

ELECTRON MICROSCOPIC OBSERVATIONS ON  
ANTIBODY-PRODUCING CELLS IN  
LYMPH AND BLOOD\*

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PLATES 20 TO 24

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In a preceding report (1) we described a method which permitted detailed investigation of the fine structure of antibody-producing cells obtained from lymph nodes. These cells, from rabbits injected with sheep erythrocytes, were recognized by the hemolytic plaques which they produced on incubation with the target erythrocytes in vitro. Such plaques were removed for study of the central cell by electron microscopy. We found that cells in the category both of lymphocytes and of plasma cells could produce antibodies of the 19S type after a single injection of the antigen. The lymphocytic cells showed an increase in structures presumably associated with synthetic functions, such as endoplasmic reticulum, Golgi body, and nucleolus, not present in the quiescent small lymphocyte. The gradations of organelle development through a series of cells which included lymphocytic and plasmacytic forms suggested that in the lymph node the antibody-producing cells underwent a progression of development leading from the small lymphocyte to the mature plasma cell, with a great variety of intermediate stages. Of these cell types some, of the plasmacytic series, have been identified in other electron microscopic studies of antibody-forming lymph node cells by Bussard and Binet (2), and by Fitch, Rowley, and Coulthard (3).

The antibody-producing cells in our earlier study (1) were obtained from the lymph node by teasing the excised organ, and thus might not represent the cells which under physiological conditions would pass into the efferent lymph and eventually into the peripheral blood. These extranodal antibody-producing cells could represent the most highly organized types, with respect to their fine structure, since they were presumably fully developed in the lymph node before leaving it to enter the efferent lymph vessel. Such cells, obtained from efferent lymph, the thoracic duct, and peripheral blood were investigated and the results are presented.

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The previous study of antibody-producing lymph node cells was largely confined to those producing 19S antibody, with no attempt to detect cells producing 7S antibody. It is possible that both types of antibody are produced in the same cells, 19S antibody synthesis preceding that of 7S (4), and that this progression in antibody production is reflected in structural changes within the cell. Therefore, in the present study cells were selected from lymph nodes and efferent lymph which produced 7S antibody, in addition to those from efferent lymph and peripheral blood which had synthesized primarily antibodies of the 19S type.

#### *Materials and Methods*

*Efferent Lymph Cells.*—Rabbits were given a single injection of 0.5 ml of 50% sheep RBC in each hind foot-pad. 4 days later the animals were anesthetized, the skin of the inner aspect of the knee incised, the semitendinosus and semimembranosus muscles cut, and each popliteal lymph node was exposed. A ligature placed around the lymphatic vessel served to distend it and to provide a better hold on it. Lymph was collected into a syringe moistened with heparin solution, 1000 USP units/ml. The lymph was centrifuged at 1200 RPM for 5 min and the sediment was resuspended in Earle's balanced salt solution for plating.

*Thoracic Duct Cells.*—Rabbits were injected with 0.3 ml of 50% sheep RBC into the mesenteric lymph nodes. 5 days later thoracic duct lymph was collected, under anesthesia, in heparin. The lymph was centrifuged at 2000 RPM for 5 min at room temperature and the cells resuspended in Earle's balanced salt solution for plating.

*Blood Leukocytes.*—Rabbits were given two intravenous injections of 4 ml of 20% sheep RBC with a 3 wk interval. 3 days after the second injection, blood was collected by cardiac puncture in one-tenth volume of a 5% solution of ethylene diamine tetra-acetate. The leukocytes were separated by a modification of the method of Agostoni and Ideo (5), in which ammonium chloride is used to lyse the red blood cells. Four volumes of a 0.83% solution of  $\text{NH}_4\text{Cl}$ , pH 7.0, were added to the blood, which was then centrifuged at 2000 RPM for 10 min at 4°C. The white cell pellet obtained was washed twice with the ammonium chloride solution and twice with medium 199.

*Hemolytic Antibody Plaque Procedure.*—Plaques due to cells producing 19S antibody were developed as described previously (1). For preparation of plaques due to 7S antibody, cells were obtained from popliteal lymph node and their efferent lymphatics 7 days after a single injection of sheep RBC into the hind foot-pad. The plates were incubated for 1 hr at 37°C under a 1 mm layer of 0.1 M 2-mercaptoethanol (2-ME). This was then removed and the plate was washed. Goat antiserum to rabbit  $\gamma$ -globulin was added, at an effective dilution, as has been used by a number of workers to enhance the production of hemolytic plaques by antibody of low hemolytic efficiency (6-9). After 30 min of incubation at 37°C this was replaced by normal guinea pig serum 1:4 as a source of complement, and a final 30 min incubation at 37°C was carried out.

