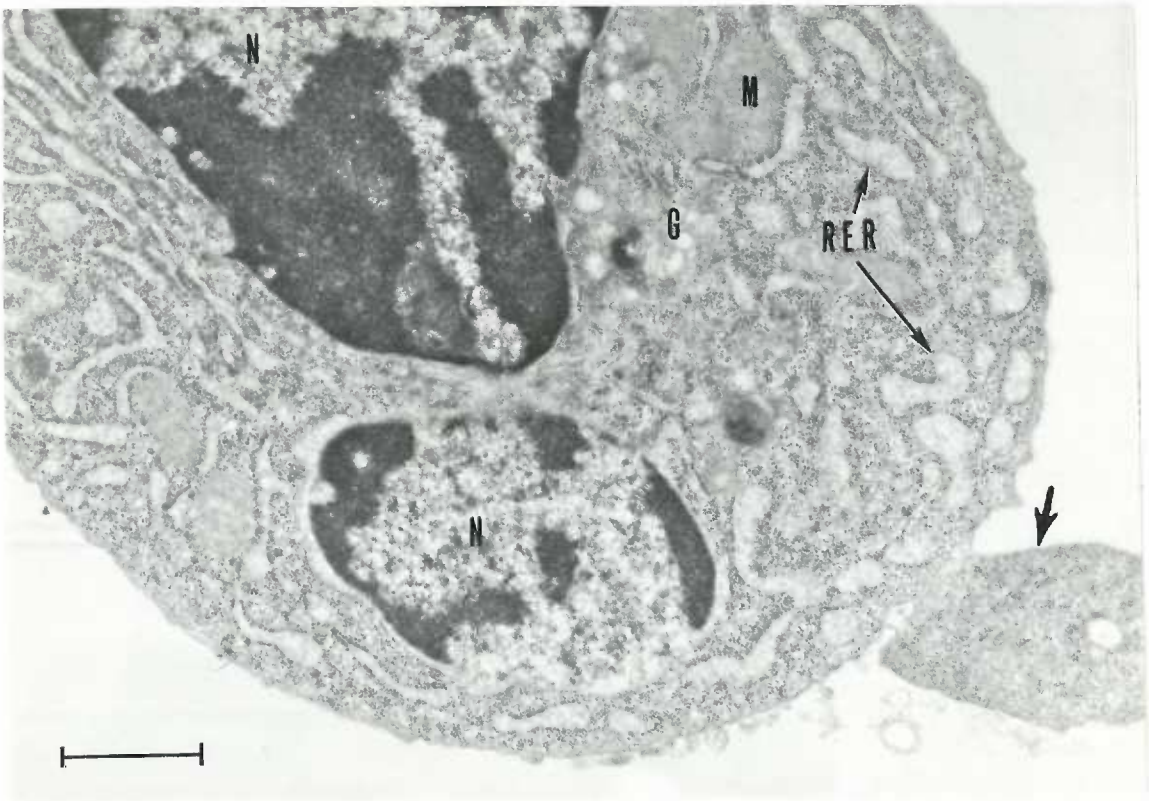
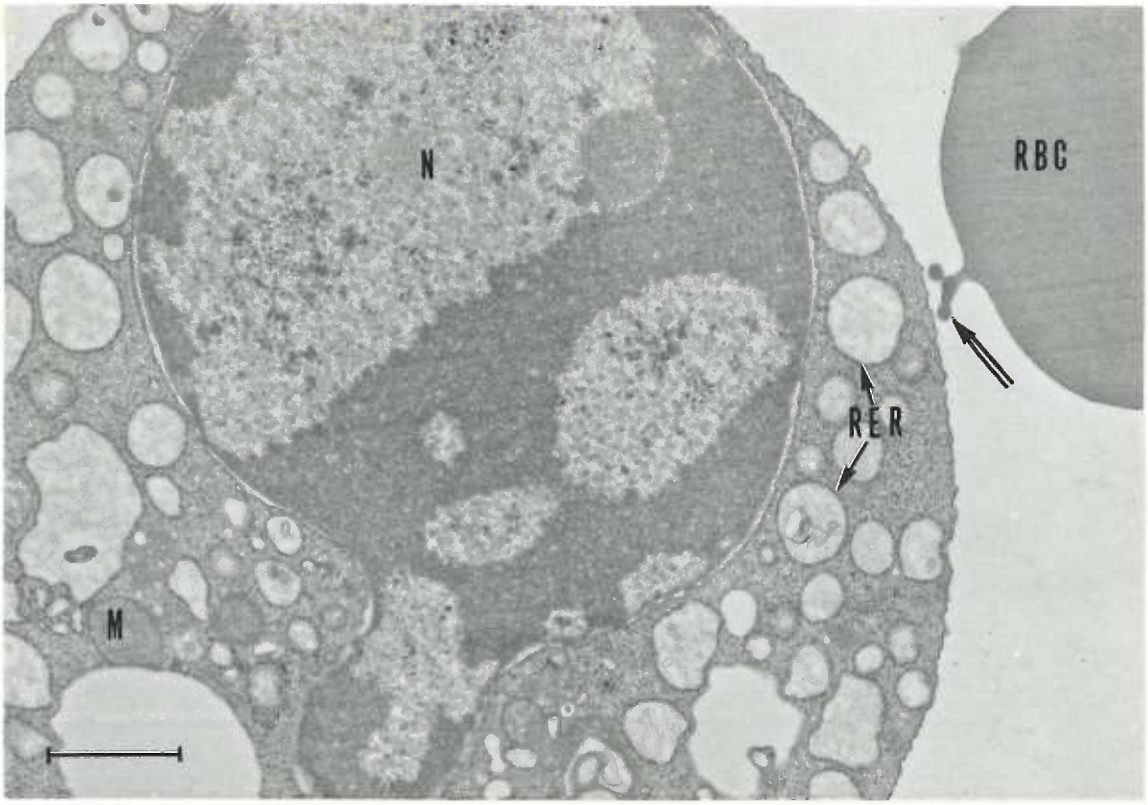

N
M
G
RER
N

Figure 29. 5 day proplasmacyte - control. This cell is very close to a red blood cell with projections almost contacting its surface. The nucleus of the proplasmacyte shows considerable chromatin clumping. The extensive RER is in rounded, distended profiles. A few small mitochondria are present.

Figure 30. 6 day plasmacyte - control. A cytoplasmic bleb of this cell is contacting a red blood cell (double arrow). A portion of the nucleus, extensive, laminated RER, and a mitochondria can be seen in the cell.


N
RBC
RER
M

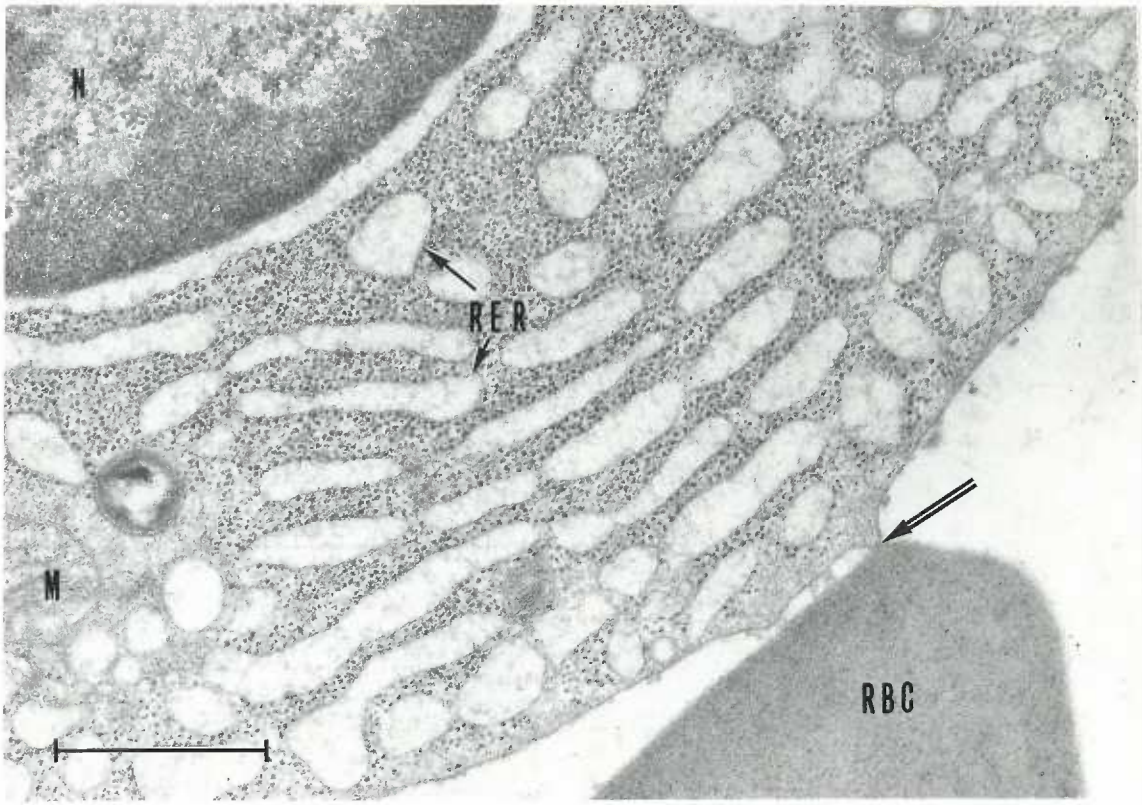

N
RER
M
RBC

Figure 31. 3 day immunoblast - D_2O. This cell has a very large, "blast-like" nucleus, with a small amount of extra-dense chromatin. A very small amount of RER is present, but the cytoplasm is packed with free ribosomes, with most being grouped into polyribosomes. A few microtubules are close to the nucleus and one mitochondria is present. A large cytoplasmic bleb can be seen at the surface of the cell.

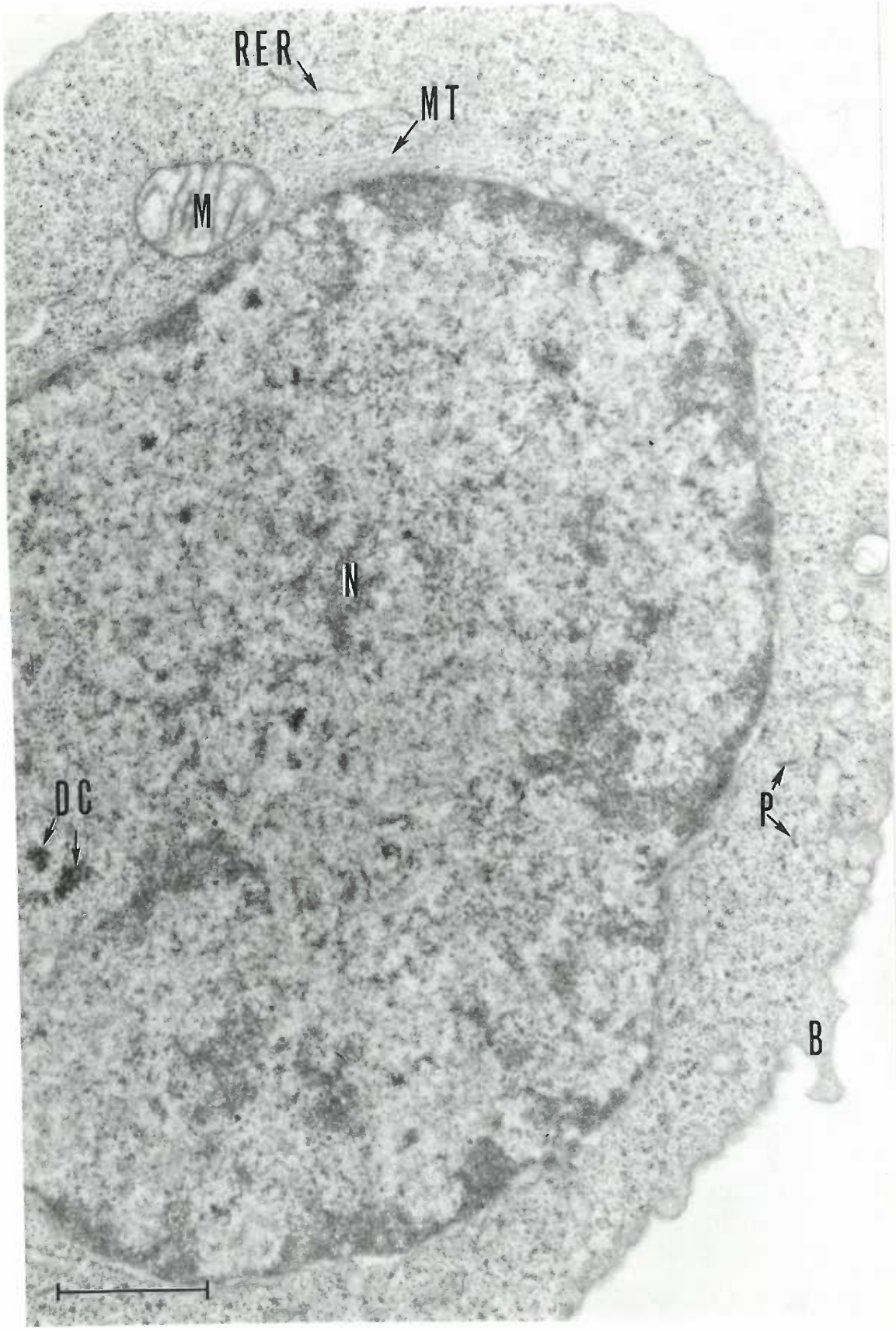

RER
MT
M
N
DC
P
B

Figure 32. 3 day plasmablast - D_2O. Virtually all the free ribosomes in this cell are grouped into spiral polyribosomes. The small amount of RER is slightly distended. Mitochondria are grouped in the center of the cell, with most having a "washed-out" appearance.

