THE ULTRASTRUCTURE AND FUNCTION OF THE CELLS IN LYMPH FOLLOWING ANTIGENIC STIMULATION

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When a lymph node is stimulated by an antigen, lymphoid blast cells and intensely basophilic cells appear in the efferent lymph (1). The precise nature of these cells and their fate and function is obscure. If these cells play any part in the events of the immune response it is most likely that they do so in situations remote from the node in which they originate, as they migrate from the stimulated node in vast numbers. In this sense it is unlikely that they would be concerned with the local events of antibody formation within the parent lymph node. Since these cells are carried away by the efferent lymph to other lymph nodes and possibly by the blood to other tissues such as the spleen, they may possess a messenger function that is important in the propagation and amplification of the immune response. Thus when an antigen enters a lymph node and is retained there, stimulated cells rather than antigen may be responsible for disseminating the immune response throughout the body thereby establishing an effective systemic immunity. In this way the cellular events of the immune response would be replicated so that each lymph node would eventually carry out and transmit instructions for antibody production following the release of stimulated cells by the node that received the initial antigenic stimulus.

If this hypothesis was correct an immune response in a peripheral lymph node would cause other nodes situated along the lymphatic chain to transmit stimulated cells to the main lymph ducts and thus eventually to the blood stream by which means they would be distributed around the body. If these stimulated cells were removed from the body as they were formed, a gross defect should occur in the systemic antibody response. Additionally these cells should be capable of invoking a systemic immune response when introduced into the circulation in the absence of antigen.

This paper records the results of experiments which have been done to test these propositions. In addition the ultrastructure of these stimulated cells has been investigated to try to relate their morphology with this proposed messen-

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ger function and to compare them with the cells in the stimulated lymph node, from which they originate.

Materials and Methods

Animals.—Merino, Merino-black face cross, Clun Forest, and Isle de France sheep were used for the experiments.

Collection of Lymph.—Lymph was collected for periods of 1–12 wk in conscious sheep in which fistulae had been established in the efferent lymphatics of the popliteal or prefemoral lymph nodes, or in the main lumbar lymph trunk. The surgical techniques for the preparation of these lymphatic fistulae have been described previously (2, 3).

Antigens.—The antigens used were suspensions of killed *Salmonella typhi* "O" organisms or washed human red blood cells. They were injected under the skin on the lateral aspect of the lower hind leg or the flank, in the regions drained by the popliteal or prefemoral lymph nodes. In some experiments an afferent lymphatic draining the lower leg was cannulated in the direction of lymph flow and the antigen injected via the cannula directly into the popliteal lymph node.

Cell Smears, Staining, and Counting.—Samples of lymph were centrifuged and the cells resuspended in a small volume of plasma. Smears were prepared and stained with Leishman's or with Wright's stain. Differential cell counts were made on stained films while total cell counts were made with a hemocytometer.

Antibody Assay.—Antibodies to human red cells were measured by direct hemagglutination. Antibodies to *Salmonella typhi* "O" organisms were measured by bacterial agglutination. The systems were incubated at 38°C for 4 hr and then allowed to stand at 4°C for 12 hr before being read.

^{131}I Labeling of Salmonella typhi Organisms.—The method used to label bacteria was that described by Webster et al. (4). Instead of dialyzing the labeled bacteria, they were centrifuged and washed 3 times with 0.9% NaCl solution, the final washing being done immediately before the bacteria were used.

Measurement of Radioactivity.—^{131}I-activity was measured in 0.2 ml samples of lymph using a thin mica end-window G-M tube.

Electron Microscopy.—Lymph cells taken for electron microscopy were collected over periods of 1–2 hr in plastic flasks containing 1–2 drops of Calciparine (Choay, Paris, 10,000 IU/ml). The lymph was centrifuged for 5 min at 1000 rpm and the plasma decanted. The cells were then resuspended and fixed in 6% glutaraldehyde in Sorensen's buffer (pH 7.2) at 5°C for 30 min, followed by 1% osmium tetroxide in Sorensen's buffer at 5°C for 30 min (5). The cells were stained with 1% uranyl acetate, for 1 hr, dehydrated in ethyl alcohol, and embedded in Epon or Araldite. Portions of lymph nodes were fixed in either 6% glutaraldehyde or in Dalton's fixative for 1–2 hr at 4°C. The pieces were stained with 1% uranyl acetate for 1 hr, dehydrated, and then embedded in Epon. Thin sections were cut with a Porter-Blum or a Huxley microtome, mounted unsupported or on formvar-coated copper grids, and stained with lead citrate (6). The sections were examined and photographed in an R.C.A. Emu 3D, or Philips EM200 electron microscope.

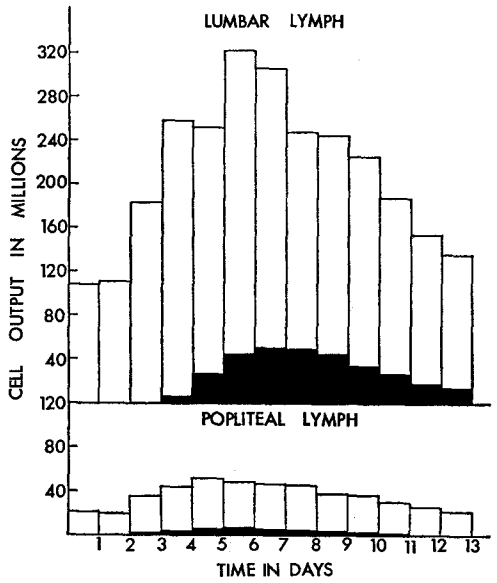
RESULTS

The Amplification of the Immune Response Within the Lymphatic System

To establish the extent to which an immune response is amplified by a series of lymph nodes along the pathway of lymphatic drainage, an experiment was done in which the output of stimulated cells in the lumbar lymph was

compared with that in the efferent lymph from the popliteal node following an antigenic challenge.

Experimental Details.—The efferent lymphatic of the popliteal node of the right leg was cannulated and at the same time the main lumbar lymph duct draining the left hind quarter was also cannulated. The lymph collected from the right leg had passed through the popliteal



TEXT-FIG. 1. The output of cells in the popliteal and lumbar lymph of a sheep following the injection of human red cells into each hind leg.

Open portion, output of total cells; and solid portion, output of basophilic cells.

node; that collected from the lumbar trunk had passed through the popliteal node and some of the nodes in the peritoneal cavity near the bifurcation of the abdominal aorta (deep inguinal, iliac, and lumbar nodes). Washed human red cells (0.5 ml of a suspension containing 5×10^7 cells) were injected subcutaneously into the lateral aspect of the lower part of both hind legs to induce primary responses in the popliteal lymph nodes. It had been shown previously (1) that foreign cells injected in this way did not pass beyond the popliteal lymph node. The cells in the popliteal and lumbar lymph were collected for 12 days following the injection of the antigen. This experiment was carried out on one Isle de France and two Merino ewes.

The results of the experiments were similar and one result is shown in Text-fig. 1. In this experiment before the antigen was injected, the output of cells in the lumbar lymph was about 4 times that in the popliteal lymph. Between 24–48 hr after the injection of the antigen the cell output from both lymphatics had begun to increase. After 96 hr the cell output from the popliteal lymph node had reached a maximum of 51.1×10^6 cells/hour of which 12% were

classified as transitional or basophilic cells. At this time the lumbar lymph had an output of 258 × 10^6 cells/hour of which 10% were transitional or basophilic cells. After this, the cell output in the popliteal lymph fell gradually. In the lumbar lymph, however, the cell output continued to rise; 144 hr after the injection of the antigen the cell output had reached a maximum of 320 × 10^6 cells/hour, 8 times that of the popliteal lymph. Of the cells in the lumbar lymph at this time 14% were transitional or basophilic cells. As in the intact animal the lumbar lymph does not pass through any other lymph nodes before entering the thoracic duct, all these cells would normally enter the blood stream directly via the thoracic duct.

The Effect of the Removal of Stimulated Lymphoid Cells from the Body on the Systemic Antibody Response

The proposition that the cells which appear in the efferent lymph following antigenic stimulation are concerned in the propagation of the immune response and in the establishment of systemic immunity was tested experimentally by making a chronic fistula in the efferent duct of a lymph node which had been stimulated by a single specific antigen and then observing the titers of specific antibody in the systemic blood.

Experimental Details.—In each of five Merino ewes the efferent duct of the popliteal node of one leg was cannulated. At the same time a perfusion cannula was inserted into one of the afferent popliteal lymph ducts of the same leg, in the direction of lymph flow. Through this cannula 2 ml of a suspension containing 6 × 10^8 killed *Salmonella typhi* "O" organisms was infused into the node over a period of 45 min. The perfusion cannula was then removed and the afferent duct tied off to prevent any of the bacterial suspension from leaking back into the tissues.