*Electron Microscopy.*—The antibody-producing cells in the center of the plaques were selected and harvested by the criteria and techniques described previously (1).

#### RESULTS

*Immunologic Data.*—Cells producing 19S antibody were found in the preparations of cells from efferent lymphatic vessels, with a frequency in the range of

20 to 48 per million. In the thoracic duct lymph such plaque-forming cells were found in the range of 9 to 33 per million, and in the blood cells in the range of 50 to 70 per million. Plaques due to 7S 2-ME-resistant antibody, obtained 7 days after the injection of sheep RBC, were produced by 40 to 51 cells per million in the case of lymph node cells and by 30 to 46 cells per million in the cells obtained from the efferent lymphatic vessel.

*Fine Structure of Antibody-Producing Cells.*—Cells producing primarily 19S antibody which were found in efferent lymph and the thoracic duct were similar in appearance. The range of development of cytoplasmic organelles, from a sparse but evident endoplasmic reticulum, to one as highly organized as in a plasma cell, was similar to that found previously in cells derived from the lymph node. However, all structures involved in active synthesis were more pronounced. The most frequent type of antibody-producing cell encountered in the lymph vessels is demonstrated in Fig. 1. This was a relatively large cell, with a diameter of  $6.5 \mu$ . The nucleus was centrally located, with an irregular outline and partially condensed chromatin. The organelles consisted of a few mitochondria and short channels of endoplasmic reticulum, most of which were widened. These channels were disorganized and sectioned at various angles. In the cytoplasmic matrix the ribosomes were aggregated into polyribosomes, a feature consistently found in these antibody-producing cells. The polyribosomes can be seen in more detail in Fig. 2.

Another type of plaque-producing cell found in these vessels showed greater resemblance to typical lymphocytes, with eccentric, deeply indented nuclei, and with all organelles, such as Golgi body and mitochondria, confined to the large pole of the cell. In these antibody-producing lymphocytes the Golgi body, when present in the section, was very large and was composed of smooth channels and vesicles. The endoplasmic reticulum, where obvious, was greatly enlarged, forming vesicles containing a grayish material. These features can be seen in Fig. 3.

The third type of cell producing 19S antibody and found in lymph vessels showed a well organized endoplasmic reticulum with flattened channels. It was the largest cell seen, with an average diameter of  $8.5 \mu$ , the cytoplasm resembling that of a plasma cell although the nucleus was similar to that of the cells described above.

Antibody-producing cells isolated from the peripheral blood had a characteristic morphology which was distinctly different from that of cells both in lymph nodes and lymphatic vessels. A representative of these cells can be seen in Fig. 4. In general they were relatively small cells with a diameter of  $5 \mu$ . The nucleus represented almost the total area of the cell. In it the chromatin was partially condensed. A nucleolus was rarely seen. The narrow ring of cytoplasm was wholly organized into parallel lamellae of endoplasmic reticulum, which were

expanded and contained granular material. The narrow spaces between these lamellae contained a profusion of polyribosomes. The mitochondria were few, and usually concentrated at one pole of the cell. A noteworthy feature was the large size of the Golgi body as compared to the mass of cytoplasm. It consisted of smooth channels and large vesicles. These details are demonstrated in Fig. 5. Occasionally the sparse cytoplasm of these cells was not as well organized, but showed widened endoplasmic reticulum with extremely large vesicles (Fig. 6).

Cells producing 7S antibody were isolated from lymph nodes and efferent lymph, and their fine structure investigated. The cells obtained from both lymph node and efferent lymph showed some small vesicles, containing granular material (Fig. 7) resembling those found in the cells producing 19S antibody. However the most characteristic features of these cells were the few long narrow channels of endoplasmic reticulum seen in Figs. 7 and 8. Few mitochondria were apparent and the Golgi body, when demonstrable, was small.

The nucleated cells outside the plaques were typical small lymphocytes, except for an occasional eosinophilic cell. In contrast to the effectively lysed red cells, presenting themselves as ghosts, always found in proximity to the antibody-producing cell within the plaque, unlysed erythrocytes surrounded the quiescent small lymphocytes outside the plaques. These cells were of an average diameter of 4.5  $\mu$ . A typical cell of this kind can be seen in Fig. 9.