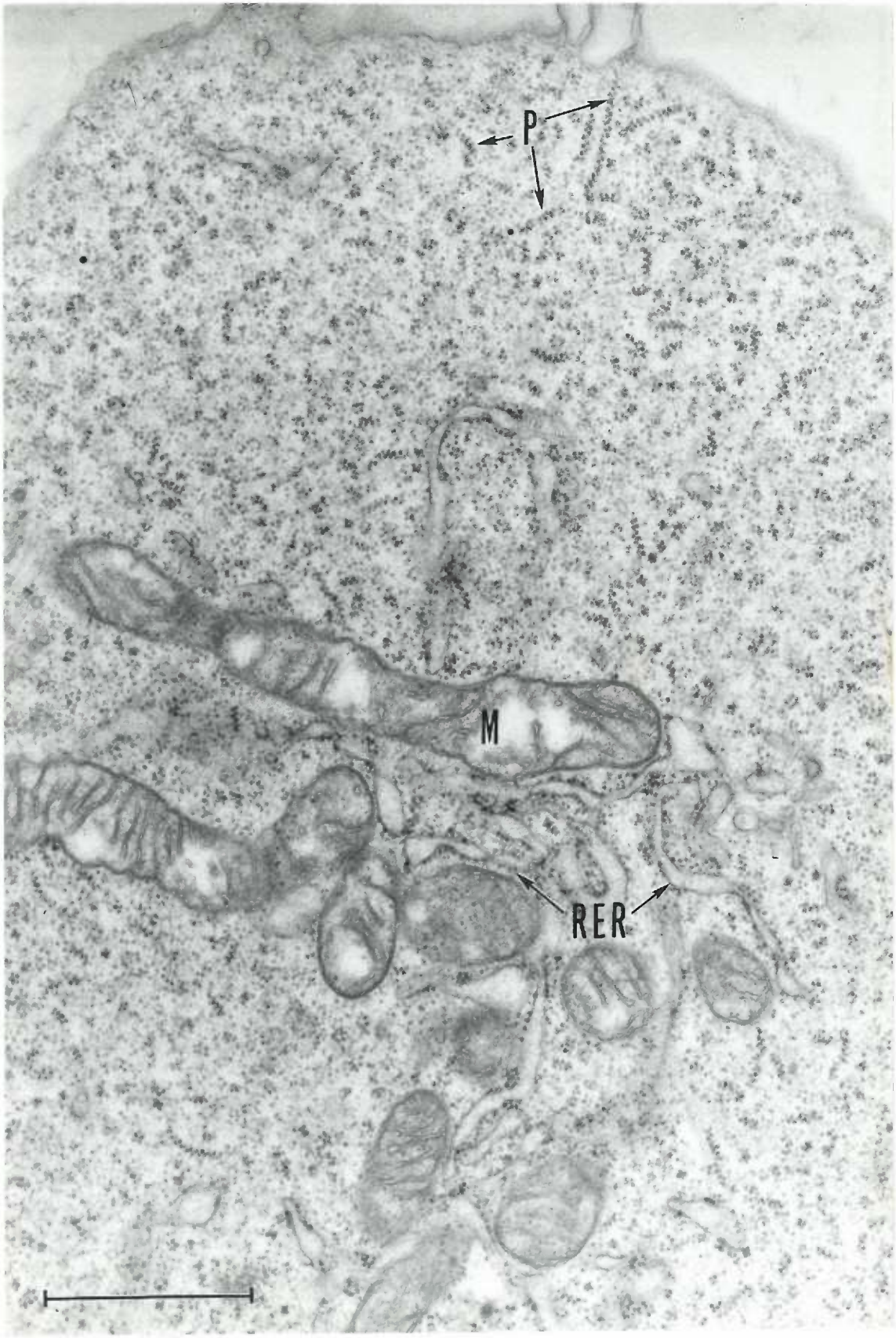

P
M
RER

Figure 33. 3 day plasmablast - D_2O. This cell has a large "blast-like" nucleus with a large nucleolus which is attached to the peripheral heterochromatin. The cytoplasm is filled with single and clustered free ribosomes. Most of the numerous mitochondria are close to the nucleus or RER which is in long, slightly distended profiles. Cytoplasmic blebs can be seen at the surface of the cell.

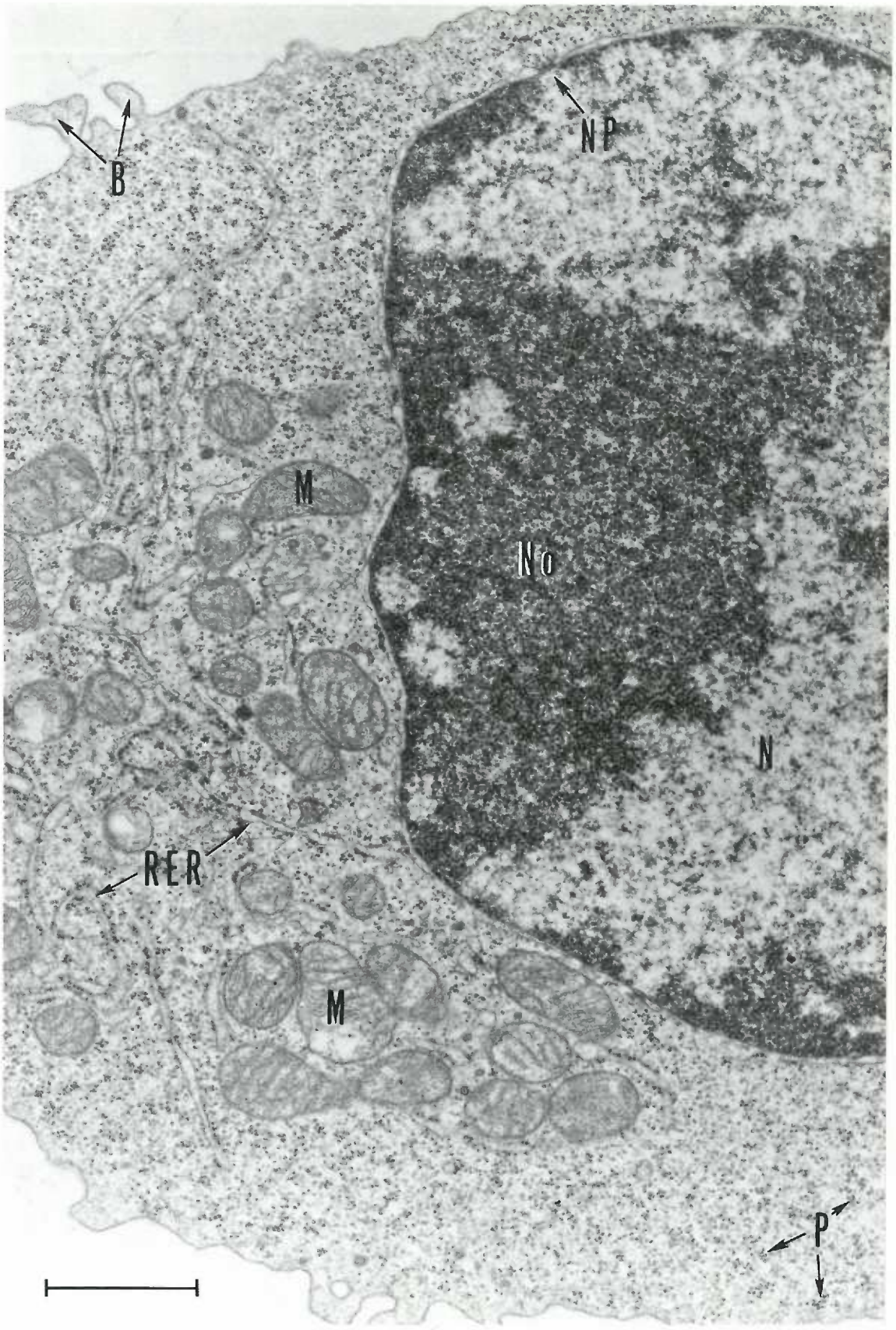

B
NP
M
No
N
RER
M
P

Figure 34. 4 day plasmablast - D_2O. The RER of this cell is in long, slightly distended profiles. There are many free ribosomes (single and in polyribosomes) in the cytoplasm. Mitochondria and cytoplasmic blebs are present. The nucleus is very large, "blast-like" and bilobed. The large nucleolus is attached to the periphery of the nucleus. Numerous nuclear pores and a few pieces of extra-dense chromatin are present.

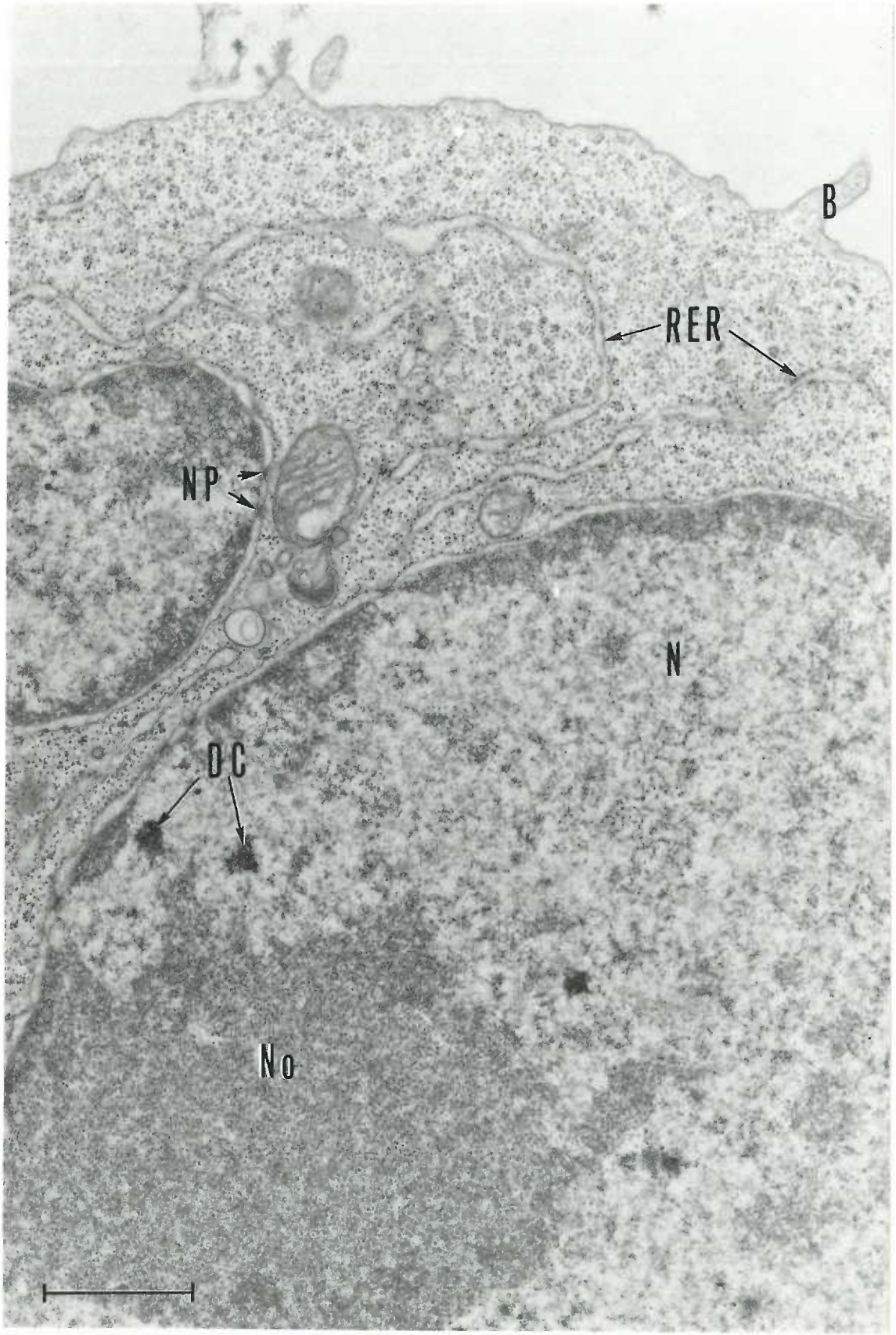

B
RER
NP
N
DC
No

Figure 35. 3 day proplasmacyte - D_2O. The amount of distention of the RER of this cell varies greatly. The cytoplasm also contains a myelin figure, numerous mitochondria and many free ribosomes. Only a small portion of the nucleus is present, and circular, tangentially cut nuclear pores can be seen.

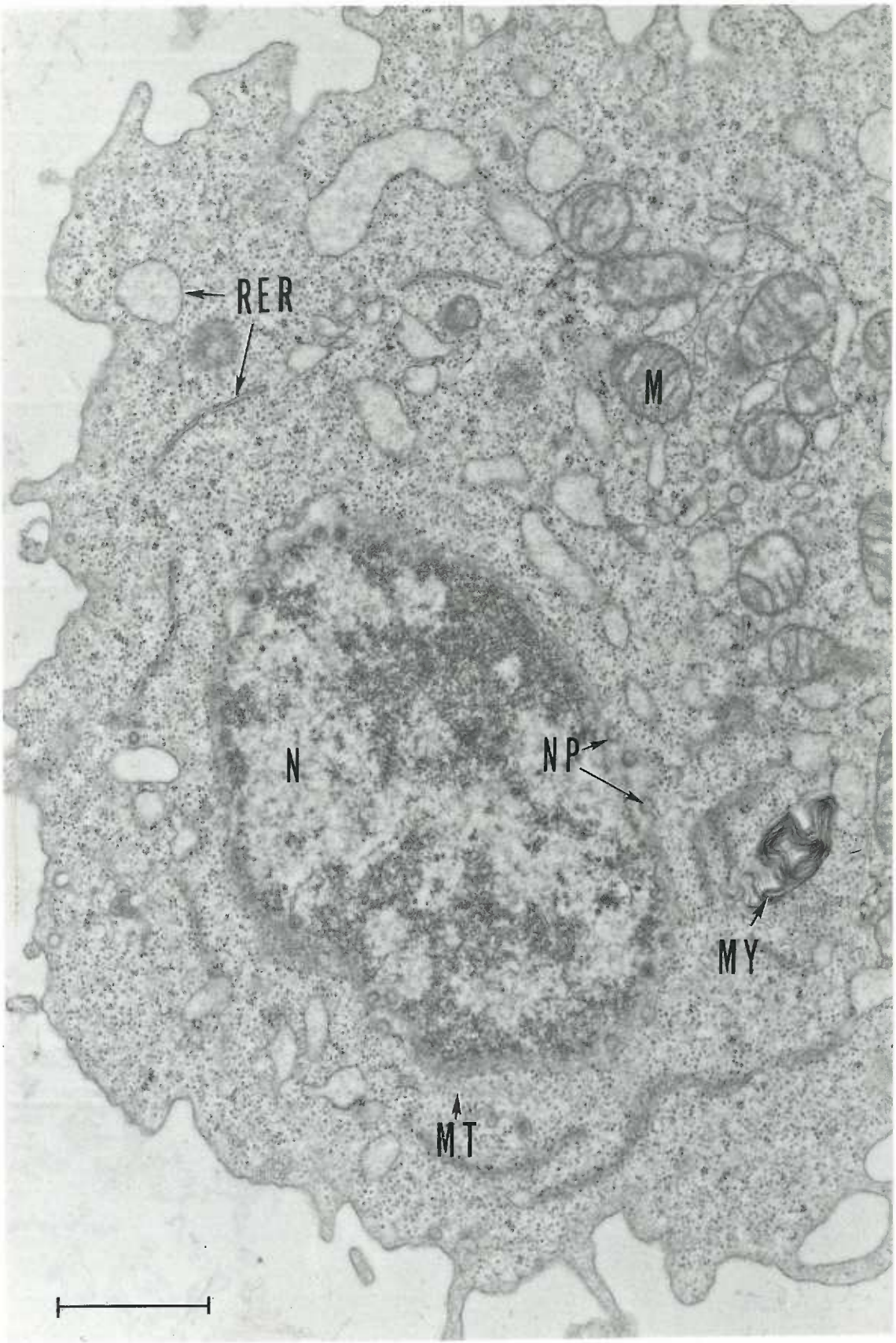

RER
M
N
NP
MY
MT

Figure 36. 3 day proplasmacyte - D_2O. This section shows a portion of red cell ghost contacting a cytoplasmic bleb. The RER is quite distended, and somewhat ordered. The 2 large mitochondria present have "washed-out" matrices. A portion of the Golgi apparatus is present. The nucleus has a large amount of heterochromatin. Nuclear pores can be seen.