In 3 of the experiments the organisms were labeled with ^{131}I. The efferent lymph was collected quantitatively throughout the period of the perfusion and for the subsequent hour and the radioactivity in the lymph was measured. In each experiment about 10^6 counts per minute of radioactivity was infused into the lymph node; the maximum amount of radioactivity recovered in the efferent lymph was 0.02% of the amount infused. It was concluded therefore that nearly all the antigen had been retained in the node. After the antigen was infused, samples of jugular venous blood and efferent lymph were collected from each ewe every day for at least 14 days. These samples were centrifuged and assayed for antibody against *Salmonella typhi* "O" organisms. Throughout the period of the experiment all the lymph from the efferent cannula, with the exception of the small samples taken for antibody assay, was collected and stored frozen. 14 days after the injection of the antigen, when the immune response had subsided, the pooled lymph was returned to the animal by injecting it into the peritoneal cavity. This was done to determine whether the defect, if any, in the systemic antibody response could be

made good by returning to the animal the antibody that had been produced by the node but which had been lost to the body through the lymphatic fistula. In each experiment the volume of lymph collected during the 14 days was about 2 liters. The lymph was twice thawed and refrozen to disrupt the lymph cells and to release any intracellular antibody they may have contained. It was filtered to remove aggregates of cell detritus, reduced to a volume of about 600 ml by pervaporation through a dialysis sac, and injected into the sheep. Daily blood samples were collected from the sheep for a further 10 days after the injection of lymph.

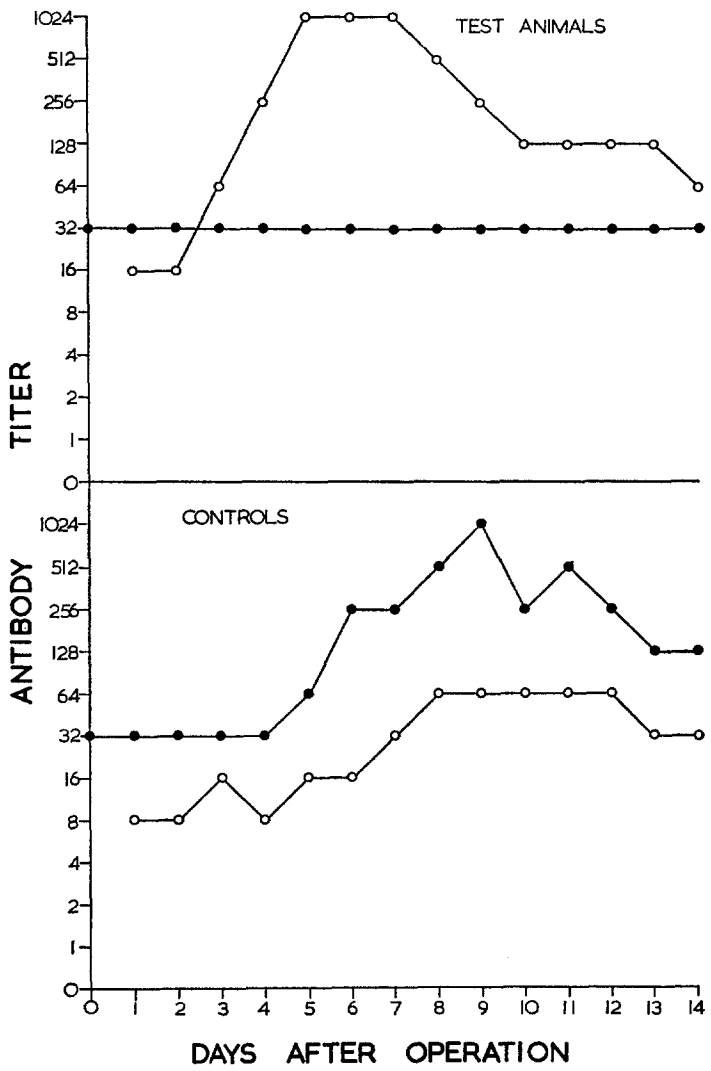
Three control experiments were done on Merino ewes in which the antigen was administered as in the test animals but the efferent duct of the stimulated node was not cannulated. The efferent duct of the contralateral (unstimulated) popliteal node was cannulated so that any effect on the systemic antibody response due to the loss of nonspecific cells and protein would be taken into account. Samples of blood and lymph for antibody assay were collected from the control animals every day.

The results of one of these experiments are shown in Text-fig. 2. Four out of the five test animals gave this result. It was found that the lymph node responded normally to the antigenic challenge and the titer of specific antibody in the lymph increased and reached a peak on the 6th day after operation. There was however, no increase in the antibody titer of the systemic blood plasma. In one ewe the antibody titer of the blood plasma increased from 1 in 16 to 1 in 256; the antibody titer of the efferent lymph plasma increased from 1 in 4 to 1 in 256. The titers in both blood and lymph reached a peak on the 6th day after the antigen was injected.

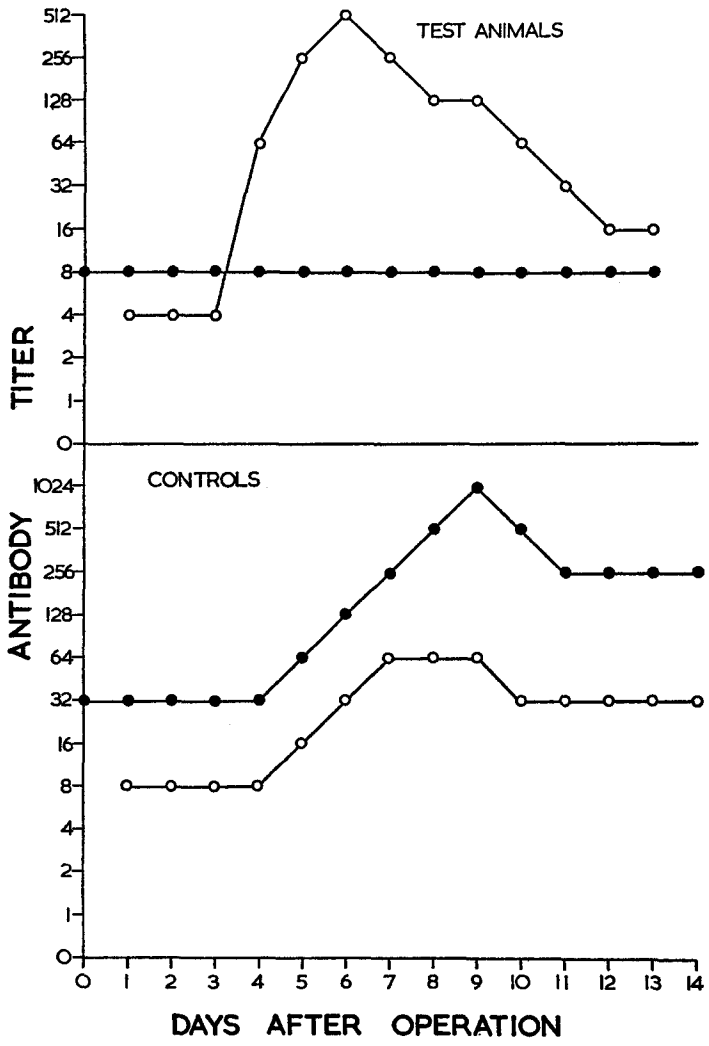
The results from the three control experiments were virtually identical. In each case the systemic antibody titer reached a maximum value of 1 in 1024 on the 9th day after the antigen was injected. The antibody titer of the lymph from the control animals reached a titer of 1 in 512, reflecting the titer in the blood plasma.

The effect of injecting the pooled efferent lymph into the test animals was the same in every case. Within 24 hr of the injection, the systemic antibody titer had increased by one dilution; this level was maintained for the next 10 days, but in no case was there any further increase.

In order to confirm these results, the experiment was repeated with a different breed of sheep and with a different lymph node. The efferent lymph ducts of the prefemoral lymph nodes of two Clun Forest wethers were cannulated and the antigen injected into the node via an afferent lymphatic in exactly the same way as described previously. Two control animals were used as before. The results are shown in Text-fig. 3. It can be seen that the results of the experiments on the Clun Forest sheep were essentially the same as those obtained with the Merinos. In the test animals there was a normal immune



TEXT-FIG. 2. The changes in the antibody titer of the systemic blood plasma and the lymph from the popliteal nodes of merino sheep following the infusion of a suspension of killed *Salmonella typhi* "O" organisms into an afferent popliteal lymphatic. In the test animals the efferent duct of the stimulated node was cannulated. In the control animals the efferent duct of the stimulated node was left intact and the efferent duct of the unstimulated node of the other leg was cannulated. ●——●, titer of antibody in blood plasma; and O——O, titer of antibody in lymph.