#### DISCUSSION

Antibody-producing cells obtained from an efferent lymphatic vessel, the thoracic duct, and peripheral blood of rabbits injected with sheep erythrocytes showed a number of morphologic features which are of interest, both within this group of cells and in comparison to cells of similar function previously isolated from lymph nodes (1). The extranodal cells described here showed some similarities and some differences when compared with the nodal cells. Cells obtained from both the lymph nodes and the extranodal sources showed considerable pleomorphism, a variety of cell types including in each case relatively simple forms with resemblances to typical lymphocytes and cell types in which the cytoplasm showed a wide range of development of organelles commonly associated with protein synthesis, such as the endoplasmic reticulum and Golgi body. Between the two sets of cells there was, however, a major difference. Among the antibody-producing cells of lymph and blood, regardless of the degree of development and organization of endoplasmic reticulum, the cells showed characteristically, small size and essentially spherical shape, approximately central position of the nucleus, and, probably most important, areas of condensed chromatin in the nucleus. In the case of the cells with the most highly developed organelles, those obtained from blood, there was the striking combination of a cell with the size and nuclear chromatin distribution of the lympho-

cyte but with the degree of organization of endoplasmic reticulum in the cytoplasm characteristic of the plasma cell, as shown in Fig. 4. Among the lymph node cells, on the other hand, with progressive development of the endoplasmic reticulum there was concomitant loss of condensation of chromatin in the nucleus, increase in size of the cell, departure from spherical shape, and eccentricity of the nucleus; that is, increasing similarity to the morphology of what is recognized as the plasma cell. In these studies, the plasma cell has not thus far been found among the antibody-producing cells of lymph or blood. Thus this cell, with its fragility and evidence for storage and holocrine release of antibody, may represent a tissue-bound cell, which, with its combination of continuous secretion and ultimate release of stored antibody, is capable of maximal short term production of antibody. On the other hand, the modified lymphocyte, which leaves the lymph node intact, retaining its differentiation of nuclear chromatin and its great numbers of cytoplasmic polyribosomes, could maintain secretion of antibody for longer periods, outside the lymph node of origin.

In the classical electron microscopic picture the lymphocyte, presumably in its resting state, is seen to be lacking in endoplasmic reticulum, its ribosomes being distributed throughout the cytoplasm. In the antibody-producing lymphocyte there is a small amount of endoplasmic reticulum, usually widened and surrounded by ribosomes. It seems unlikely, however, that these small areas could account for the considerable rate of antibody secretion by these cells, since in the earlier study (1) the plaques containing these cells were as large as those produced by cells with fully developed endoplasmic reticulum. The ribosomal particles in the cytoplasmic matrix must, then, have contributed substantially to the amount of antibody secreted, and, indeed, they were aggregated into polyribosomes in all the cells which have been examined. Consistent with this is the observation that these polyribosomal aggregates were far more pronounced in lymphocytes found in the center of plaques than in the small quiescent lymphocytes outside or near the edge of the plaques, an example of which can be seen in Fig. 9. How a cell possessing only a minimal amount of endoplasmic reticulum releases the protein synthesized on its free polyribosomes is not known.

Many of the antibody-producing cells observed in this investigation did not fit well into the recognized categories of cells of the lymphatic system. It must, however, be remembered that these cells were selected on the basis of their function, and constituted only a very small fraction of the total of cells obtained from lymph and blood. The chances that these cell types would have been found in electron microscope studies of random cells of blood or lymph are indeed minute.

The insight gained by this and the foregoing study on the fine structure of

antibody-producing cells is limited by the total number of cells investigated, and the fact that the response was to one antigen, sheep erythrocytes, in the rabbit. It remains to be seen whether variations in antigens, the schedule of their injection, and other hosts will result in emergence of different cell types. This seems unlikely, because changes in the basic structural feature of cells must be limited, unless cells other than those of the lymphatic system are involved in the synthesis of antibody.

#### SUMMARY

Antibody-producing cells have been identified among cells obtained from efferent lymphatic vessels, the thoracic duct, and peripheral blood. These cells, which produced plaques of hemolysis and which were quite rare (20 to 50 per million), due in most instances to 19S antibody, were located and studied by electron microscopy.

Of the antibody-producing cells found in these three sites there were several features common to all: small size (5 to 8  $\mu$ ), generally spherical shape, approximately central position of the nucleus, and retention in the nucleus of the condensations of chromatin characteristic of the lymphocyte.