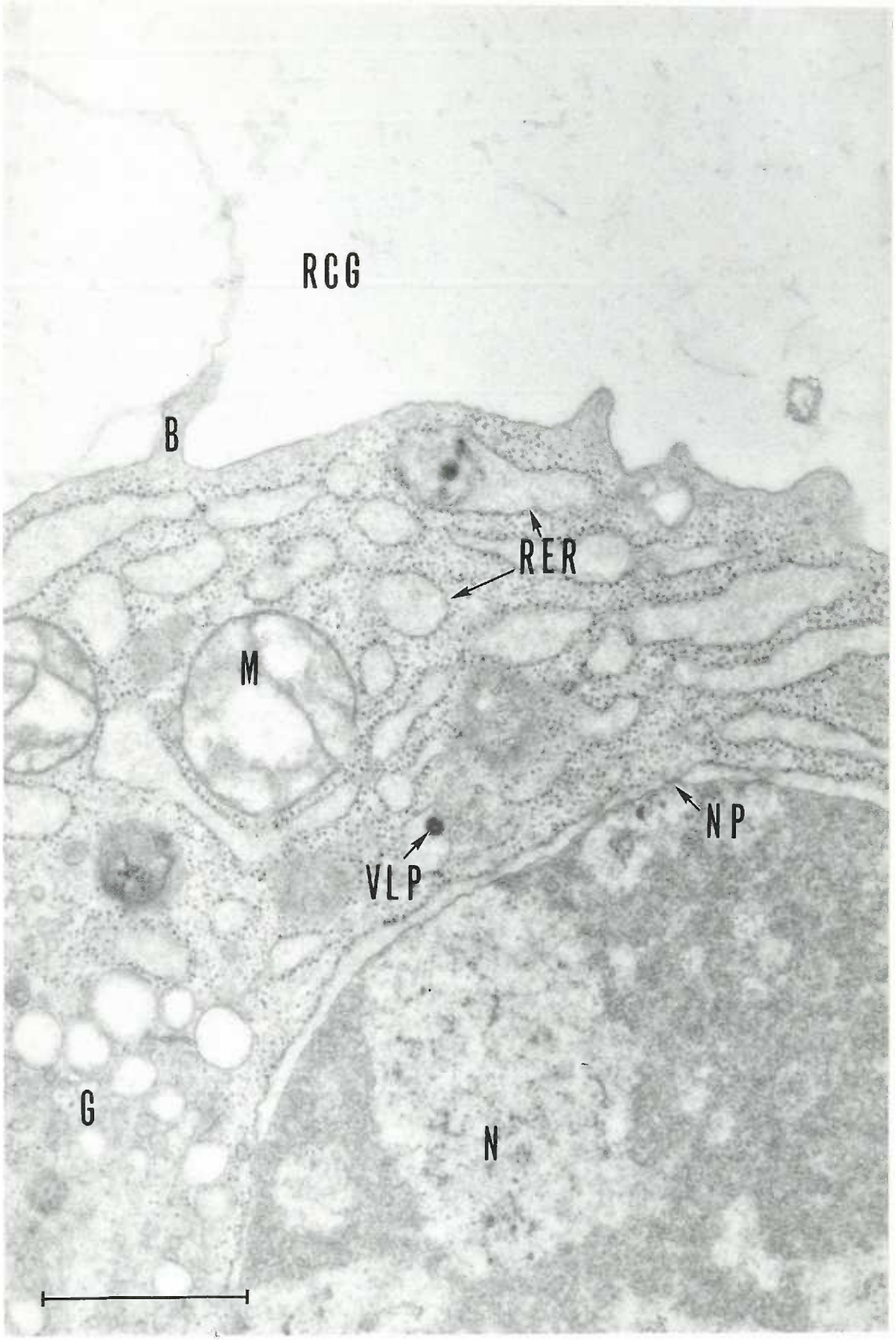
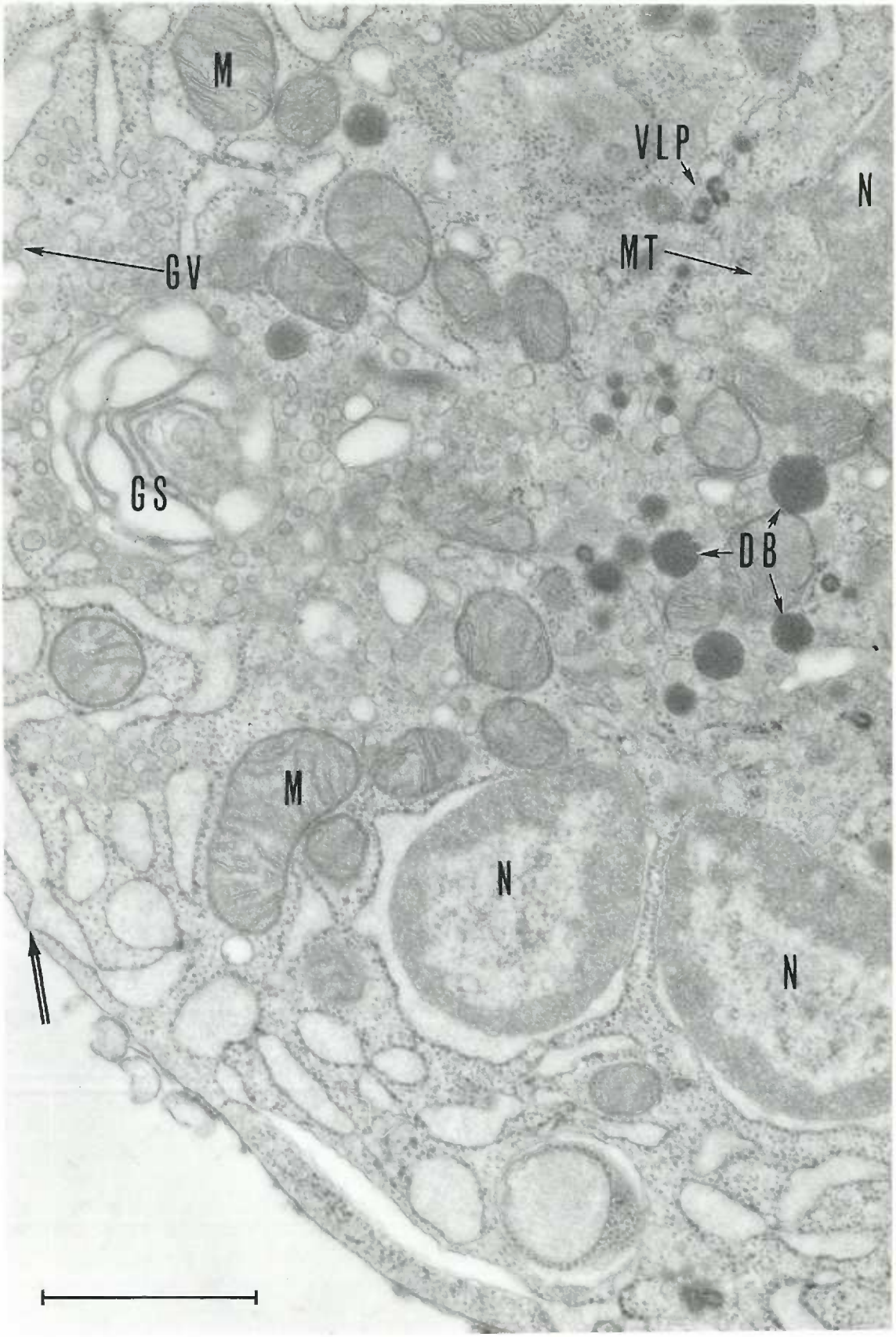
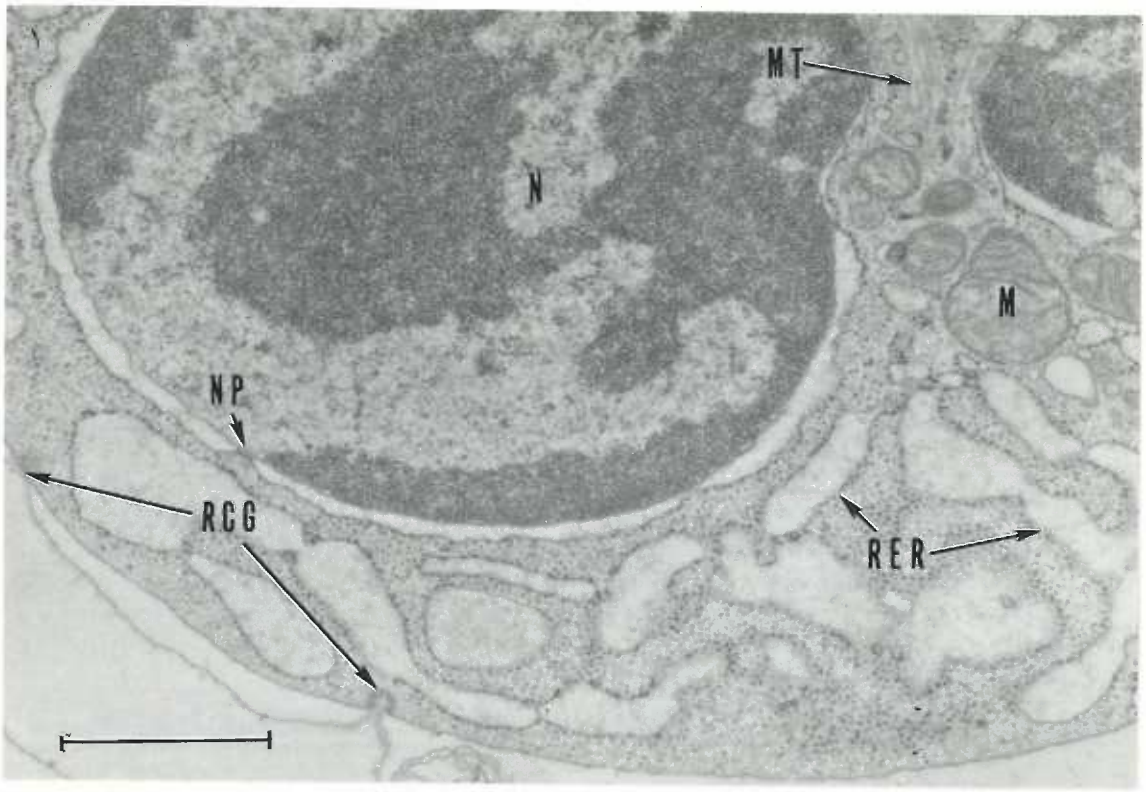

RCG
B
RER
M
NP
VLP
G
N

Figure 37. 4 day plasmacyte - D_2O. The Golgi apparatus of this cell is very large, showing saccules, vesicles and dense bodies. VLPs are also in the area of the Golgi apparatus. A bleb in the RER protruding into the area of Golgi vesicles can be seen. Numerous mitochondria are located in and around the Golgi apparatus. One portion of RER membrane is very close to the cell membrane (double arrow).


M
VLP
N
MT
GV
GS
DB
M
N
N

Figures 38 and 39. 4 day plasmacyte - D_2O. The red cell ghost next to this cell makes contact with the peripheral cell membrane in 2 places, with one portion of the red cell ghost being within a micropinocytotic process. The point of contact in fig. 39 is the same micropinocytotic process seen in fig. 38. RER is distended and quite ordered. The nucleus has a considerable amount of clumped chromatin and nuclear pores can be seen. A group of microtubules are close to the nucleus (seen in cross-section in fig. 39).


MT
N
M
NP
RCG
RER

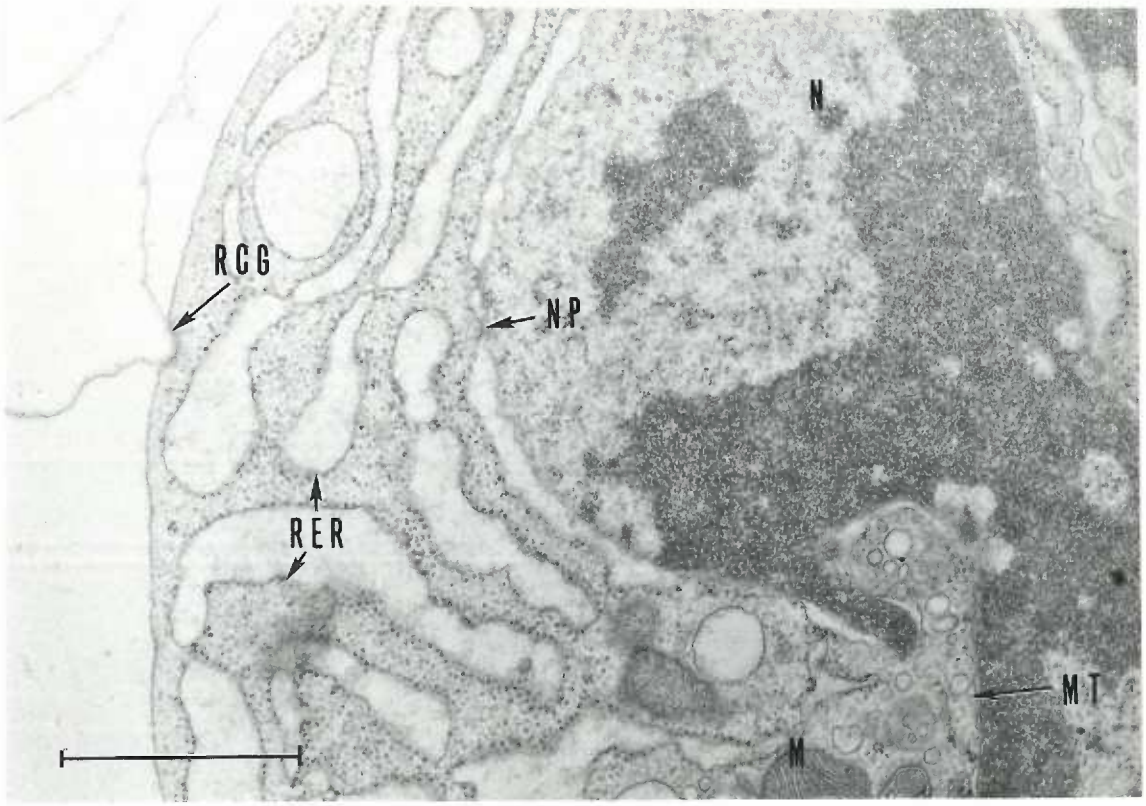

N
RCG
NP
RER
MT
M

Figure 40. 6 day plasmacyte - D_2O. The RER of this cell is very extensive, but in much shorter segments than most plasmacytes. One segment is very close to the cell membrane (double arrow). Many intracisternal VLPs are present. Small, moderately dense mitochondria are scattered in the cell. A small portion of Golgi apparatus is close to the nucleus. it contains a large dense body. The peripheral nucleus has large clumps of heterochromatin and a small nucleolus. Most of the nucleolus is surrounded by heterochromatin.

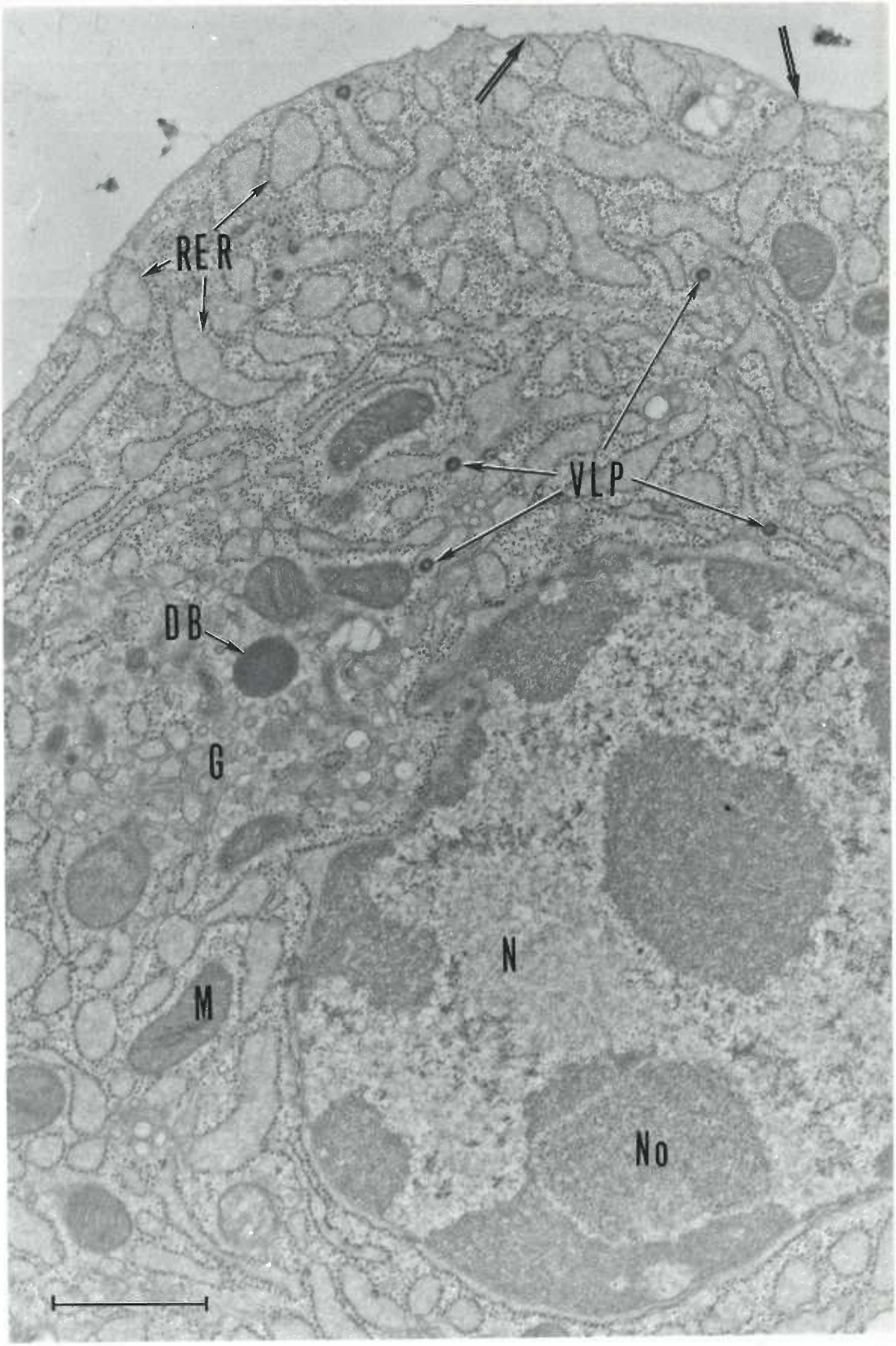

RER
VLP
DB
G
N
M
No

Figure 41. 5 day APC - D_2O. This APC has many long RER profiles. The amount of distention is quite even. The cytoplasm also contains mitochondria, dense bodies, and a few free ribosomes. Nuclear heterochromatin is pyknotic in appearance and sharply divided from euchromatin. Two nucleoli are present.