TEXT-FIG. 3. The changes in the antibody titer of the systemic blood plasma and the lymph from the prefemoral nodes of Clun Forest sheep following the infusion of a suspension of killed *Salmonella typhi* "O" organisms into an afferent prefemoral lymphatic. In the test animals the efferent duct of the stimulated node was cannulated. In the control animals the efferent duct of the stimulated node was left intact and the efferent duct of the unstimulated node in the other flank was cannulated. ●——●, titer of antibody in the blood plasma; and O——O, titer of antibody in the lymph.

response in the lymph plasma but no systemic antibody response whatsoever. It seems unlikely that this defect could be explained solely on the basis of loss of antibody via the lymphatic fistula. The maximum systemic antibody response in the control animals occurred 9 days after the antigen was administered whereas antibody production by the node that received the antigen was maximal within 6 days.

These findings suggested that in the control experiments, antibody production occurred in sites other than the initially stimulated node and that the stimulated cells which were released into the efferent lymph from this node were instrumental in initiating this process. These cells do not appear in the lymph for a few days after antigenic stimulation (1) and so the cellular immune response and antibody production in other sites of the body lags behind the stimulated node. This finding was in accord with the results of the previous experiments where the cellular response occurred later and was more prolonged in the lumbar lymph than in the popliteal lymph.

The Transfer of Immunity by the Cells in the Efferent Lymph from an Antigenically Stimulated Lymph Node

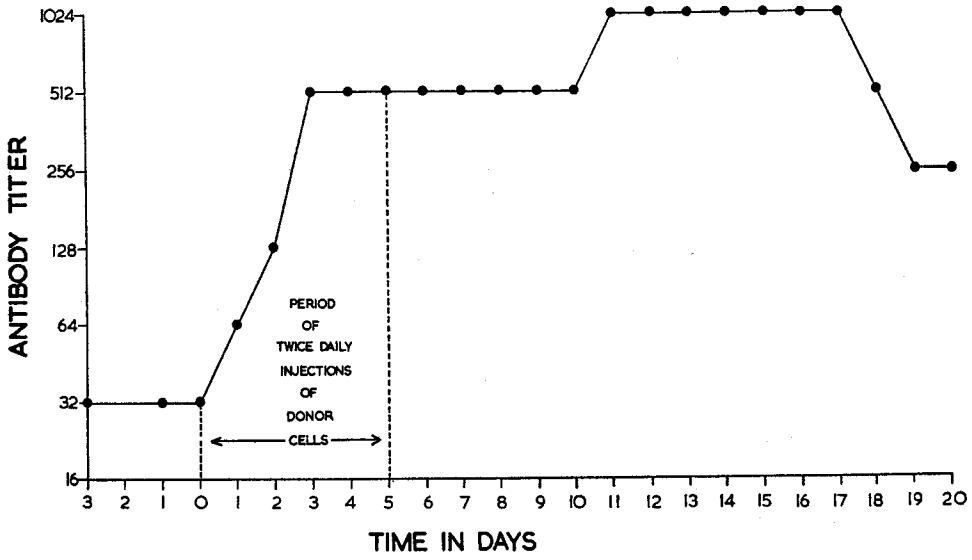
In order to establish conclusively that one of the functions of the cells released into the efferent lymph by a stimulated lymph node is to propagate the immune response it must be shown that these cells are able, in the absence of antigen, to initiate a normal immune response.

Experimental Details.—Twin adult Merino–black face wethers were used for this experiment. They were the survivors of a triplet birth and at birth anastomatic connections were present between their placental circulations. Slee (7) showed by the survival of skin homografts that they were tolerant of one another's tissues, and Tucker (8) also demonstrated erythrocyte mosaicism in these sheep which involved both the cell antigens and the hemoglobin types. In view of the fact that each sheep had two populations of red cells, one belonging to itself and one derived from its twin, it was assumed that they would be tolerant of one another's lymphoid cells and that cells could be transferred from one sheep to the other and survive to function normally even though the sheep were not syngeneic.

In one of the twins the efferent ducts of both prefemoral nodes were cannulated. At the same time a suspension of *Salmonella typhi* "O" organisms was infused into each lymph node via an afferent cannula as described previously. The lymph from both efferent cannulae was collected quantitatively over 12-hr periods. At the end of each collection period the lymph from both sides was pooled, the volume measured and total and differential white cell counts were made. The cells collected during the first 48 hr after the infusion of the antigen were discarded. During this time there were some neutrophil polymorphonuclear leukocytes in the lymph (1) which might conceivably have contained fragments of antigen.

The cells in the lymph collected between 48 and 170 hr after the operation

were washed and injected into the jugular vein of the recipient twin. Many of these cells were basophilic cells showing vigorous mitotic activity. The cells were washed in the following way: to each collection of lymph an equal volume of phosphate-buffered saline (pH 7.4) was added and the cells deposited from the diluted lymph by centrifugation at 10°C for 10 min. The supernatant was discarded and the cells then resuspended in a volume of sterile Hank's solution equal to the original volume of the lymph collection. After a second centrifuga-



TEXT-FIG. 4. The titer of antibody against *Salmonella typhi* "O" antigen in the blood plasma of a merino-blackface sheep before, during and after a 5 day period during which the sheep received intravenous injections of washed lymph cells from its chimeric twin. The lymph cells from the twin were collected from bilateral fistulae in the efferent ducts of the prefemoral lymph nodes after these nodes had been stimulated with killed *Salmonella typhi* "O" organisms.

tion the cells were resuspended in 20 ml Hank's solution and then injected immediately into the recipient twin. During the 5 day period over which the injections were given the recipient twin received a total of 6.31×10^{10} cells of which approximately 10% (6.14×10^{9}) were transitional blast cells or basophilic cells; the other 90% of cells were normal lymphocytes. Daily blood samples were collected from the jugular vein of the recipient twin over a period of 24 days before, during, and after the period of twice daily injections of donor cells. The blood plasma was collected following centrifugation and assayed for antibody against *Salmonella typhi* "O" organisms.

The result of this experiment is shown in Text-fig. 4. It can be seen that the titer of antibody against *Salmonella typhi* "O" organisms in the blood plasma

of the recipient twin began to rise a day after the first injection of donor cells but did not reach a maximum value until 6 days after the last injection. The immune response was a vigorous one, and was more prolonged than the response of the control animals in the previous experiments. There was no doubt that the active antibody formation which took place in the recipient twin following the injection of the donor cells, occurred too rapidly to be explained in terms of the inadvertent transfer of antigen.

The Ultrastructure of the Cells Released from a Lymph Node following Antigenic Stimulation

The results of the previous experiments suggested that at least some of the cells which appear in lymph following an antigenic stimulus play an important part in propagating the immune response. The fine structure of these lymphoid cells was investigated so as to establish their morphological characteristics.

Experimental Details.—The efferent ducts of the popliteal lymph nodes of two Isle de France ewes were cannulated and 0.5 ml of a suspension containing 5×10^7 washed human red blood cells was injected into the leg of each animal. One ewe was given two injections of red cells 10 days apart to provoke both primary and secondary immune responses in the node. The lymph cells coming from the node were collected throughout these two responses and the cells examined by light, phase, and electron microscopy. In the second sheep so as to ensure maximum antigenic stimulation of the lymph node, 4 successive injections of red cells were given into the leg at 10-day intervals. The lymph was collected over a period of 36 days, and representative samples of all the cells were examined. At the end of the 36 days, i.e. 5 days after the fourth injection of antigen, the popliteal node was removed together with an unstimulated prefemoral node on the opposite side and examined by light and electron microscopy.

Light Microscopy.—Before the lymph node was stimulated essentially all the cells in the efferent lymph were normal lymphocytes. After antigenic stimulation different types of cells appeared in the lymph. In the case of the ewe which was given four successive injections of antigen, at the height of the third and fourth response only about 30% of the cells in the lymph were normal lymphocytes; the rest were large cells showing varying degrees of basophilia. The appearance of these cells in Leishman stained films has been described previously (1).

Examples of these cells, as seen by phase microscopy are shown in Figs. 1 *a*–1 *d*. The large basophilic cells were distinguished by their large nucleoli and numerous mitochondria (Figs. 1 *a* and 1 *b*). These cells moved very actively (Figs. 1 *b* and 1 *c*) and were frequently found in mitosis (Fig. 1 *d*).

Electron Microscopy.—The electron microscopic appearance of a normal lymphocyte is shown in Fig. 2. The lymphocytes contained several large mitochondria, a few Golgi membranes and paired centrioles. Scattered throughout

the cytoplasm of the cells were many single, free ribosomes and occasionally one or two unorganized pieces of ergastoplasm. The nucleus showed a characteristic indentation near the region of the centrosome and the nuclear chromatin was clumped around the nuclear membrane.