The differences among the cells of these sources were largely in the relative amount and state of organization of the organelles of the cytoplasm. In cells of the efferent lymphatic vessel and the thoracic duct, the endoplasmic reticulum showed a range from relative scarcity to considerable numbers of well organized channels. Between these extremes were cells with a considerable amount of endoplasmic reticulum, the channels disorganized and sectioned at various angles. The cytoplasmic matrix of all of these contained a profusion of polyribosomes. Antibody-producing cells obtained from peripheral blood showed, around the roughly spherical nucleus, a ring of cytoplasm which was narrow, but wholly organized into parallel lamellae of endoplasmic reticulum, with many polyribosomes between these, and a large Golgi body.

Some similarities and some differences of these cells, in comparison with antibody-producing cells obtained from lymph nodes, have been indicated.

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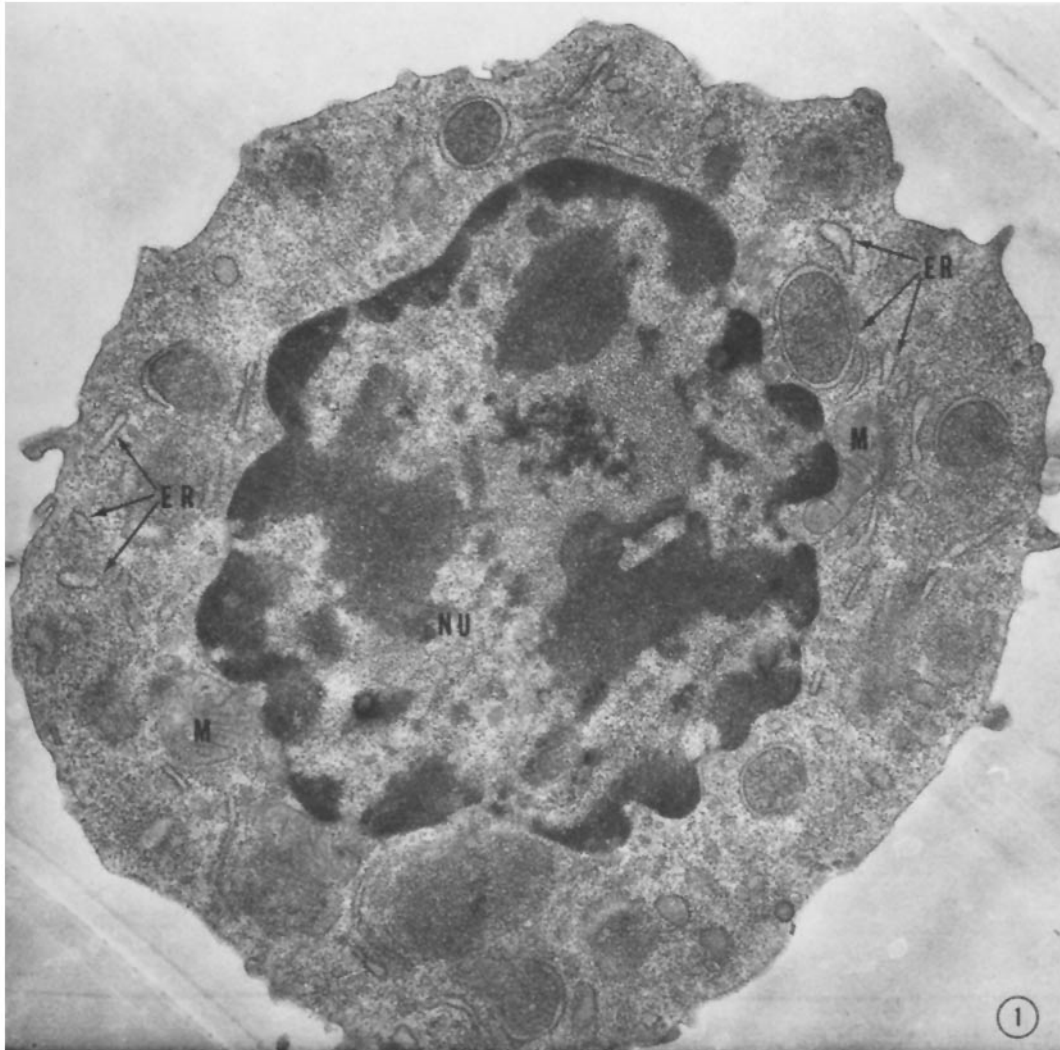
## EXPLANATION OF PLATES

<i>NU</i> , nucleus	<i>ER</i> , endoplasmic reticulum
<i>M</i> , mitochondria	<i>G</i> , Golgi body

## PLATE 20

FIG. 1. Large antibody-producing cell most commonly found in efferent lymph and thoracic duct. Central location of nucleus with irregular outline and condensed chromatin. Short and widened channels of endoplasmic reticulum (*ER*) sectioned at various angles.  $\times 20,000$ .