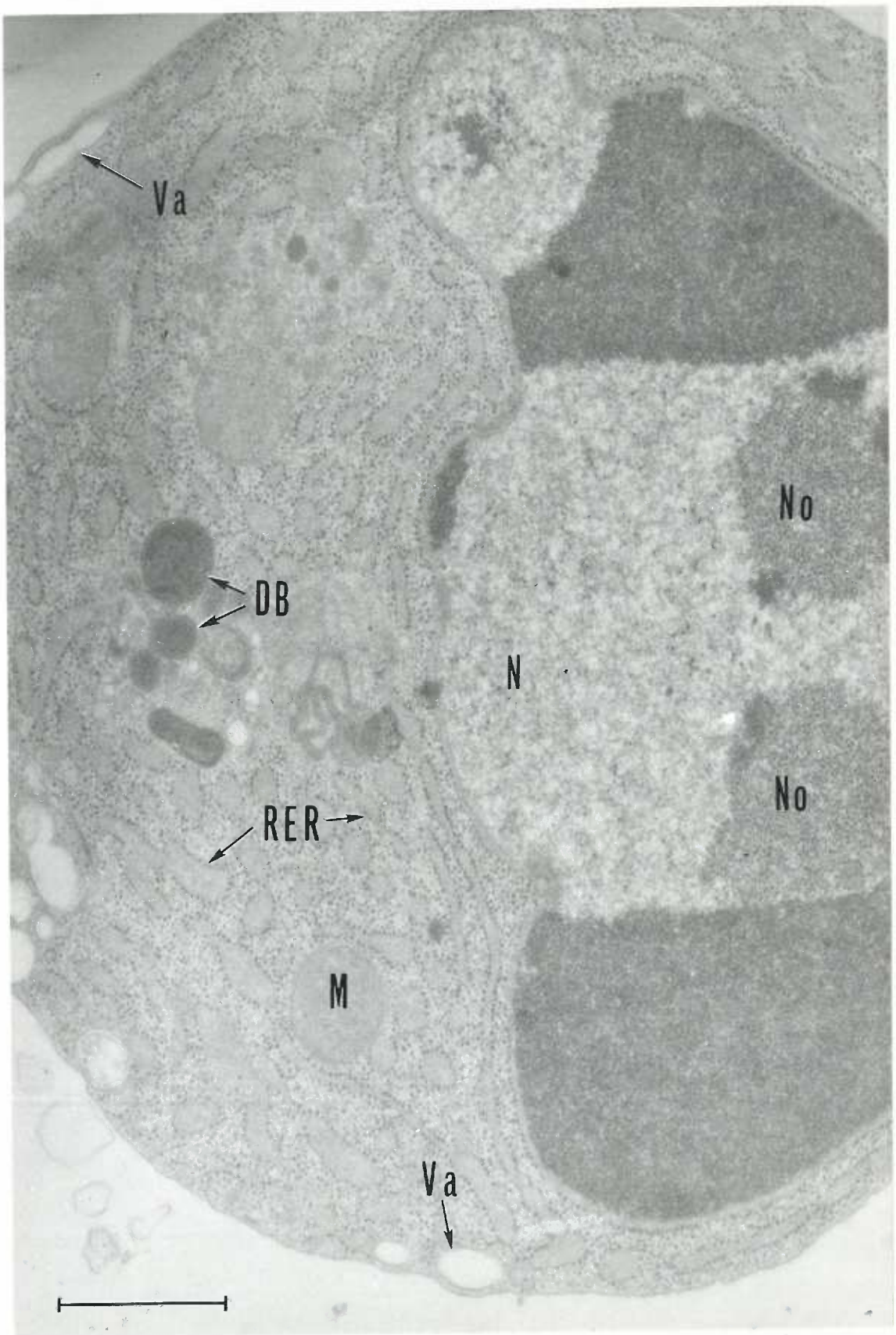

Va
No
DB
N
No
RER
M
Va

Figure 42. 6 day APC - D_2O. This cell has very circular, evenly-distended RER, suggesting a tubular shape. The nuclear envelope is also broken up into rounded profiles. Part of the pyknotic nucleus has no nuclear envelope.

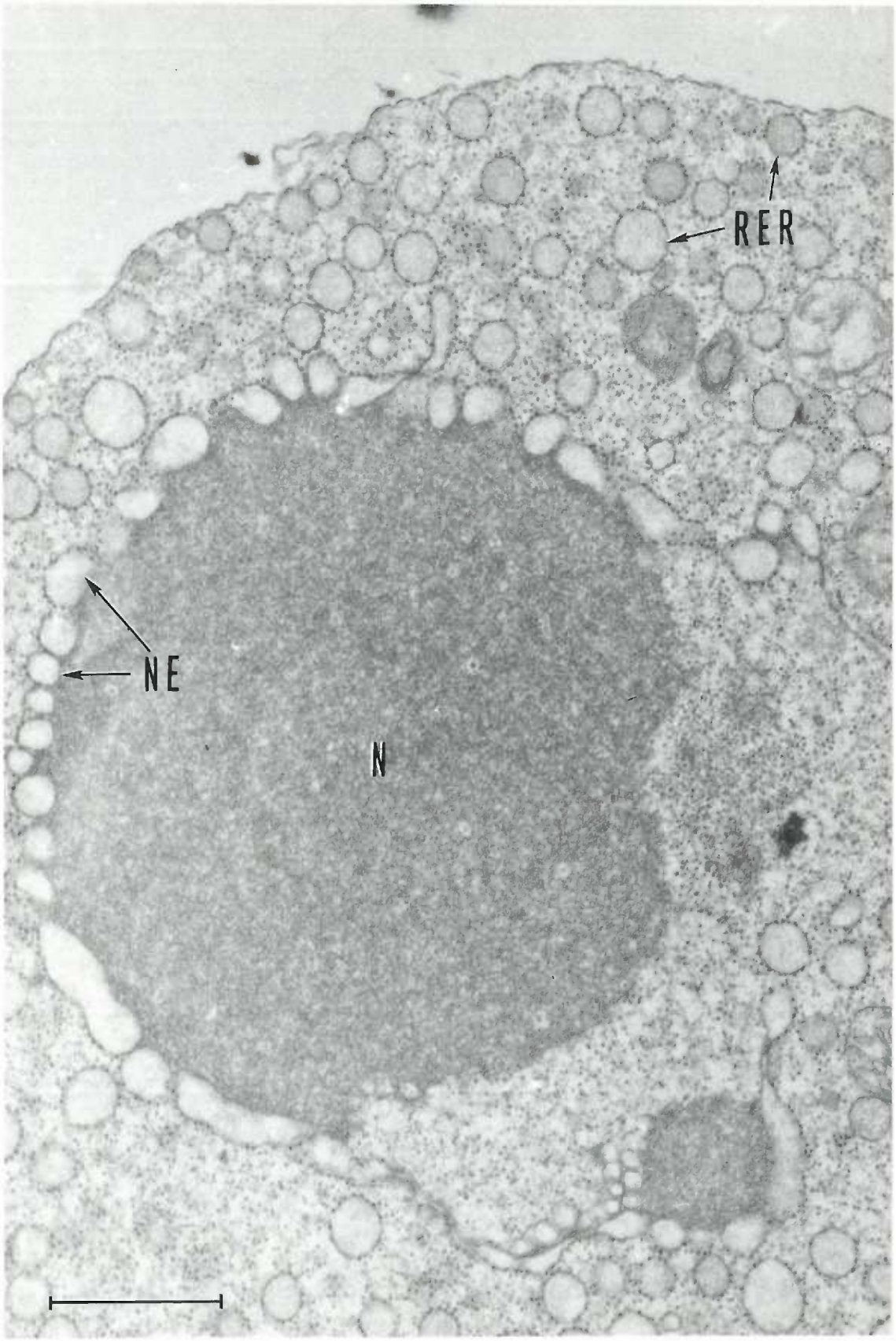

RER
NE
N

Figure 43. 6 day APC - D_2O. This APC has both circular and elongated RER profiles. The cytoplasm also contains mitochondria and a few free ribosomes. The nuclear envelope is seen both in contact with the nucleus and in the cytoplasm. Both membranes of that portion in the cytoplasm are coated with ribosomes. All of the nucleus has a pyknotic appearance. Long villus-like cytoplasmic blebs appear to be forming vacuoles.

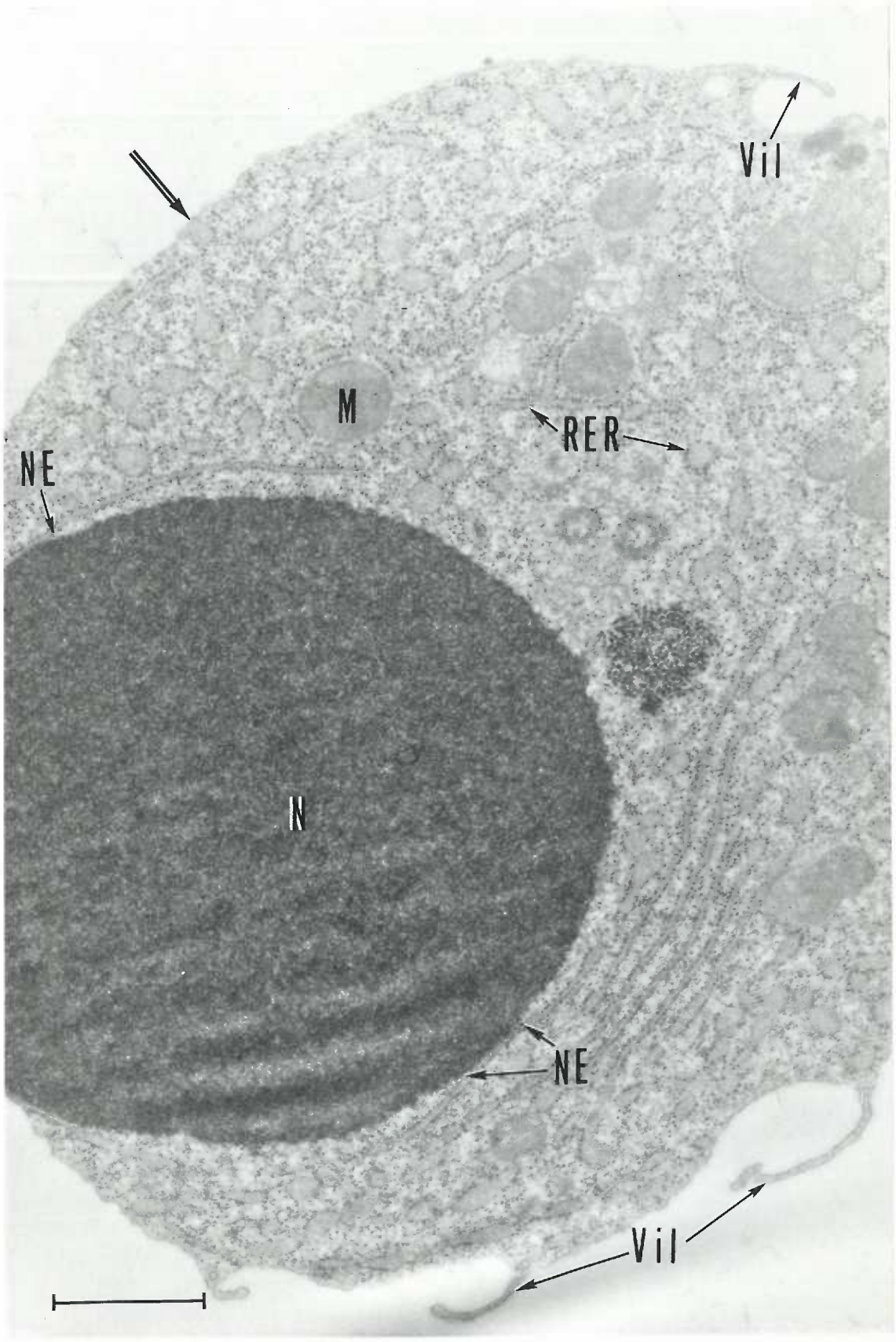

Vil
M
RER
NE
N
NE
Vil

Figure 44. 5 day lysed cell - D_2O. Nucleus, nucleolus, VLPs and disrupted mtiochondria are recognizable features of this lysed plaque-forming cell.