After antigenic stimulation, the basophilic cells which appeared in the lymph were readily identified in the electron microscope. These cells were large and many had the characteristics of primitive undifferentiated cells. The cytoplasm of those cells judged to be the least differentiated contained many ribosomes; these occurred for the most part as monoribosomes although some polyribosomal clusters were present together with a small amount of ergastoplasm showing no apparent organization (Figs. 3 and 4). Throughout the cytoplasm of these cells bundles of microfibrils occurred frequently; these bundles were arranged so as to appear to traverse the cytoplasm in several planes. Many of these large cells were fixed in bizarre shapes and were thought to be the same cells as those which appeared actively motile in the phase microscope preparations. The nuclei of these cells contained one or more nucleoli and their nuclear chromatin was scanty.

Other cells had a more differentiated appearance (Figs. 5 and 6). In these cells many of the ribosomes occurred in aggregates or in rosettes and in some cases in short chains and spirals (Figs. 6 and 7). The rudimentary ergastoplasm was arranged in some cases as short pieces of reticulum localized in one part of the cell, while in others it appeared more generally distributed throughout the cytoplasm (Fig. 5). In occasional cells the ergastoplasm showed some orientation around the nucleus but this was seen infrequently (Fig. 6). The Golgi zone was well developed in these cells and microfibrils and microtubules were often present. The nucleus contained one or two nucleoli and clumped chromatin.

When the stimulated popliteal lymph nodes were examined they were found to contain many cells which were classical mature plasma cells and plasmablasts. The popliteal lymph node removed after four successive stimulations with human red cells weighed 5.4 g, about five times heavier than a normal unstimulated node. In parts of this node practically all the cells were mature plasma cells in which the cytoplasm was filled with dilated ergastoplasmic sacs arranged in concentric lamellae. There were also many plasmablasts in which the ergastoplasm also showed an obvious concentric arrangement around the nucleus. Cells with no developed ergastoplasm which were similar to the basophilic cells seen in efferent lymph were also present in the node and these were often situated alongside plasma cells.

The concentric arrangement of the ergastoplasm in a classical plasma cell is compared with the ergastoplasm of a cell in efferent lymph in Figs. 8 *a* and 8 *b*.

DISCUSSION

The results of our experiments suggest that the cells which appear in the efferent lymph coming from an antigenically stimulated lymph node are responsible for propagating and amplifying the immune response throughout the body. Before considering the mechanisms involved in detail it is necessary to make one or two more immediate interpretations of the results. It is apparent that in the majority of cases the development of a systemic immune response following the localization of antigenic material in a single lymph node depends on the existence of an intact lymphatic pathway between that node and the rest of the body. Since it is the cells released by the node, rather than humoral factors, which appear to be the prime movers in the propagation of the immune response it follows that in general these cells depend exclusively on the lymphatic system for their immediate transport. This is important as it would appear to eliminate the possibility of the direct entry of stimulated lymphocytes into the blood from within the lymph node. If a significant number of immunologically active cells were able to enter the blood directly it would have been impossible for the immune response to remain localized within the node as it did in our experiments.

There are, however, anatomical situations where lymphatic-venous connections occur in lymph nodes. In the course of over 100 operations on single lymph nodes one case was found where a popliteal lymph node had a common lymphatic-venous drainage. Although such obvious examples are rare, lesser degrees of direct lymphatic-venous communication within lymph nodes may occur more frequently (9, 10) and certainly such connections develop in cases of lymphatic obstruction (11). A situation such as this may explain the result of the one experiment where the immune response was not localized completely within the lymphatic system.

The mechanism whereby a systemic immune response follows a localized antigenic stimulus has not been investigated sufficiently for a comprehensive explanation of the process to be made. McMaster and Hudack (12) showed that following a single intradermal injection of killed bacteria into the ears of mice, antibodies were present in extracts of the lymph nodes which drained the injection site, in higher titer either than in the blood or in extracts of other lymph nodes. No one doubts that in these circumstances the regional lymph node is the site where the earlier phases of antibody production occur. However, the mechanism which leads subsequently to the production of antibody at sites remote from the site of localization of the antigen, and also to the development of widespread secondary reactivity, is not understood in detail. Sjovall (13) suggested that sensitized lymphocytes might spread the immune response from one node to another but he had no experimental evidence to support this view. More recently evidence implicating free floating

cells in the events of the immune response has been published. Wesslen (14) obtained cells from the thoracic duct of immunized rabbits; he claimed that at the time of collection these cells contained no specific antibody but produced antibody when cultured in vitro. Hulliger and Sorkin (15) reported that some of the cells in the buffy coat from the blood of immunized rabbits formed specific antibody when incubated at 37°C for 3 hr. This was confirmed by Kearney and Halliday (16) who by means of the Jerne plaque technique enumerated antibody-forming cells in the peripheral blood taken from rabbits during secondary immune responses. It seems likely therefore that free floating cells may be concerned in the cellular events of antibody formation. As the most obvious result of antigenic stimulation was the appearance of large numbers of basophilic, rapidly dividing blast cells in the lymph, a good case can be made for assigning to them the function of propagating the immune response although there is also the possibility that among the normal population of lymphocytes some sensitized but morphologically unchanged cells may also play an important part. It remains to consider the mechanisms that could be involved.

There are three possible ways in which the cells released from a stimulated lymph node could propagate the immune response. Firstly, they could act merely as vehicles for the transport of antigen; secondly, they could themselves proliferate and differentiate into active antibody-producing cells; or thirdly, they could act by actually transferring information to other cells which would then be stimulated to produce antibody.

The first of these possibilities seems unlikely. The cells in the lymph from a stimulated node are virtually entirely lymphoid or plasmoid in character and such cells are generally regarded as being incapable of phagocytosis. However, it is possible that such cells do contain a few molecules of the antigen which provoked their formation. According to Wellensiek and Coons (17) molecules of a ferritin antigen were demonstrable by immunofluorescence and electron microscopy, not only in the phagocytic cells of lymph nodes stimulated with this antigen but also in cells of the plasma series concerned with the production of specific antibody. However, even if the lymph cells did contain a few molecules of antigen, it is almost certain that this was not enough to stimulate a widespread immune response as, in our experiments, the injection of the lymph which contained the disrupted lymph cells was not followed by active antibody production in the recipient sheep.

The second possibility, i.e. that the lymph cells ultimately colonize other aggregations of lymphoid tissue and proliferate and differentiate into antibody-forming cells, is at first sight the most obvious explanation of the results. Many experiments have been performed in which cells teased from a stimulated lymph node or spleen have been used to transfer adoptive immunity

from one animal to another. Such experiments are too numerous to cite in detail but those of Harris and Harris (18) are among the most typical and best known. The successful transfer of immunity in these experiments has been explained in terms of the survival and proliferation within the host of donor cells already committed to the ultimate production of antibody. This view is supported by the fact that the process is most efficient where there is least genetic disparity between the donor and host or where the host-against-graft reaction is inhibited by irradiation. The cells used in these types of experiments were obtained from lymph nodes or spleens and these cells must necessarily have been a mixture of lymphocytes, phagocytic cells, and also immature and mature plasma cells. Antibody production would be the inevitable consequence of the survival of such cells within the host.

The situation in our experiments may be quite different. There is no evidence to show whether or not the basophilic cells coming from a stimulated node ultimately develop into classical plasma cells in some other situation. Hall and Morris (1) described these cells as plasmablasts, immature, and mature plasma cells on the basis of their appearance in Leishman films and on the finding that these cells contained antibody. This classification is not correct in terms of their ultrastructural characteristics. The electron microscopic features of cells in the plasma cell series have been described in detail by Braunsteiner and Pakesch (19); Thiery (20); and Bernhard and Granboulan (21). The characteristic feature of these cells is the abundant, highly organized, lamellar ergastoplasm similar to that found in cells actively engaged in protein synthesis and secretion such as the acinar cells of the pancreas, and parotid gland (Porter (22)). The basophilic cells found in the efferent lymph of the popliteal lymph node during the immune responses to human red cells did not have this feature at any stage.

De Petris and Karlsbad (23) described basophilic blast cells in lymph nodes following antigenic stimulation which contained antibody. These cells resembled closely the cells found in lymph in that they had no organized endoplasmic reticulum but contained many polyribosomes in their cytoplasm. As these cells in lymph also contain antibody (Hall and Morris (1)) and have been shown to be secreting antibody (Cunningham, Smith, and Mercer (32)), it is likely that the synthesis of specific antibody may take place in the absence of an organized endoplasmic reticulum, presumably as a function of the ribosomal clusters and spirals distributed throughout the cytoplasm.

Hall and Morris (24) showed that the basophilic cells of efferent lymph are formed in the lymph node where they are found together with classical plasmablasts and plasma cells. Whether or not these cells have a common progenitor in the lymph node is not known. While it is likely that these basophilic cells undergo differentiation which leads to the formation of plasma cells, it may

be that in the early stages of differentiation at least, these cells have both lymphocytic and plasmocytic potentialities. In any event the classical plasma cells represent a residential population of antibody-forming cells within the lymph node while the itinerant basophilic cells in the lymph are a population of cells involved in both antibody synthesis and in the propagation of the immune response. The possibility exists also that the basophilic cells may produce a different type of antibody from that produced by classical plasma cells and there is some evidence that cells of this type may be concerned with the production of 19S antibody in the early phase of the immune response whereas the classical plasma cells may be responsible for most of the 7S antibody production. (Schoenberg, Rupp, and Moore (25); and Schoenberg, Stavitsky, Moore, and Freeman (26)).