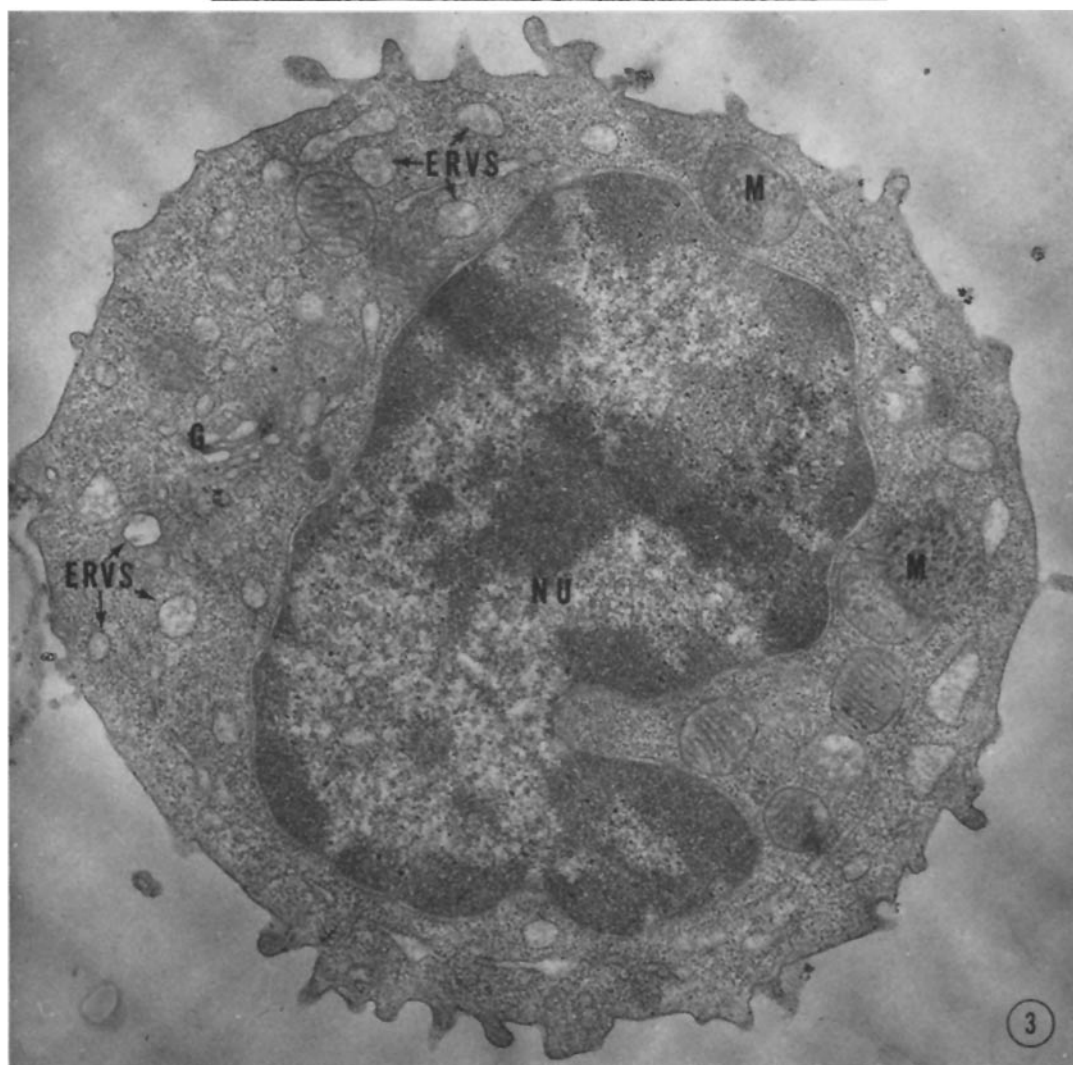
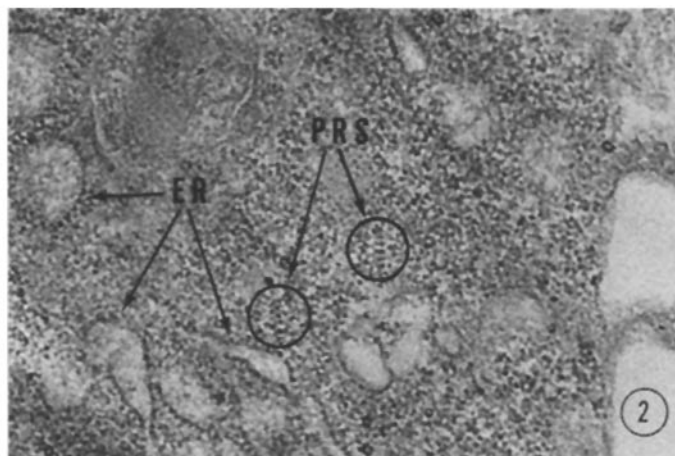