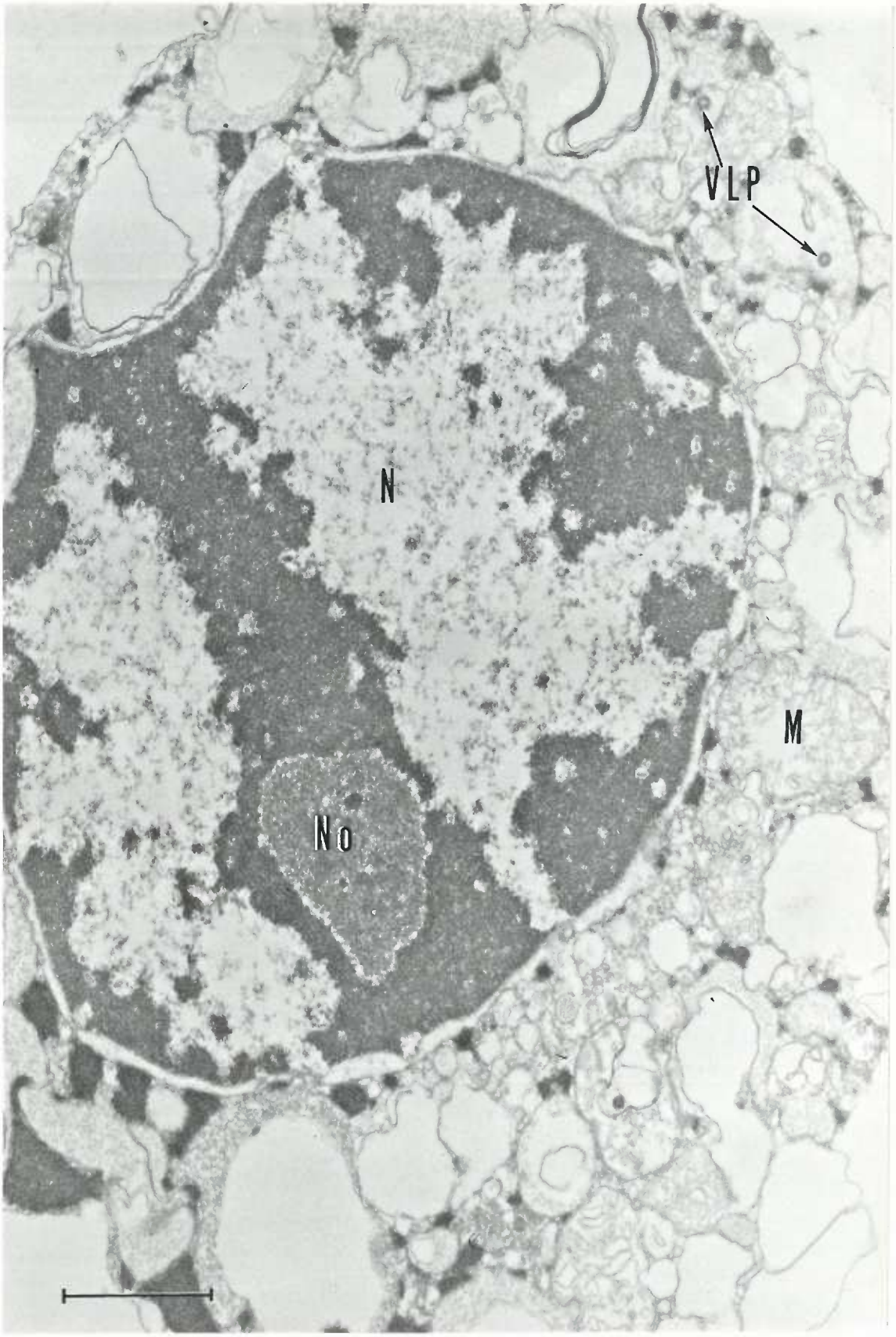

VLP
N
M
No

Figure 45. 4 day plasmablast - PHA. The nucleus of this cell is large and "blast-like". It contains a central nucleolus and a myelin figure next to the nuclear envelope. Most of the RER is in long, very narrow profiles. A group of microtubules cut in cross-section can be seen close to the nucleus.

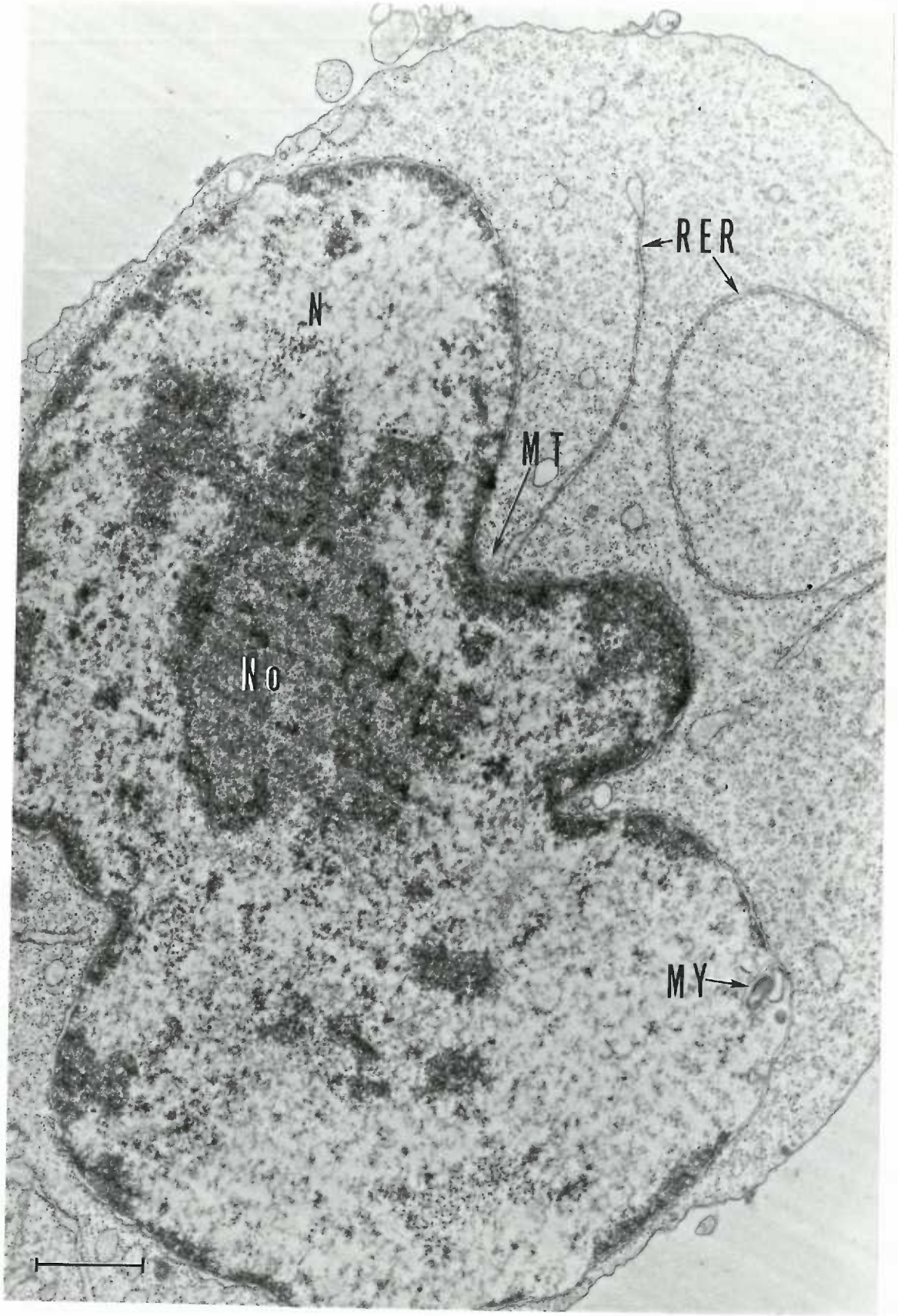

RER
N
MT
No
MY

Figure 46. 5 day proplasmacyte - PHA. The mitochondria of this cell have dense matrices and slightly distended cristae. The RER is quite distended. The cytoplasm also contains a large Golgi apparatus, microfibrils and microtubules. Two nuclear lobes are present.

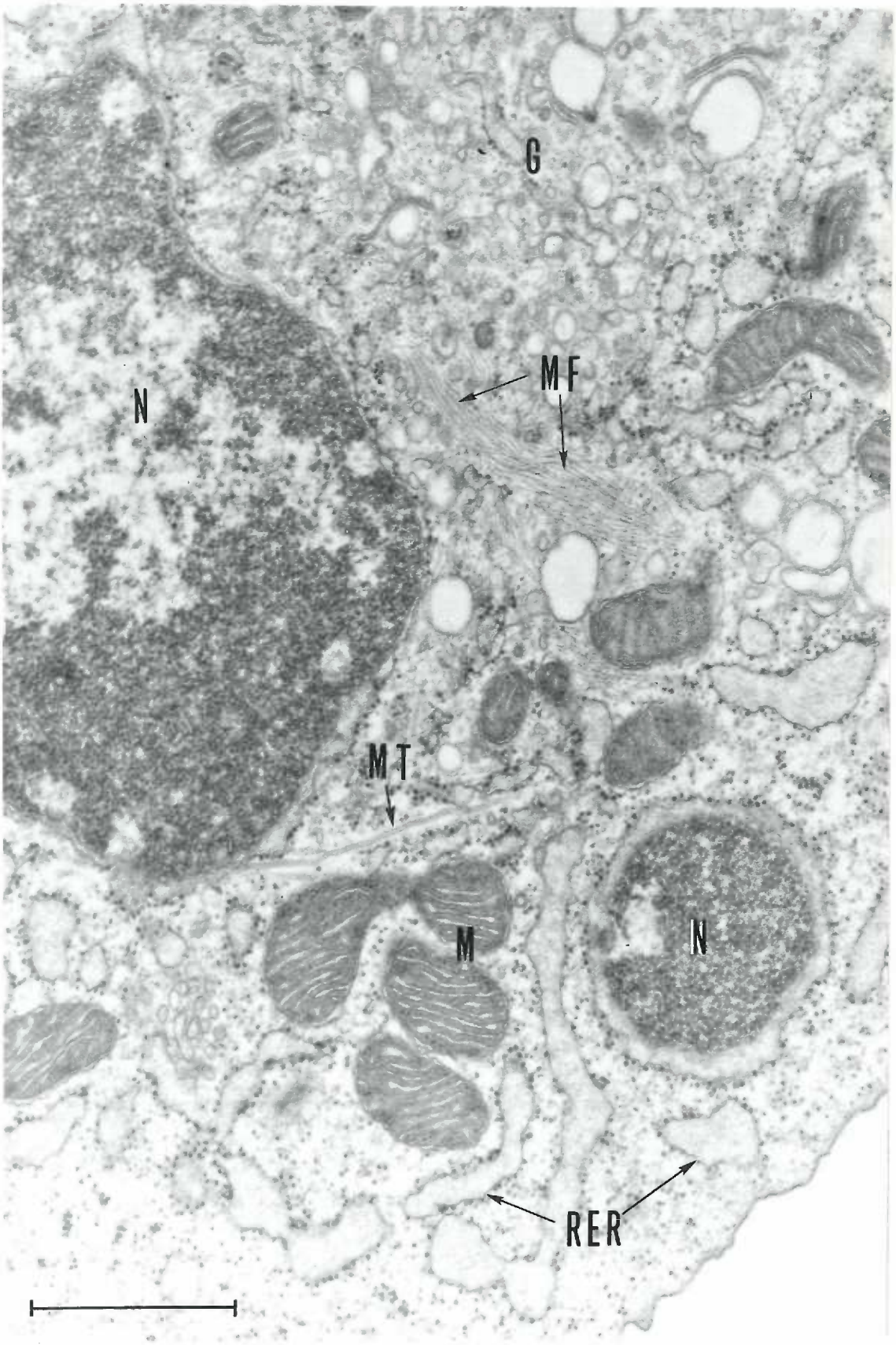

G
N
MF
MT
M
N
RER

Figure 47. 4 day proplasmacyte - PHA. The RER of this cell is quite distended. There are numerous free ribosomes in the cytoplasm. Mitochondria are clost to the large Golgi apparatus. A large bundle of microfibrils and numerous microtubules are present.

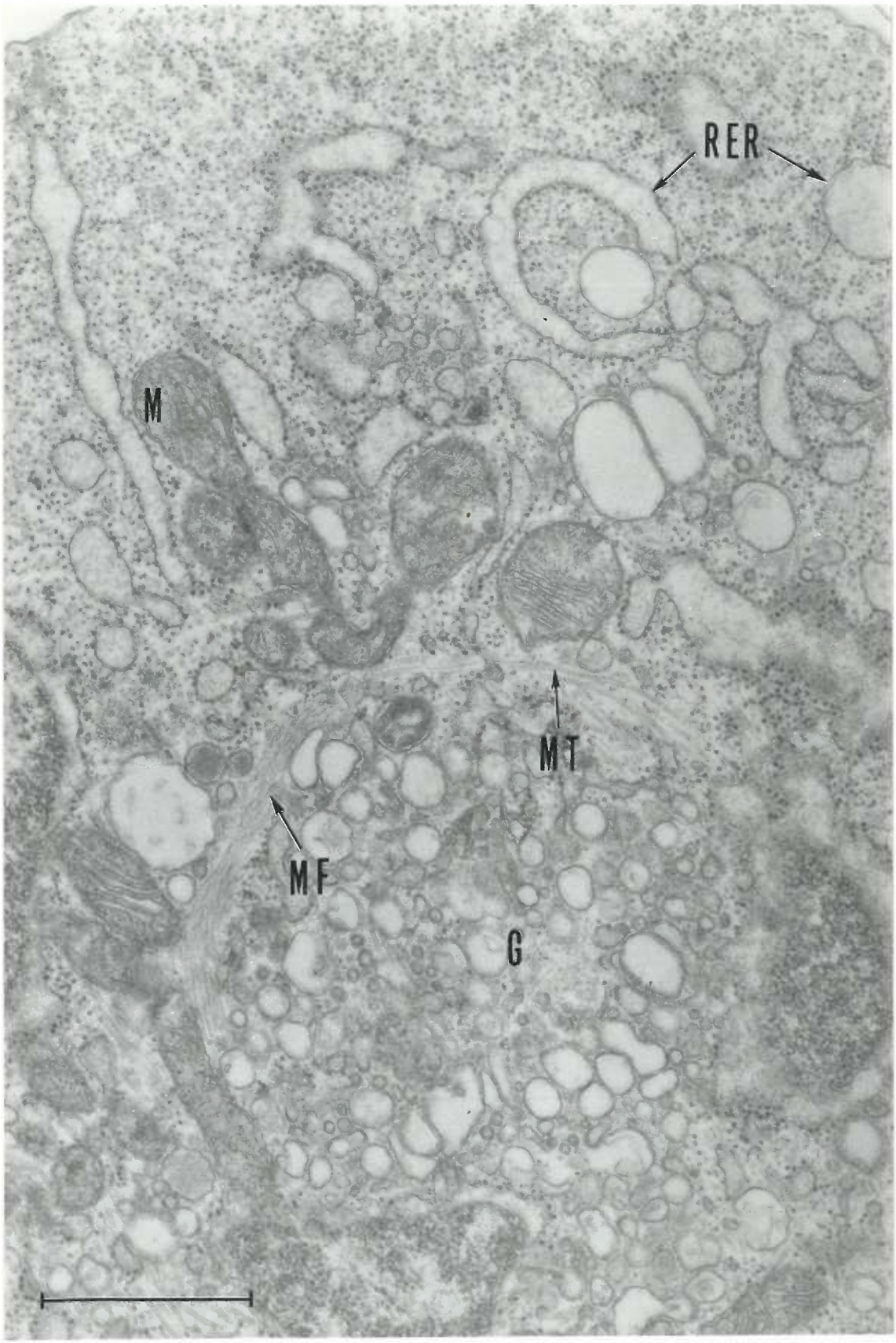

RER
M
MT
MF
G

Figure 48. 5 day proplasmacyte - PHA. The nucleus of this cell is peripheral, but contains only a small amount of heterochromatin. Most of the RER profiles are rounded. One piece of RER is in contact with the cell membrane (double arrow). Only a few mitochondria and a small amount of Golgi apparatus are present.