The failure of classical plasma cells to leave lymph nodes has been commented on previously (Reinhardt and Yoffey (27)). Their absence from lymph may be due to the fact that these cells for the most part have special anatomical relationships within the node, and are often found grouped in so called plasmocytic islets in the medullary cords associated with macrophages or reticular cells. This situation however does not hold in all cases. Following the injection of red cell antigens under the skin classical plasma cells and plasmablasts have been found in afferent lymph (G. Moreno, M. Bessis, and B. Morris. 1966. Data to be published.) and when influenza virus has been used as an antigen classical plasma cells and plasmablasts have been found in efferent lymph during the secondary response (J. B. Smith and B. Morris. 1966. Unpublished observations). However even in these two instances, apart from lymphocytes, basophilic cells without organized endoplasmic reticulum are much more common.

The other possible way in which these cells could express a messenger function would be by some interaction occurring between their RNA and effector cells in other situations. There are some experimental observations which suggest that a direct cell to cell transfer of information may take place. Fishman et al. (28, 29) claimed to have induced the production of specific antibody in lymphoid cells by supplying them with RNA extracted from macrophages which had previously ingested the antigen. Several workers have demonstrated an intimate anatomical relationship between these two types of cells which makes this transfer feasible from a morphological point of view (Thiery (20); White (30); Schoenberg et al. (33); and McFarland and Heilman (31)).

Such experiments bear mainly on the initial transfer of information from the phagocytic cells, which first received the antigen, to the lymphoid cells which subsequently make the specific antibody. However, there are situations, as in our own experiments, where antibody production appears to take place at sites remote from the site of antigen localization. If an RNA-protein com-

plex of some sort is necessary to initiate antibody production there must be some means of conveying such a complex round the body in a protected form. It seems unlikely that the macrophages themselves can do this because they are conspicuously absent from intermediate and central lymph and from the blood. However, the basophilic cells in the lymph from stimulated nodes would seem to be the ideal cell for this purpose. Their existence as free floating cells gives them mobility; they are extremely motile, and they contain a relatively large amount of RNA. They thus have all the properties which one would expect to find in a messenger cell that expresses its function in the above manner. Furthermore, whatever the exact function of these basophilic cells in the lymph may be, there is no doubt that they appear in all the types of immune responses which we have so far studied. We have observed them coming from nodes stimulated with soluble proteins, soluble extracts of bacteria and helminths, killed bacteria, viruses, heterologous red cells, homologous lymphocytes, homologous skin grafts, and heterologous tumor implants. As far as is known at the moment the cells seen in the lymph following these various stimuli are always of the same type, although their numbers and the times at which they appear may vary somewhat in the different experimental situations.

SUMMARY

When a lymph node receives an antigenic stimulus the cell population in the efferent lymph changes and large basophilic cells appear. During a secondary immune response cells of this type may account for over 50% of the cells present in lymph.

When examined by electron microscopy, many of these cells were found to be primitive undifferentiated blast cells with many free ribosomes in their cytoplasm and only an occasional piece of endoplasmic reticulum. Their nuclear chromatin was sparse and the nuclei contained several nucleoli. Many other cells which were judged to be more differentiated had large numbers of ribosomes arranged in clusters which took the form of rosettes or spirals. These cells also had more ergastoplasm but this occurred usually in the form of short pieces of disorganized endoplasmic reticulum. No cells with the ultrastructure of classical plasma cells were found in efferent lymph although these cells were abundant in the stimulated lymph nodes.

It was shown that when the lymph which contained these cells was collected quantitatively no systemic immunity developed even though a vigorous immune response took place in the lymph node with the formation of many plasma cells. Failure of the systemic immune response to develop could not be explained merely in terms of the loss of antibody. It was concluded that these basophilic cells rather than antigen are responsible for propagating the

immune response throughout the body and that they depend on an intact lymphatic pathway for their immediate transport. This view was supported by experiments which showed that these cells are capable of initiating immune responses in other lymph nodes of the same animal and of transferring active immunity between chimeric twins.

The most likely explanation of these results is that the basophilic lymphoid cells carry out their messenger function by developing into plasma cells at sites remote from the site at which antigen is localized. However this has yet to be proven and the possibility remains that these mobile, highly motile, RNA-rich cells may express their messenger function by transferring information to other effector cells.

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BIBLIOGRAPHY

1. Hall, J. G., and B. Morris. 1963. The lymph-borne cells of the immune response. *Quart. J. Exp. Physiol.* **48:** 235.
2. Lascelles, A. K., and B. Morris. 1961. Surgical techniques for the collection of lymph from unanaesthetized sheep. *Quart. J. Exp. Physiol.* **46:** 199.
3. Hall, J. G., and B. Morris. 1962. The output of cells in lymph from the popliteal node of sheep. *Quart. J. Exp. Physiol.* **47:** 360.
4. Webster, R. G., W. G. Laver, and S. Fazekas de St. Groth. 1962. Methods in immunochemistry of viruses: 3. Simple techniques for labelling antibodies with ^{131}I and ^{35}S. *Australian J. Exp. Biol. and Med. Sci.* **40:** 321.
5. Sabatini, D. D., K. Bensch, and R. J. Barnett. 1963. The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. *J. Cell Biol.* **17:** 19.
6. Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy *J. Cell Biol.* **17:** 208.
7. Slee, J. 1963. Immunological tolerance between litter-mates in sheep. *Nature.* **200:** 654.
8. Tucker, E. M. 1964. European Society for Animal Blood Groups Research. Symposium of the Czechoslovakian Academy of Science. Prague. in press.
9. Schulze, W. 1925. Untersuchungen über die Capillaren und postcapillaren Venen lymphatischer Organe. *Z. ges. Anat. Ergebn. Anat. Entw. Gesch.* **76:** 421.
10. Rusznyak, I., M. Foldi, and G. Szabo. 1960. Lymphatics and Lymph Circulation. Pergammon Press, N.Y.
11. Heath, T. J. 1964. Pathways of intestinal lymph drainage in normal sheep following thoracic duct occlusion. *Am. J. Anat.* **115:** 569.

12. McMaster, P. D., and S. S. Hudack. 1935. The formation of agglutinins within lymph nodes. *J. Exp. Med.* **61:** 783.
13. Sjovall, H. 1936. Experimentelle Untersuchungen über das Blut und die blut bildenden Organe—Besonders das lymphatische Gewebe—des Kaninchens bei wiederholten Aderlässen. *Acta Pathol. Microbiol. Scand. Suppl.* **27:** 1.
14. Wesslen, T. 1952. Studies on the role of lymphocytes in antibody production. *Acta Dermato-Venereol.* **32:** 265.
15. Hulliger, L., and E. Sorkin. 1963. Synthesis of antibodies by blood leucocytes of the rabbit. *Nature.* **198:** 299.
16. Kearney, R., and W. J. Halliday. 1965. Enumeration of antibody forming cells in the peripheral blood of immunized rabbits. *J. Immunol.* **95:** 109.
17. Wellensiek, H. J., and A. H. Coons. 1964. Studies on antibody production. The cellular localization of antigen molecules (ferritin) in the secondary response. *J. Exp. Med.* **119:** 685.
18. Harris, T. N., and S. Harris. 1960. Lymph node cell transfer in relation to antibody formation. *Ciba Found. Symp., Cellular Aspects Immunity.* 172.
19. Braunsteiner, H., and F. Pakesch. 1955. Electron microscopy and the functional significance of a new cellular structure in plasmocytes. *Blood.* **10:** 650.
20. Thiery, J. P. 1960. Microcinematographic contribution to the study of plasma cells. *Ciba Found. Symp., Cellular Aspects Immunity.* 53.
21. Bernhard, W., and N. Granboulan. 1960. Ultrastructure of immunologically competent cells. *Ciba Found. Symp., Cellular Aspects Immunity.* 92.
22. Porter, K. R. 1954. Electronmicroscopy of basophilic components of cytoplasm. *J. Histochem. Cytochem.* **2:** 346.
23. De Petris, S., and G. Karlsbad. 1965. Localization of antibodies by electron microscopy in developing antibody producing cells. *J. Cell. Biol.* **26:** 759.
24. Hall, J. G., and B. Morris. 1965. The origin of the cells in the efferent lymph from a single lymph node. *J. Exp. Med.* **121:** 901.
25. Schoenberg, M. D., J. C. Rupp, and R. D. Moore. 1964. The cellular response of the spleen and its relationship to the circulating 19S and 7S antibody in the antigenically stimulated rabbit. *Brit. J. Exp. Pathol.*, **45:** 111.
26. Schoenberg, M. D., A. B. Stavitsky, R. D. Moore, and M. J. Freeman. 1965. Cellular sites of synthesis of rabbit immunoglobulins during primary response to diphtheria toxoid–Freund's adjuvant. *J. Exp. Med.* **121:** 577.
27. Reinhardt, W. O., and J. M. Yoffey. 1957. Lymphocyte content of lymph from the thoracic and cervical ducts in guinea pigs. *J. Physiol. (London).* **136:** 227.
28. Fishman, M., and F. L. Adler. 1963. Antibody formation initiated *in vitro*. II. Antibody synthesis in x-irradiated recipients of diffusion chambers containing nucleic acid derived from macrophages incubated with antigen. *J. Exp. Med.* **117:** 595.
29. Fishman, M., R. A. Hammarton, and V. P. Bond. 1963. *In vitro* transfer of macrophage RNA to lymph node cells. *Nature.* **198:** 549.
30. White, R. G. 1963. Functional recognition of immunologically competent cells by means of the fluorescent antibody technique. *Ciba Found. Study Group 16.* 6.