(Hummeler et al.: Antibody-producing cells in lymph and blood)

PLATE 21

FIG. 2. Aggregation of ribosomes into polyribosomes (*PRS*) in the cytoplasm of the antibody-producing cell in Fig. 1.  $\times 36,000$ .

FIG. 3. Small antibody-producing cell from efferent lymph. Slightly eccentric and indented nucleus with condensed chromatin. The Golgi body is well developed with many smooth vesicles. The endoplasmic reticulum (*ER*) is disorganized and in places widened to give an appearance of vesicles (*ERVS*) which contain amorphous material.  $\times 22,000$ .

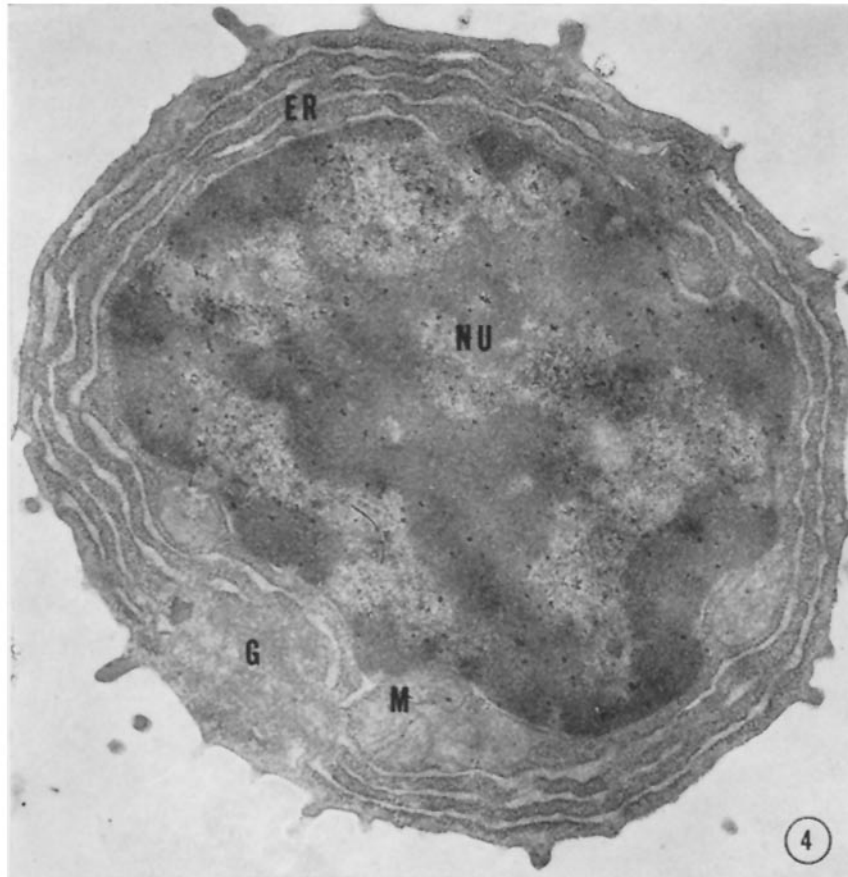


(Hummeler et al.: Antibody-producing cells in lymph and blood)

PLATE 22

FIG. 4. Small antibody-producing cell from the blood. The large central nucleus shows an irregular outline and condensed chromatin. The relatively small cytoplasm contains few mitochondria and a large, well developed Golgi body, and is organized into parallel lamellae of widened endoplasmic reticulum (*ER*).  $\times 21,000$ .