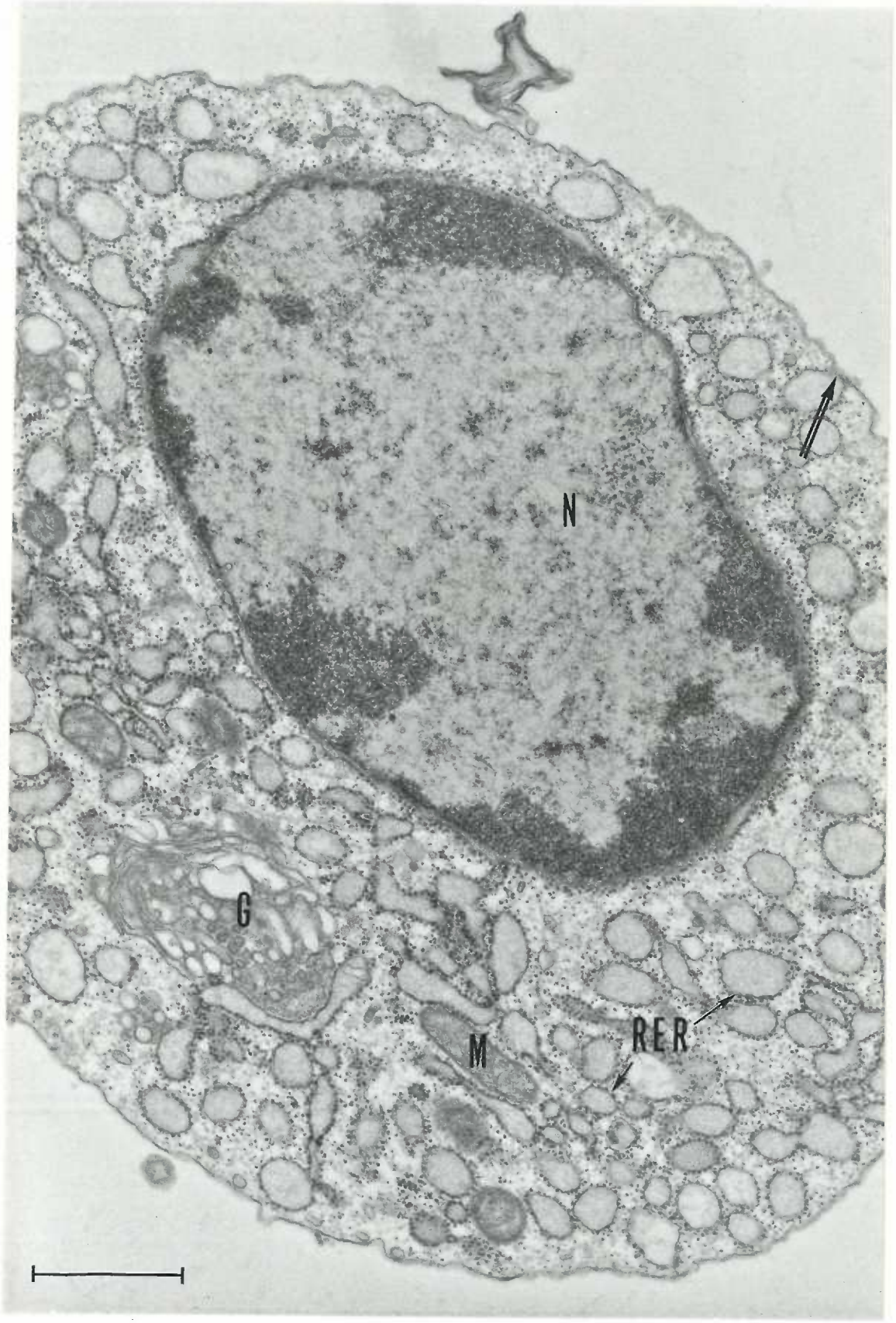

N
G
M
RER

Figure 49. 5 day proplasmacyte - PHA. One portion of the RER of this cell appears to be fused with the cell membrane (double arrow). Mitochondria and numerous single free ribosomes are in the cytoplasm. A nuclear hof cut in cross-section can be seen in the nucleus.

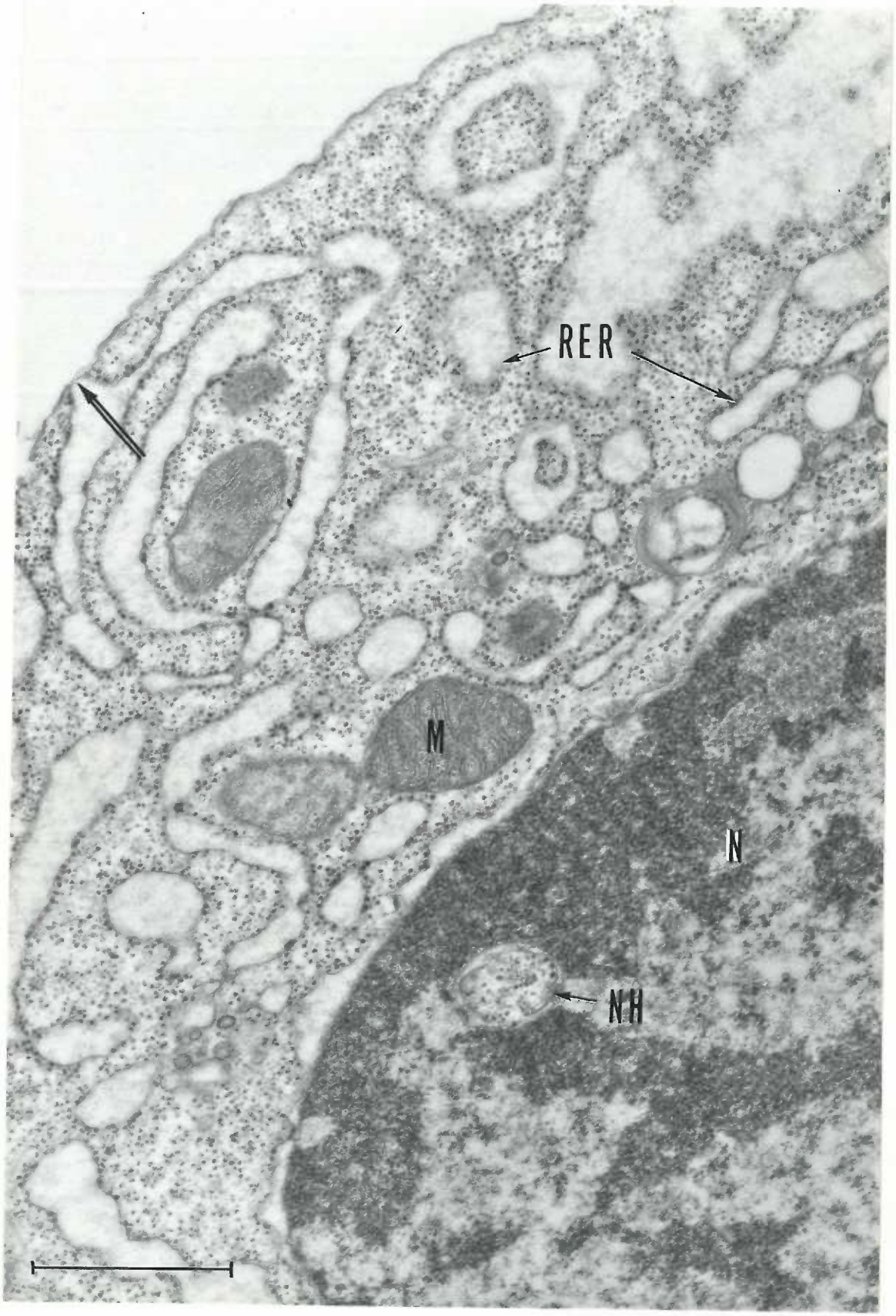

RER
M
N
NH

Figure 50. 5 day proplasmacyte - PHA. Four nuclear lobes can be seen in this cell, with microtubules cut in cross-section between the lobes. The RER is quite distended. A small amount of Golgi apparatus can be seen close to the nucleus.

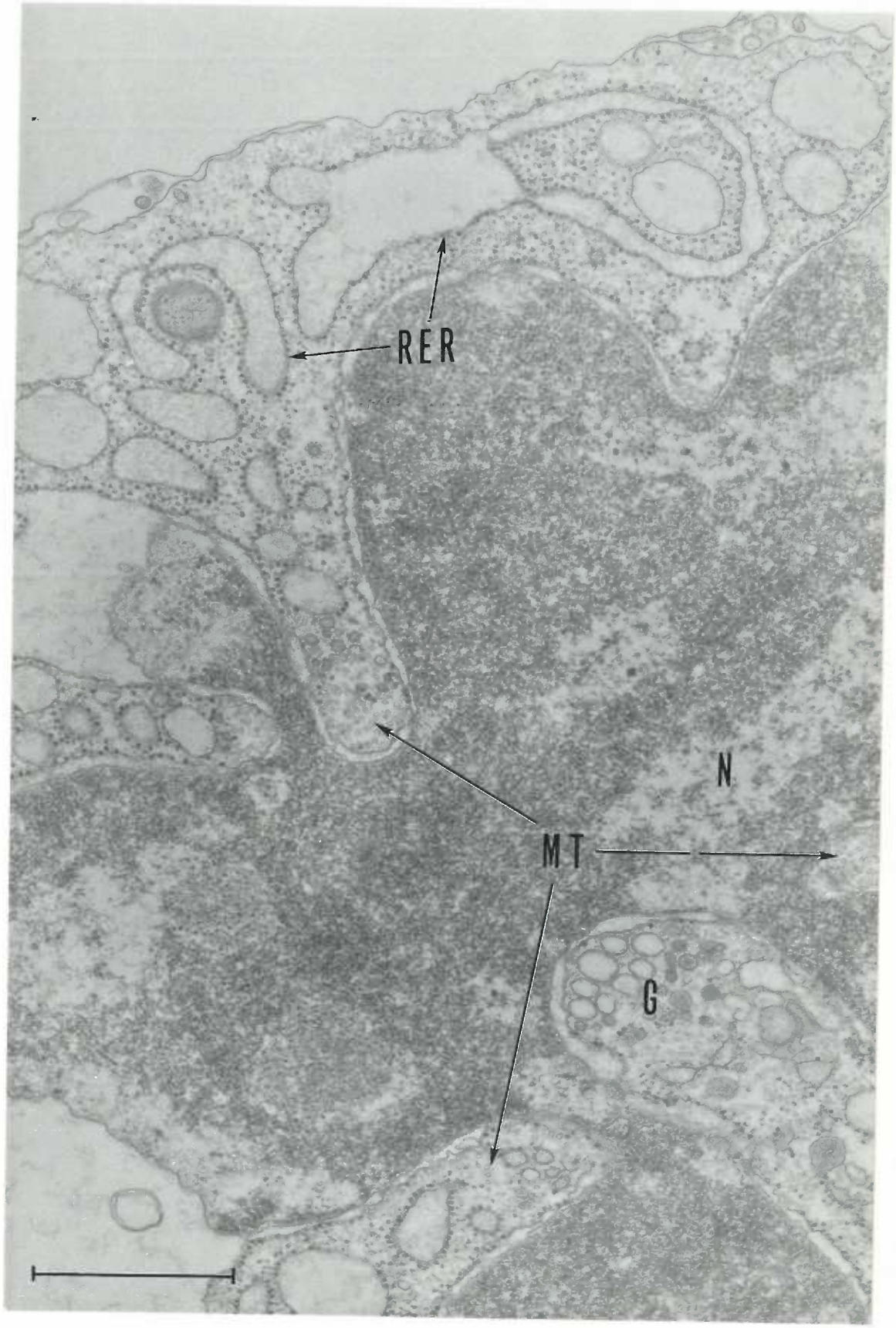

RER
N
MT
G

Figure 51. 4 day plasmacyte - PHA. The nucleus of this cell contains a large amount of heterochromatin. A number of nuclear pores can be seen. Large mitochondria are close to the nucleus and Golgi vesicles. Two Golgi vesicles are continuous with a Golgi vacuole and a portion of RER (double arrows).

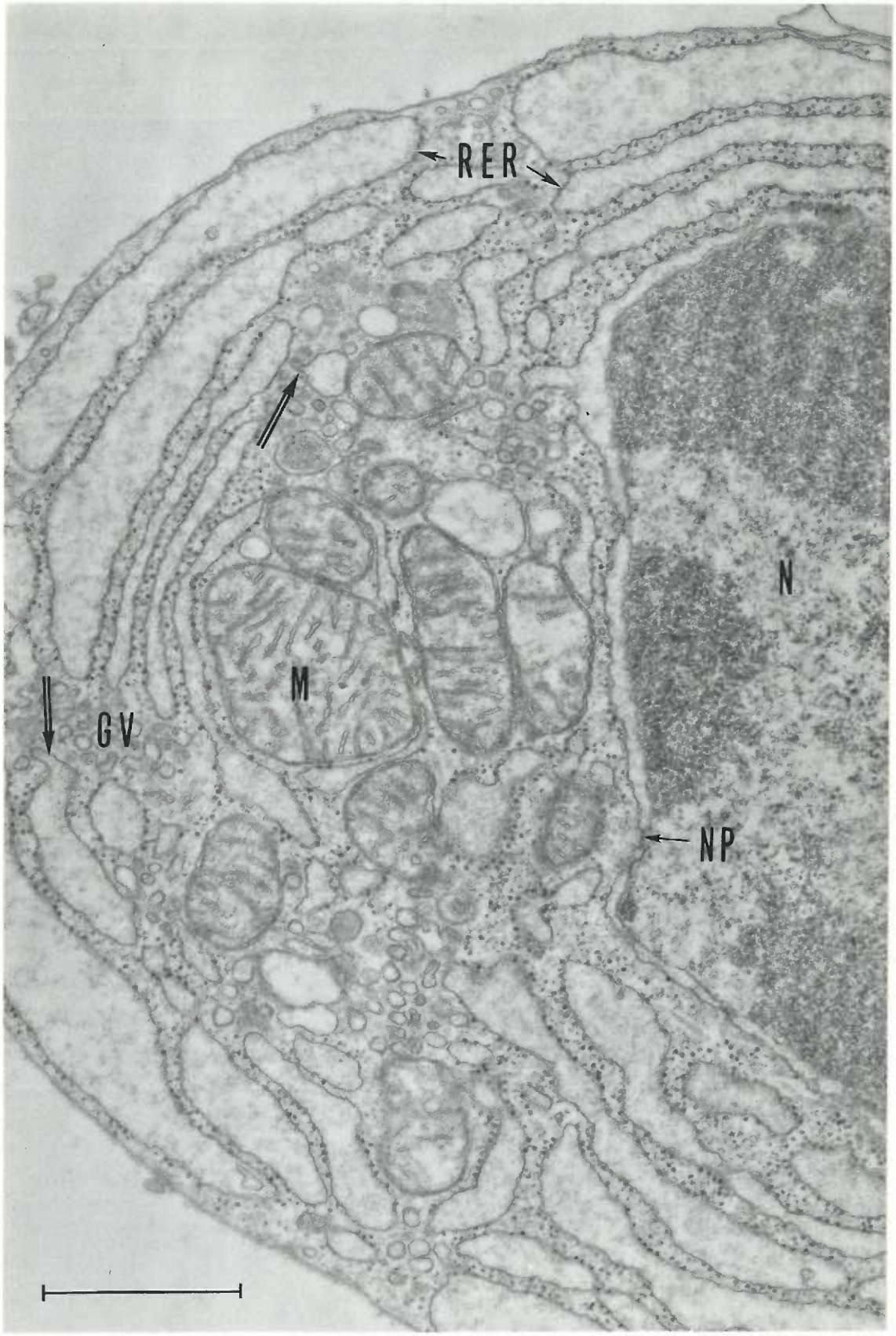

RER
N
M
GV
NP

Figure 52. 5 day APC - PHA. This cell has a very pyknotic appearing nucleus. The extensive RER is seen mostly in rounded profiles. A small amount of Golgi apparatus is present. Three processes (Pr) from the adjacent red blood cell are very close to the membrane of this cell.