31. McFarland, W., and D. H. Heilman. 1965. Lymphocyte foot appendage. Its role in lymphocyte function and in immunological reactions. *Nature.* **205:** 887.
32. Cunningham, A. J., J. B. Smith, and E. H. Mercer. 1966. Antibody formation by single cells from lymph nodes and efferent lymph of sheep, *J. Exp. Med.* **124:** 701.
33. Schoenberg, M. D., V. R. Mumaw, R. D. Moore, and A. S. Weisberger. 1964. Cytoplasmic interaction between macrophages and lymphocytic cells in antibody synthesis. *Science.* **143:** 964.

EXPLANATION OF PLATES

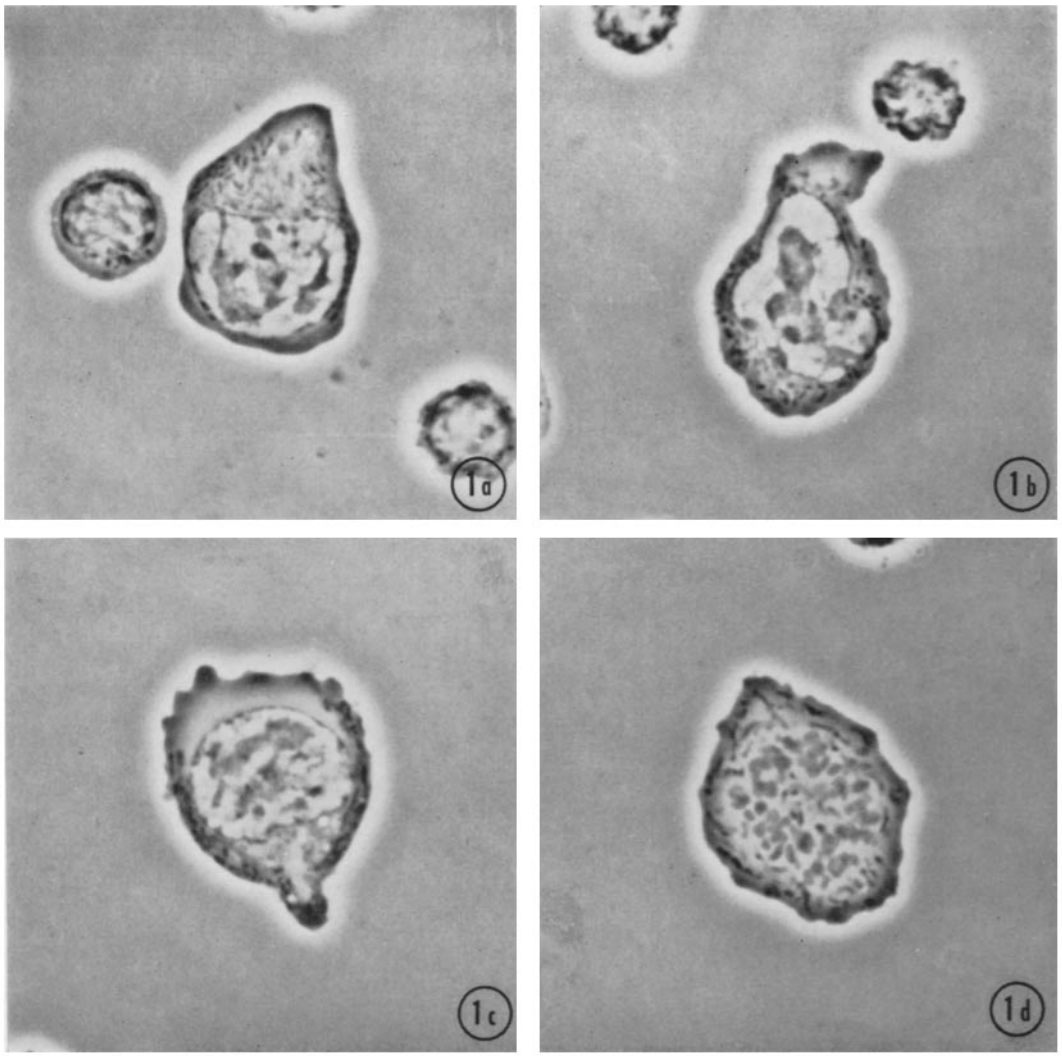
PLATE 6

FIGS. 1 *a*–1 *d*. Phase microscope pictures of cells found in the efferent lymph of t popliteal node following antigenic stimulation. × 1500.

FIG. 1 *a*. Two small lymphocytes and a large blast cell.

FIGS. 1 *b* and 1 *c*. Large, actively motile basophilic blast cells.

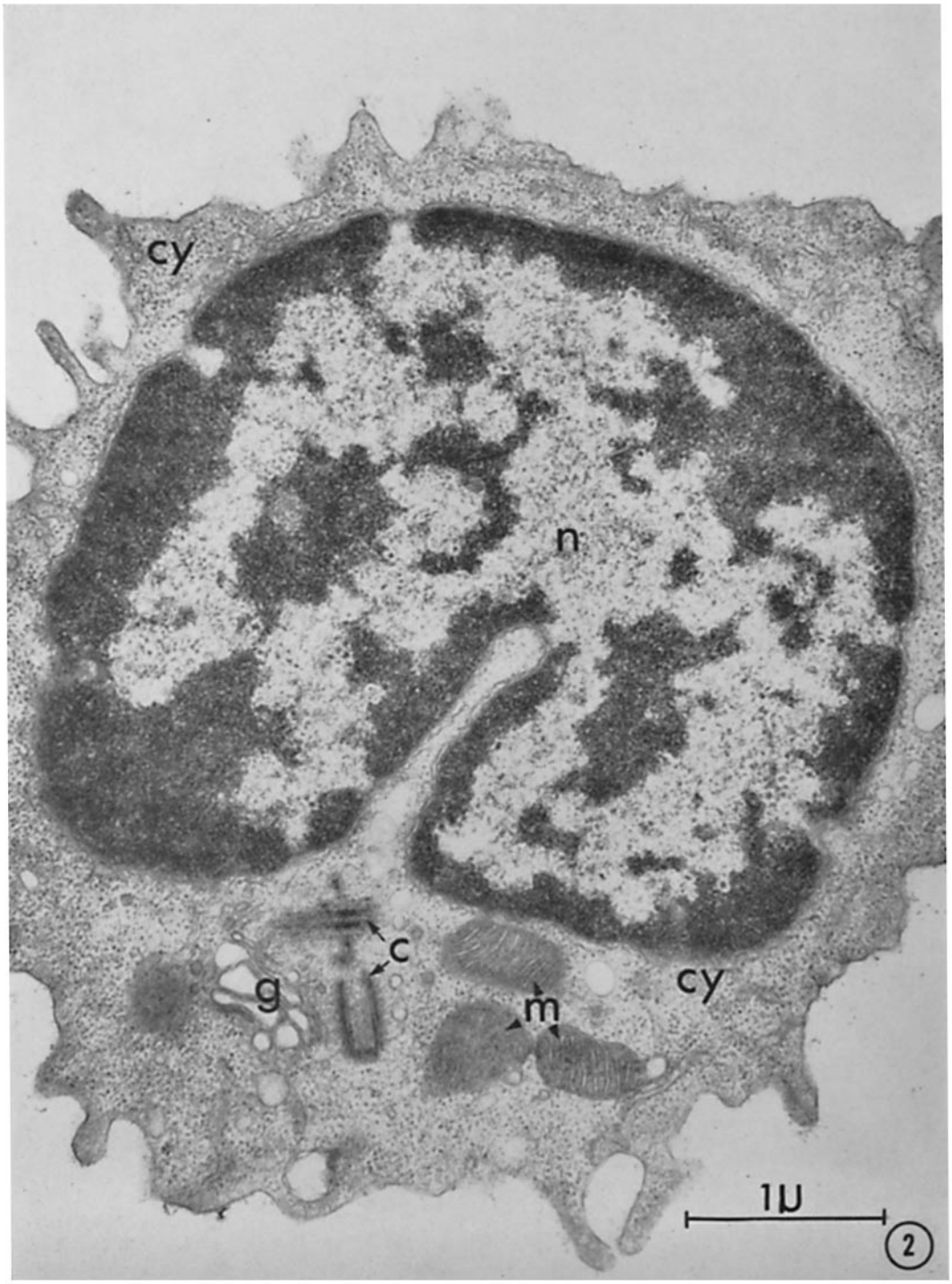
FIG. 1 *d*. Large blast cell in mitosis.