FIG. 5. Details of the Golgi body and the endoplasmic reticulum (*ER*) of the cell shown in Fig. 4. Direct connection between the endoplasmic reticulum (*ER*) channels and the smooth vesicles of the Golgi body are indicated by arrows.  $\times 35,000$ .



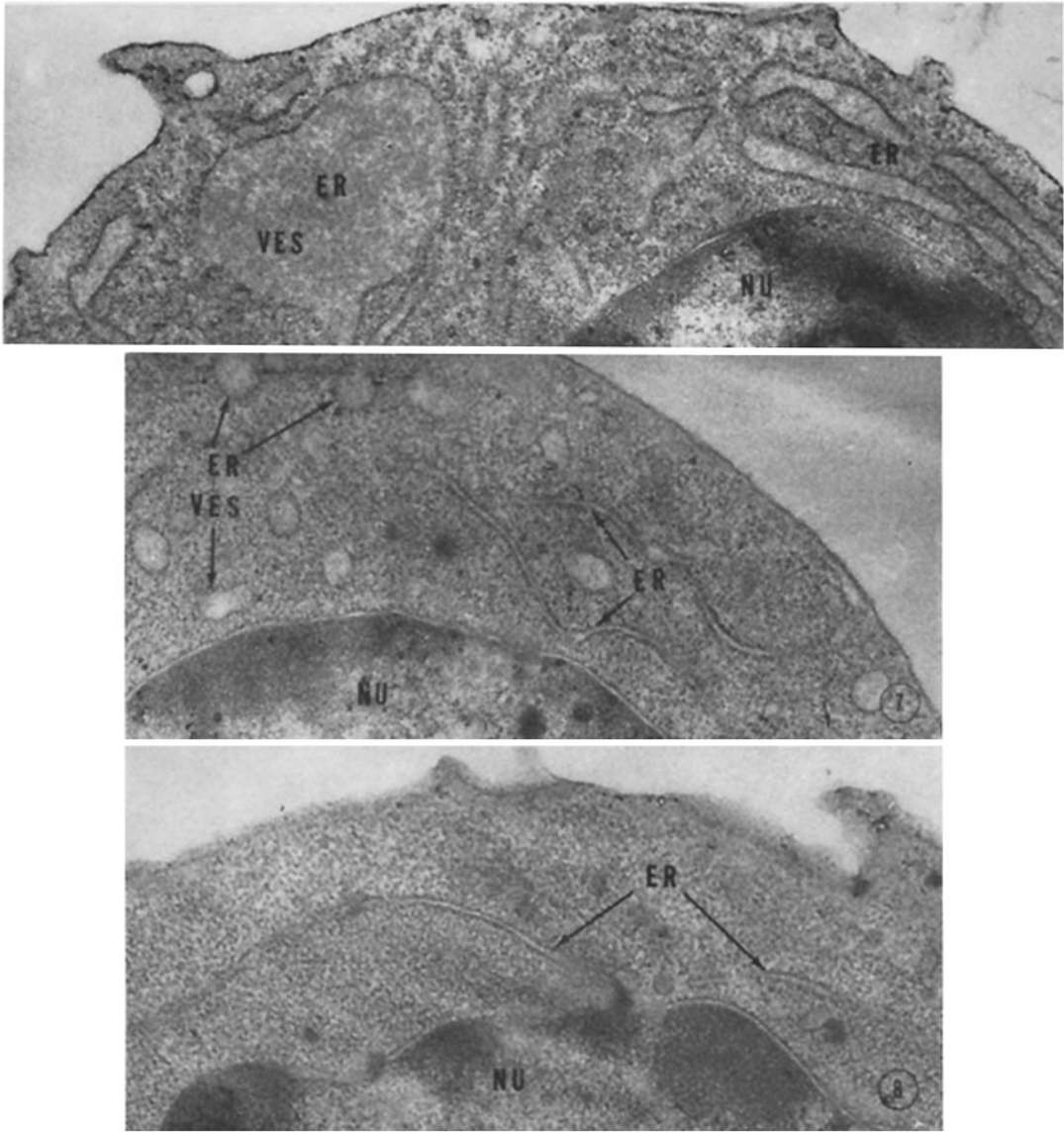
(Hummeler et al.: Antibody-producing cells in lymph and blood)

PLATE 23

FIG. 6. Cytoplasmic details in antibody-producing cell from the blood. In contrast to the cell shown in Fig. 4, the endoplasmic reticulum (*ER*) is disorganized. All channels are widened, occasionally forming a large vesicle (*ER VES*) which contains amorphous material.  $\times 31,000$ .