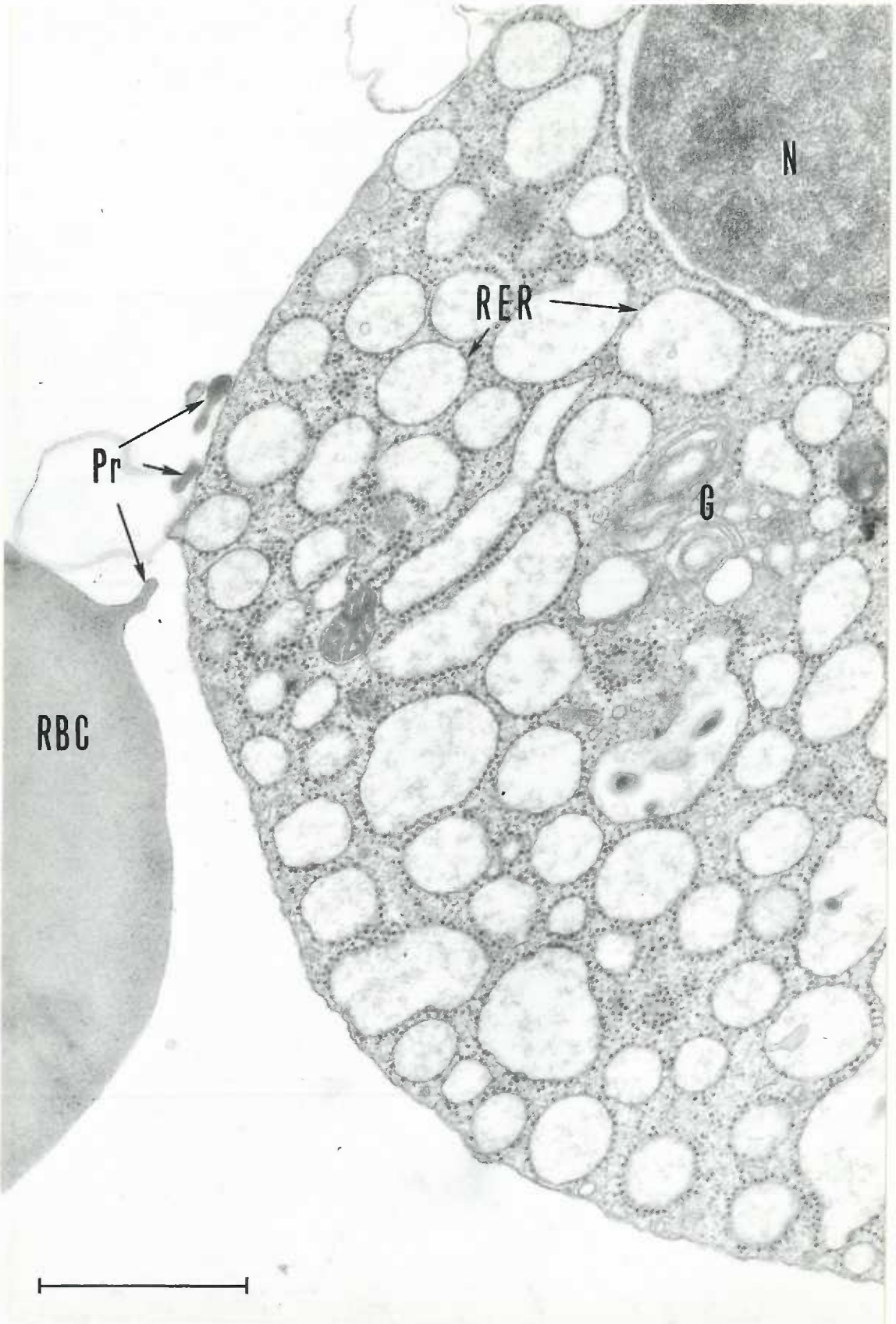

N
RER
Pr
G
RBC

Figure 53. 5 day APC - PHA. All the RER profiles of this cell are rounded. The nucleus is very pyknotic-appearing, and has no nuclear envelope. One large mitochondria can be seen.

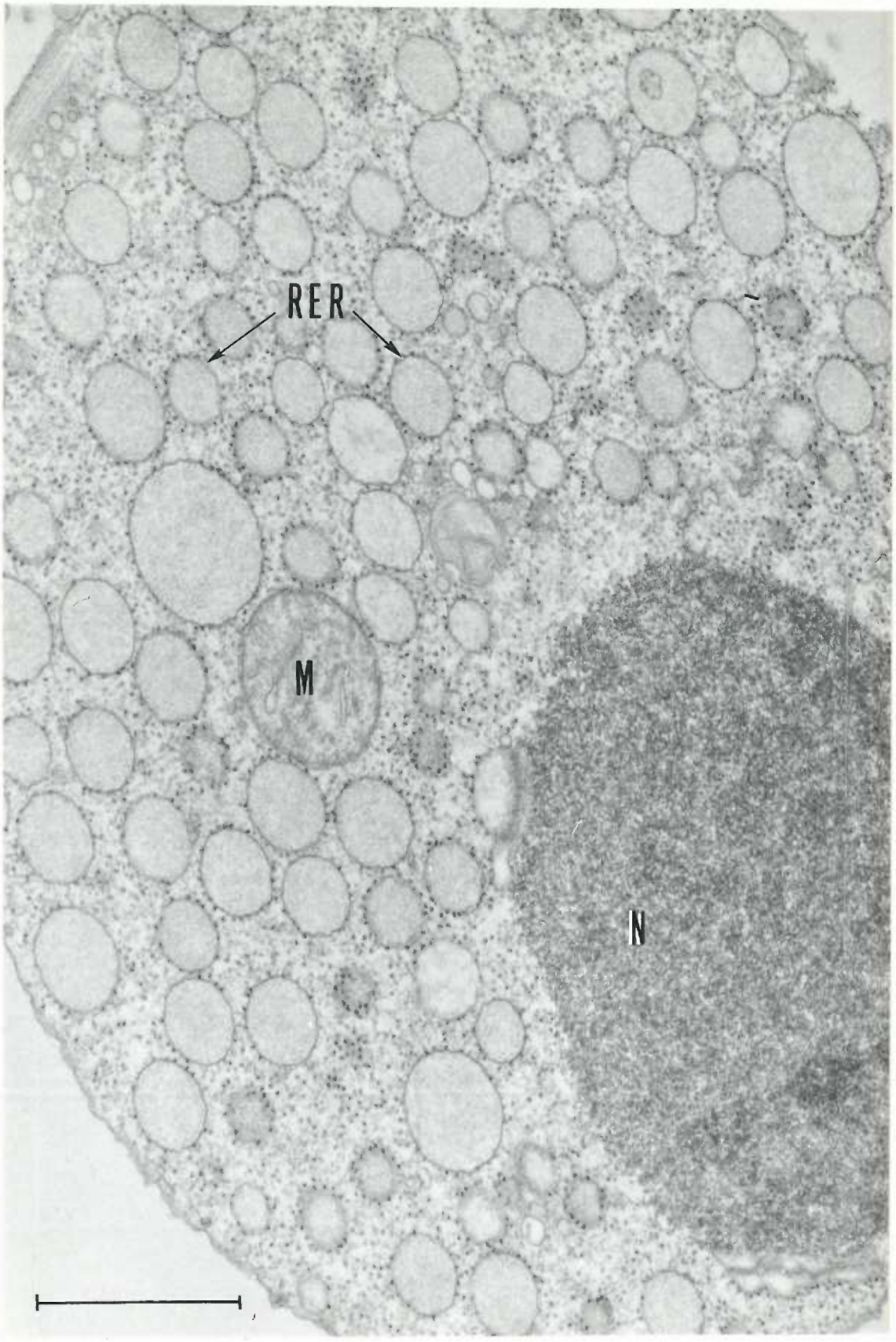

RER
M
N

Figure 54. 4 day plasmablast - ALS. The nucleus of this cell is very large and "blast-like". A few microtubules cut in cross-section can be seen close to the nucleus. There is only a small amount of RER, but it is moderately distended. The cytoplasm is full of free ribosomes, with many being grouped into polyribosomes.

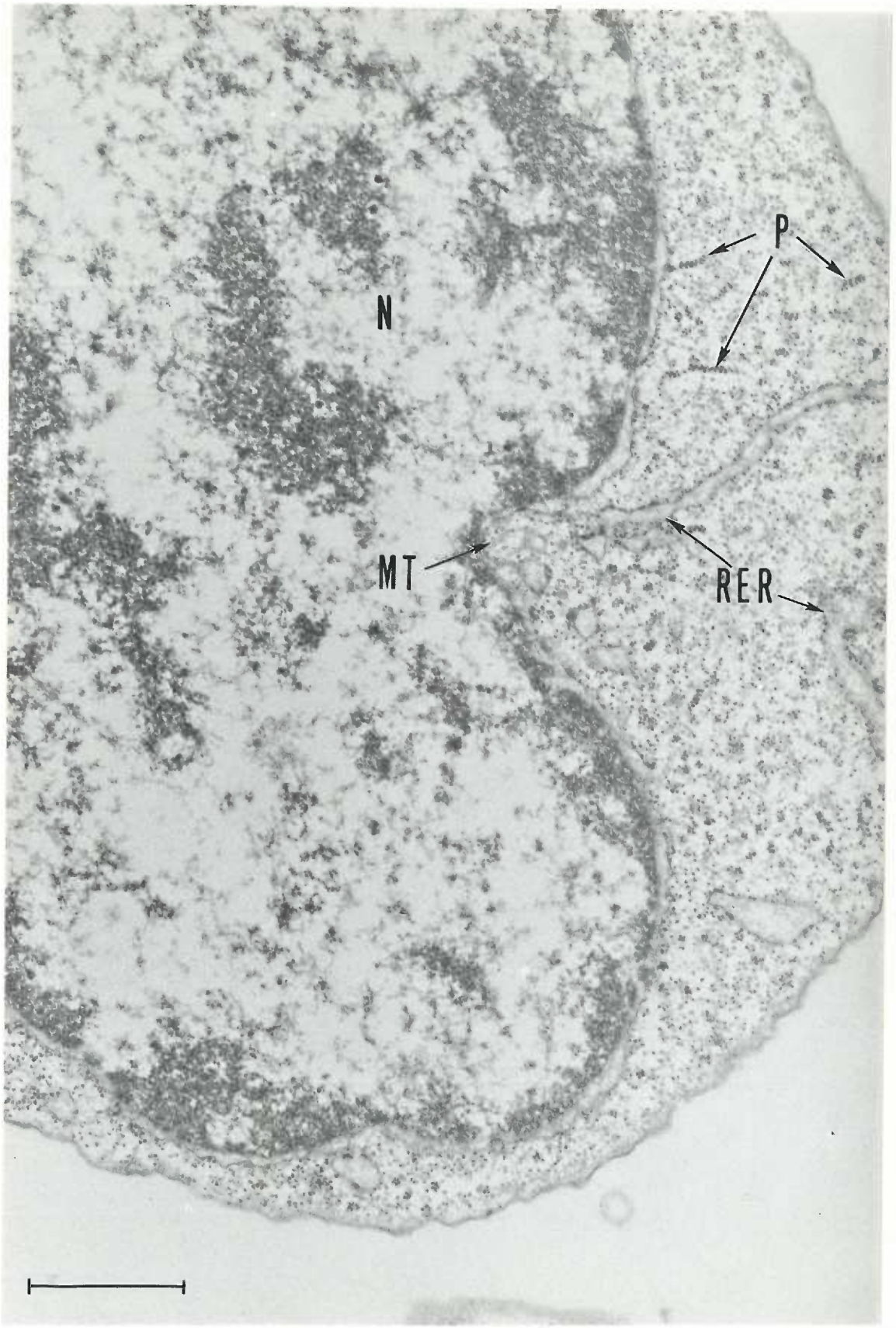

P
N
MT
RER

Figure 55. 4 day plasmablast - ALS. The nucleus of this cell is rounded and slightly peripheral. A number of nuclear pores are present. The cytoplasm contains numerous mitochondria, Golgi apparatus, a small amount of RER, and free ribosomes.

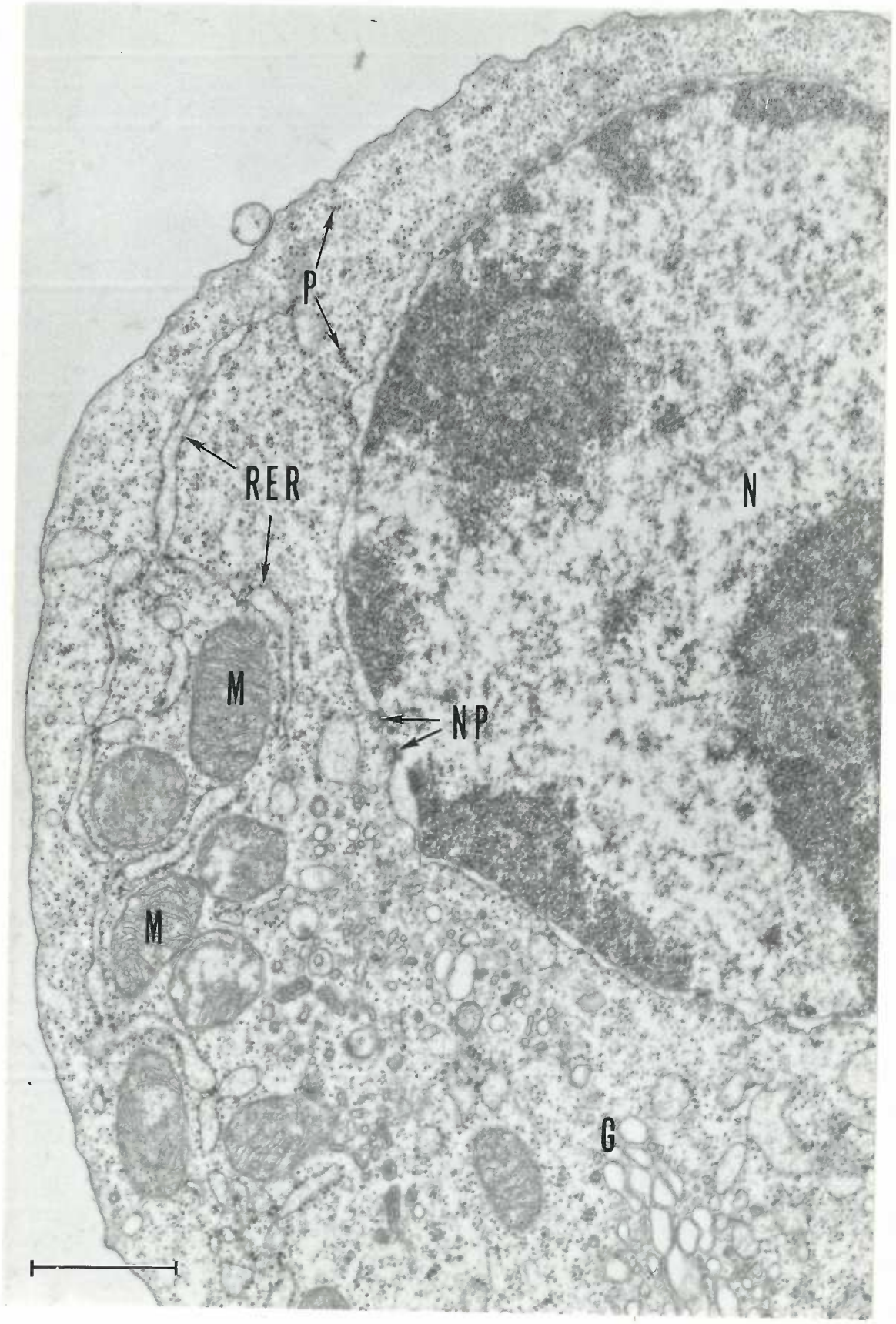

P
RER
N
M
NP
M
G

Figure 56, 4 day plasmablast - ALS. The mitochondria of this cell have distended cristae and dense matrices. Two of them appear to have broken up into dense globules (double arrows). The RER is in long narrow profiles. Part of the nucleolus and a nuclear hof cut in cross section can be seen in the large nucleus.