(Hall et al.: Ultrastructure and function of lymph cells)

PLATE 7

FIG. 2. A normal lymphocyte of a sheep. This cell comprises about 95% of the cells in the efferent lymph coming from an unstimulated lymph node. Nucleus (*n*), golgi bodies (*g*), centrioles (*c*), mitochondria (*m*), and cytoplasm with monoribosomes (*cy*). × 30,000.

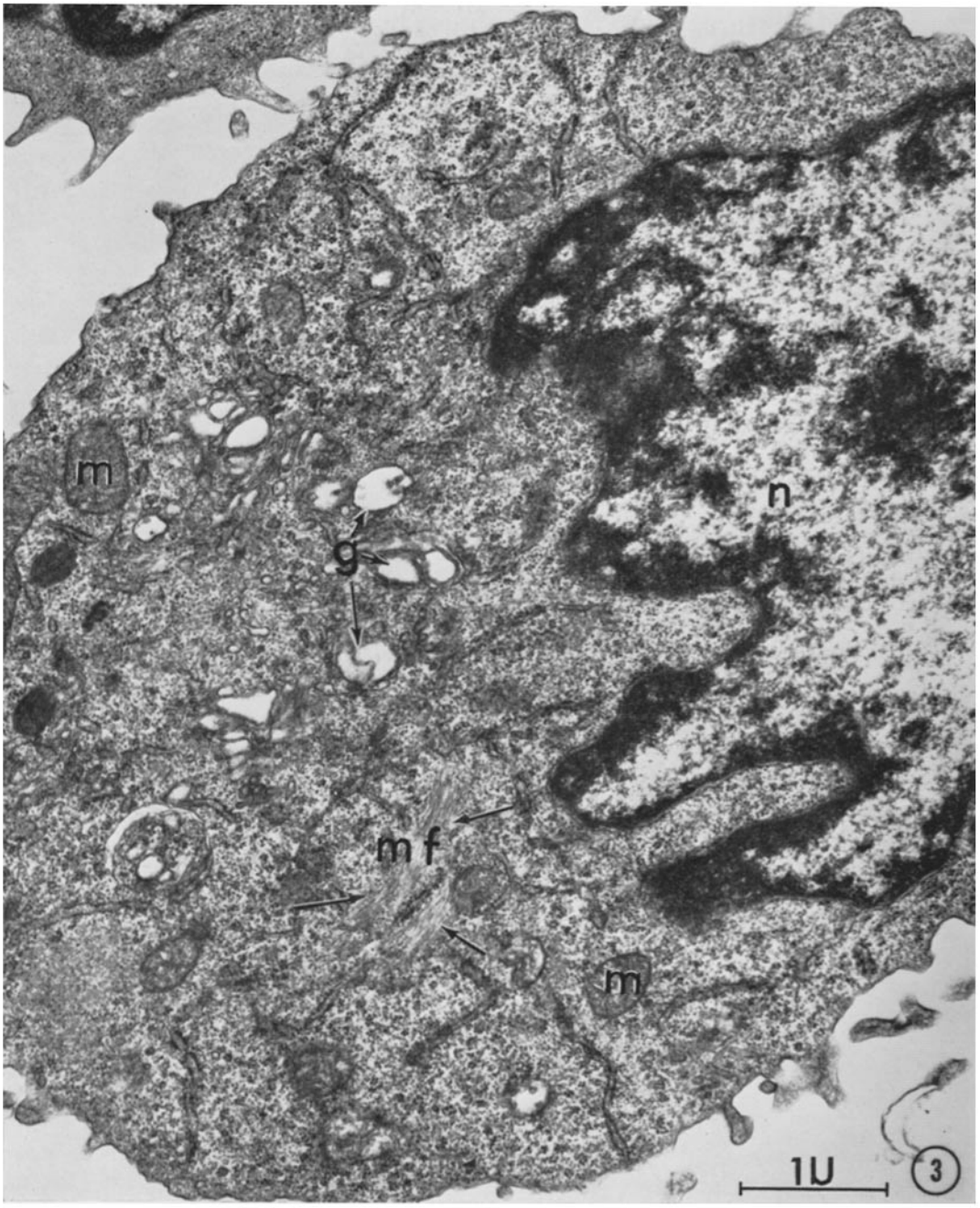


(Hall et al.: Ultrastructure and function of lymph cells)

PLATE 8

FIGS. 3–8. Cells collected from the efferent lymph of the popliteal lymph node during an immune response.

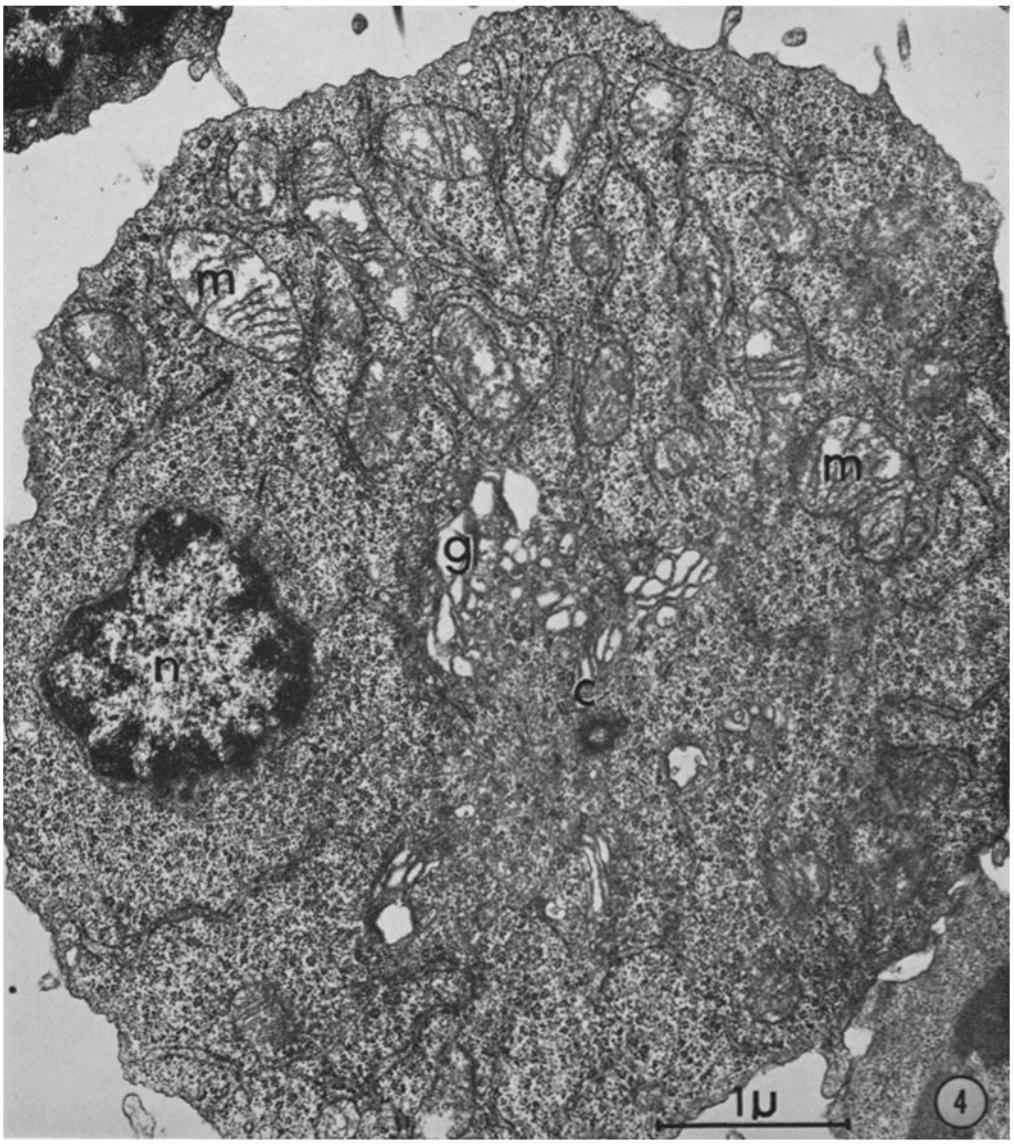
FIG. 3. A large blast cell which appears in lymph early in the immune response. This cell contains a few isolated pieces of endoplasmic reticulum and some polyribosomes. A bundle of microfibrils is indicated by the arrows. Nucleus (*n*), microfibrils (*mf*), mitochondrion (*m*), and golgi bodies (*g*). × 21,000.