FIG. 7. Cytoplasm of a lymph node cell producing 7S antibody. Vesicular formation of endoplasmic reticulum (*ER*) is predominant (*ER VES*) but long narrow channels are also evident.  $\times 31,000$ .

FIG. 8. Cytoplasm of a lymph cell producing 7S antibody. No vesicular structures are apparent. The endoplasmic reticulum (*ER*) consists of long and narrow channels which are sparse.  $\times 31,000$ .

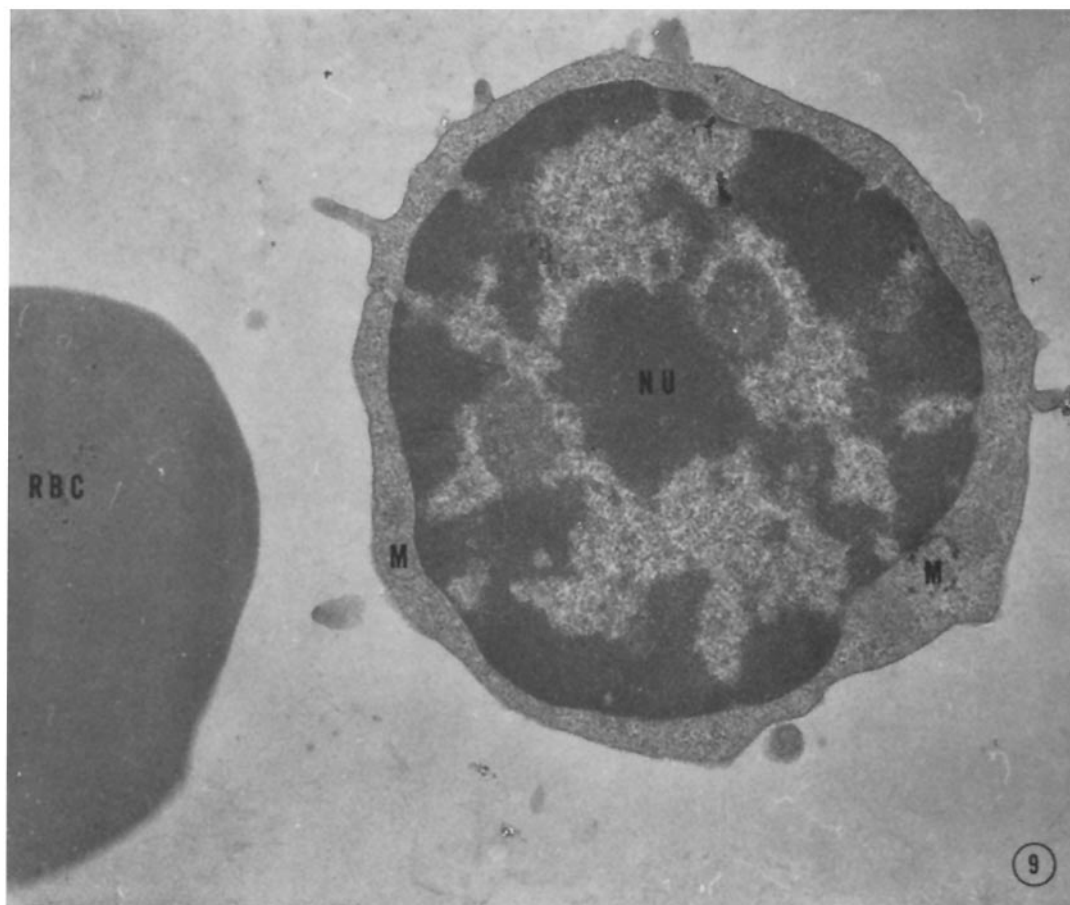


(Hummeler et al.: Antibody-producing cells in lymph and blood)

PLATE 24

FIG. 9. Small lymphocyte outside the hemolytic plaque. The sheep erythrocyte (*RBC*) is not lysed, indicating the quiescent state of the nucleated cell. This cell has a small amount of cytoplasm containing an occasional mitochondrion. No endoplasmic reticulum (*ER*) is evident.  $\times 20,000$ .





(Hummeler et al.: Antibody-producing cells in lymph and blood)