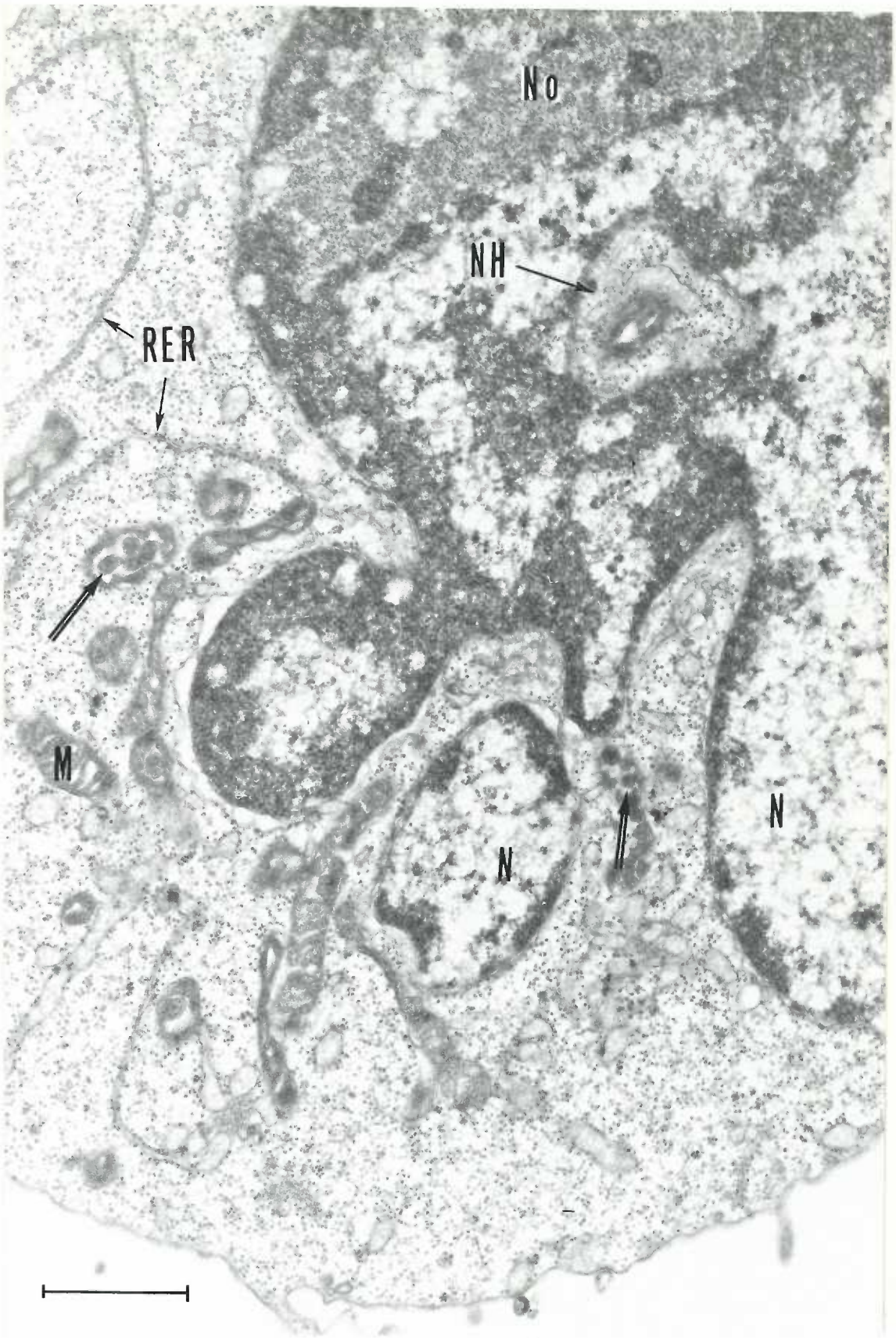

No
NH
RER
M
N
N

Figure 57. 4 day plasmablast - ALS. The nucleus of this cell is very large and "blast-like", and contains a nucleolus surrounded by a thin rim of heterochromatin. A small Golgi apparatus is close to the nucleus. A number of dense bodies are next to the Golgi apparatus. Numerous mitochondria and RER profiles are also present.

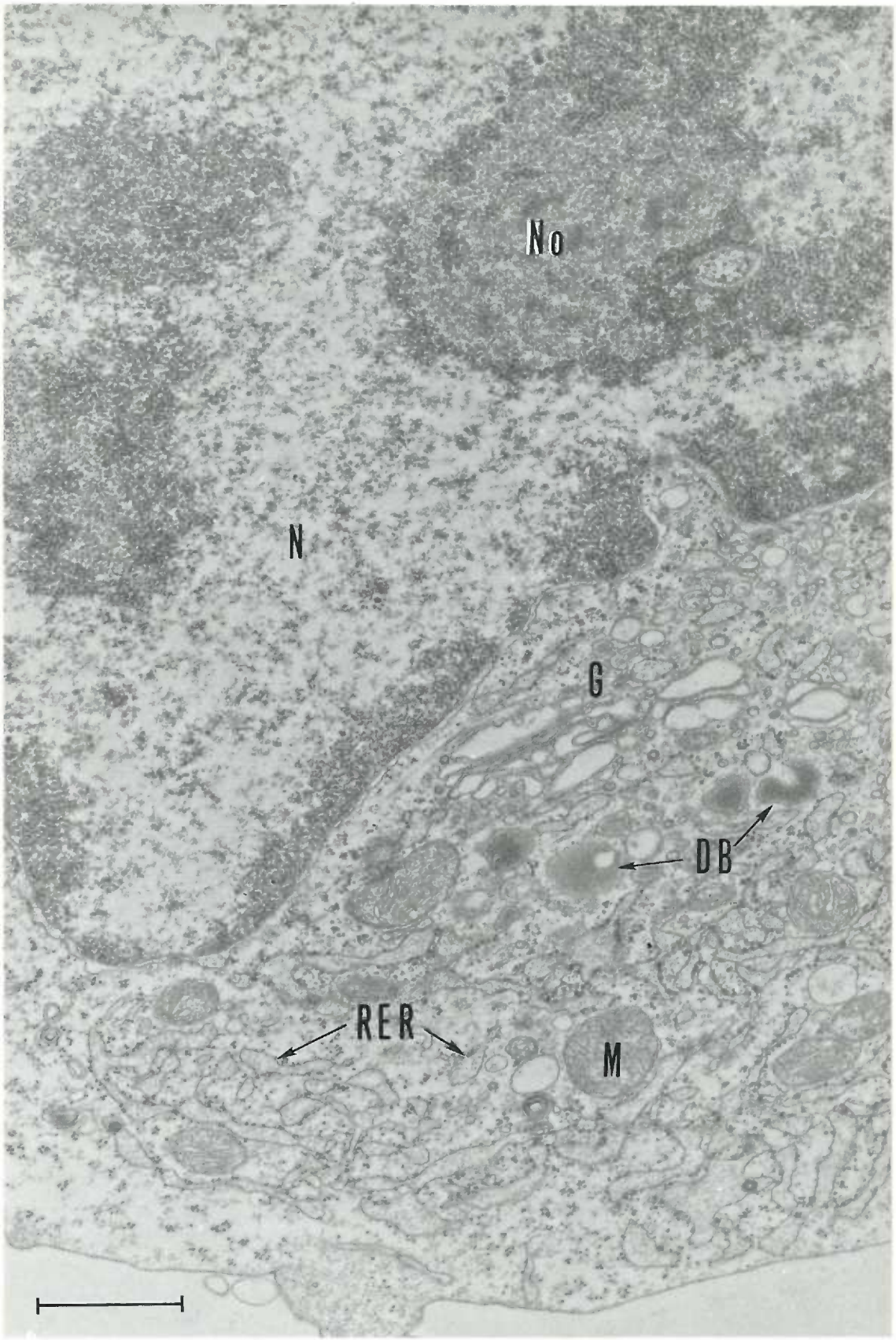

No
N
G
DB
RER
M

Figure 58. 4 day proplasmacyte - ALS. The RER in this cell is quite extensive, but only moderately distended. One portion of RER is very close to the cell membrane (double arrow). Microtubules are associated with the centriole, which is located in the Golgi apparatus. Two large dense bodies and numerous mitochondria are present.

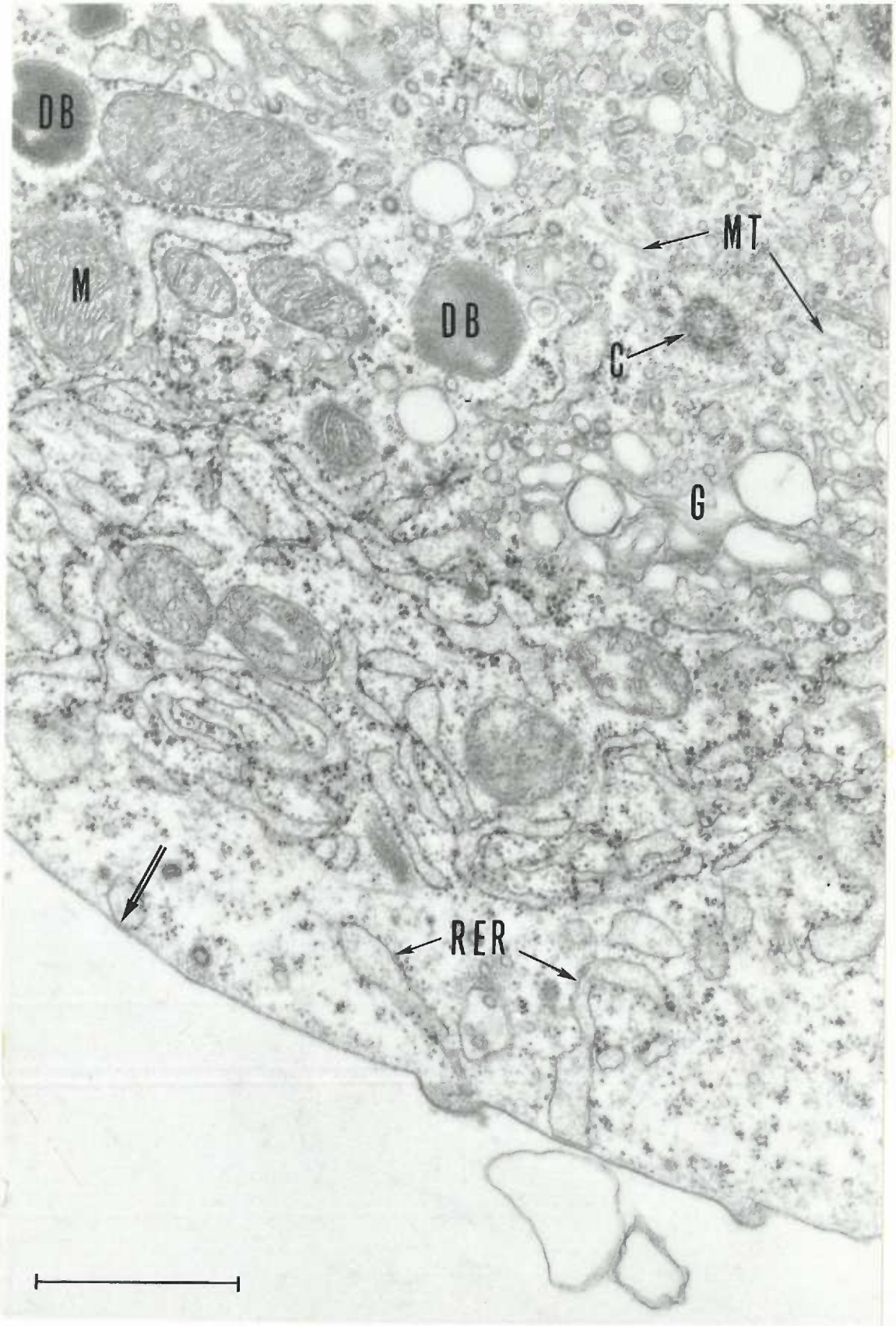
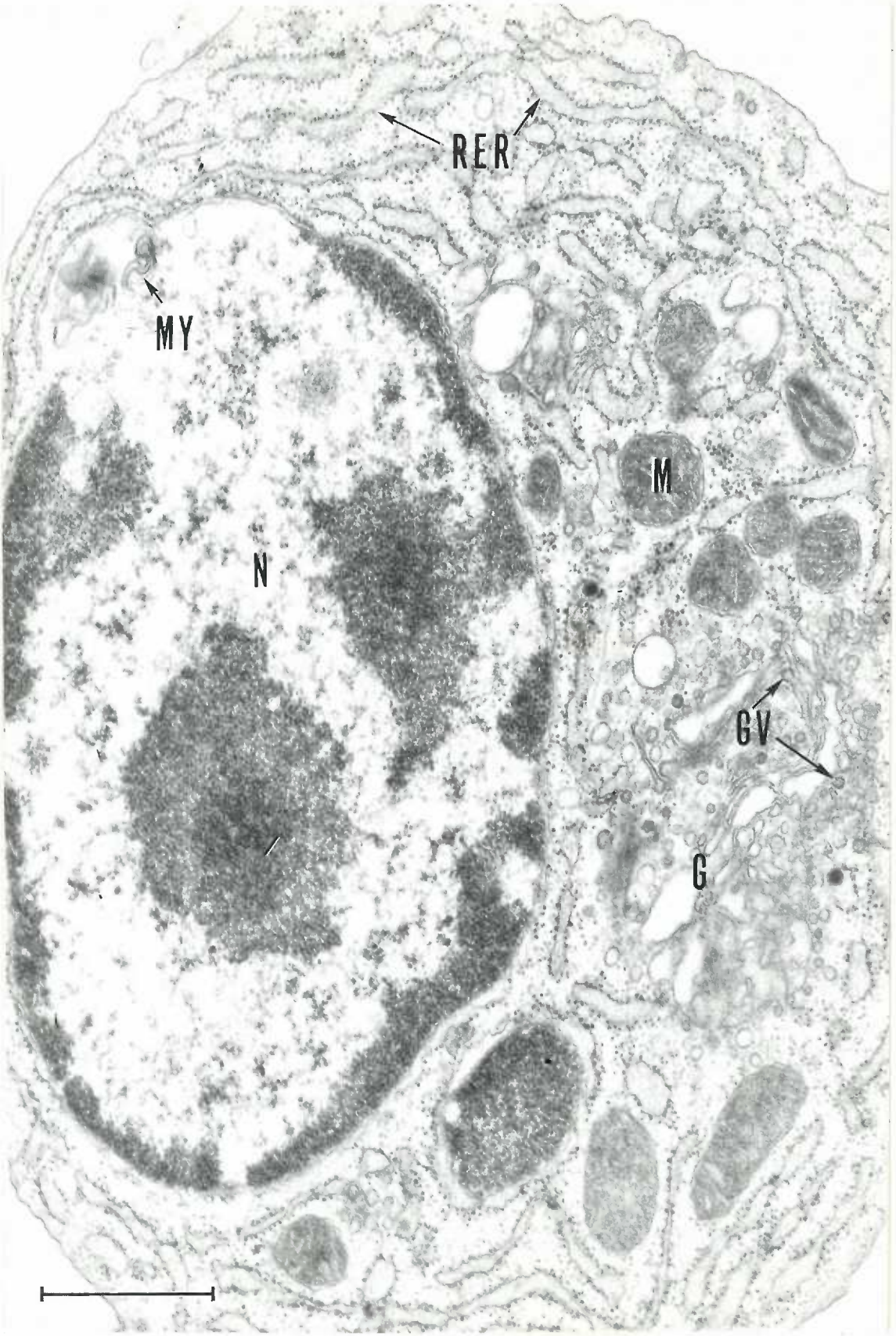

DB
M
DB
MT
C
G
RER

Figure 59. 4 day plasmacyte - ALS. The nucleus is peripheral and contains a myelin figure close to the nuclear envelope. The RER is extensive and well ordered. The numerous mitochondria have dense matrices. Golgi vesicles can be seen connected to Golgi saccules.


RER
MY
M
N
GV
G