(Hall et al.: Ultrastructure and function of lymph cells)

PLATE 9

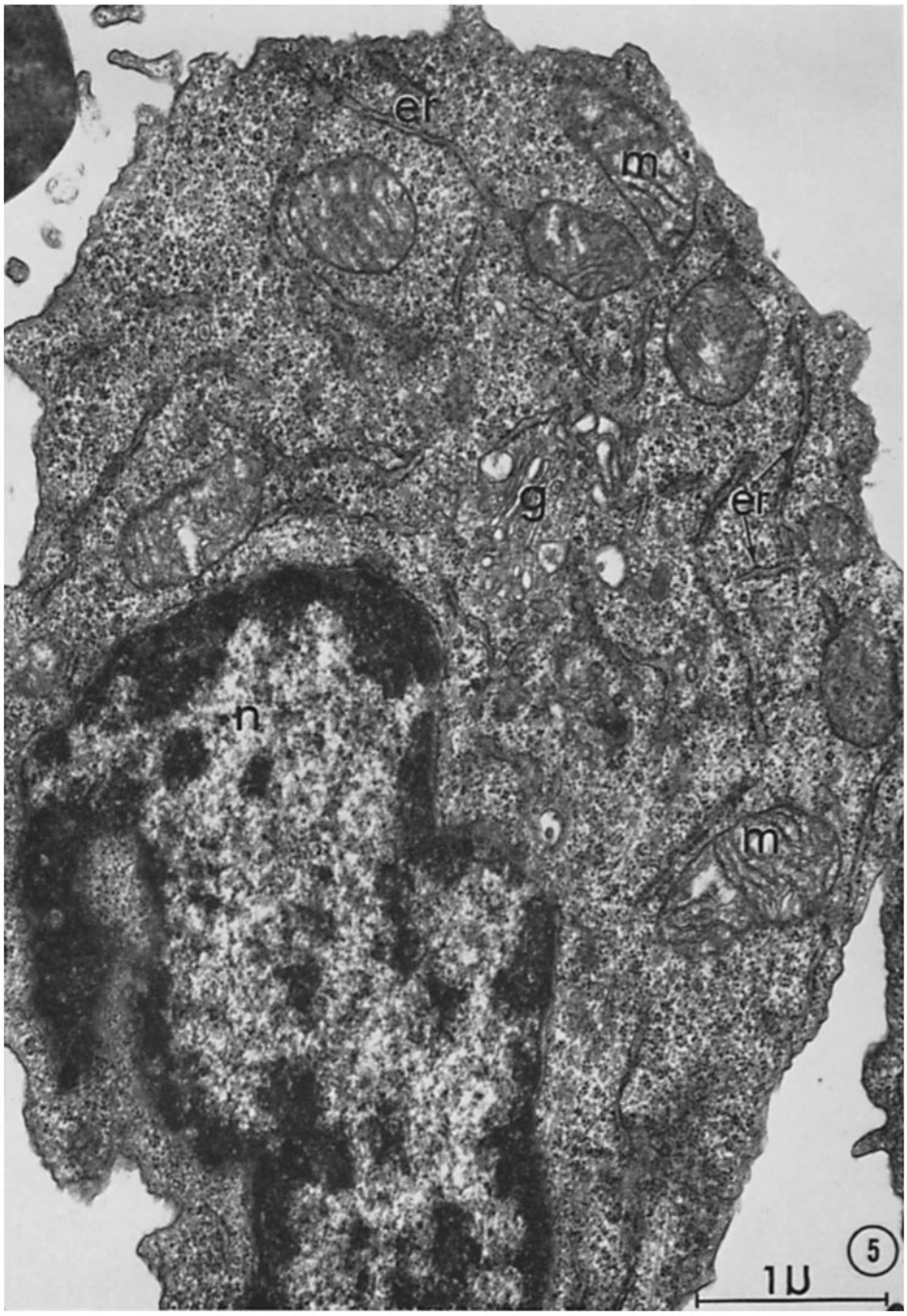
FIG. 4. The cytoplasm of a basophilic blast cell containing many free polyribosomes. The plane of the section is through the region of the centrosome. Nucleus (*n*), mitochondrion (*m*), golgi bodies (*g*), and centriole (*c*). × 25,000.



(Hall et al.: Ultrastructure and function of lymph cells)

PLATE 10

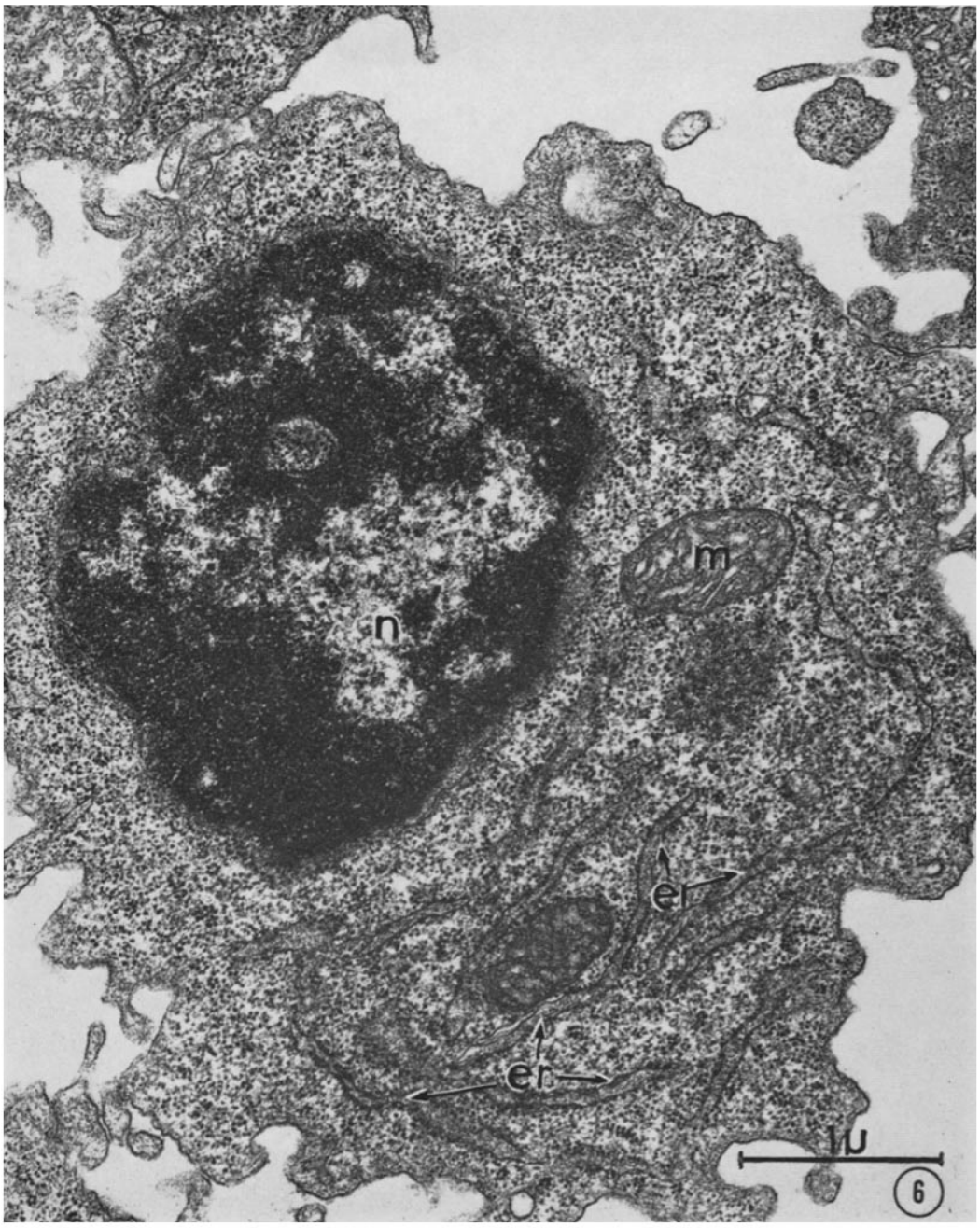
FIG. 5. A basophilic cell with a rudimentary endoplasmic reticulum. The cytoplasm contains many polyribosomes. Nucleus (*n*), mitochondrion (*m*), endoplasmic reticulum (*er*), and golgi bodies (*g*). × 26,000.



(Hall et al.: Ultrastructure and function of lymph cells)

PLATE 11

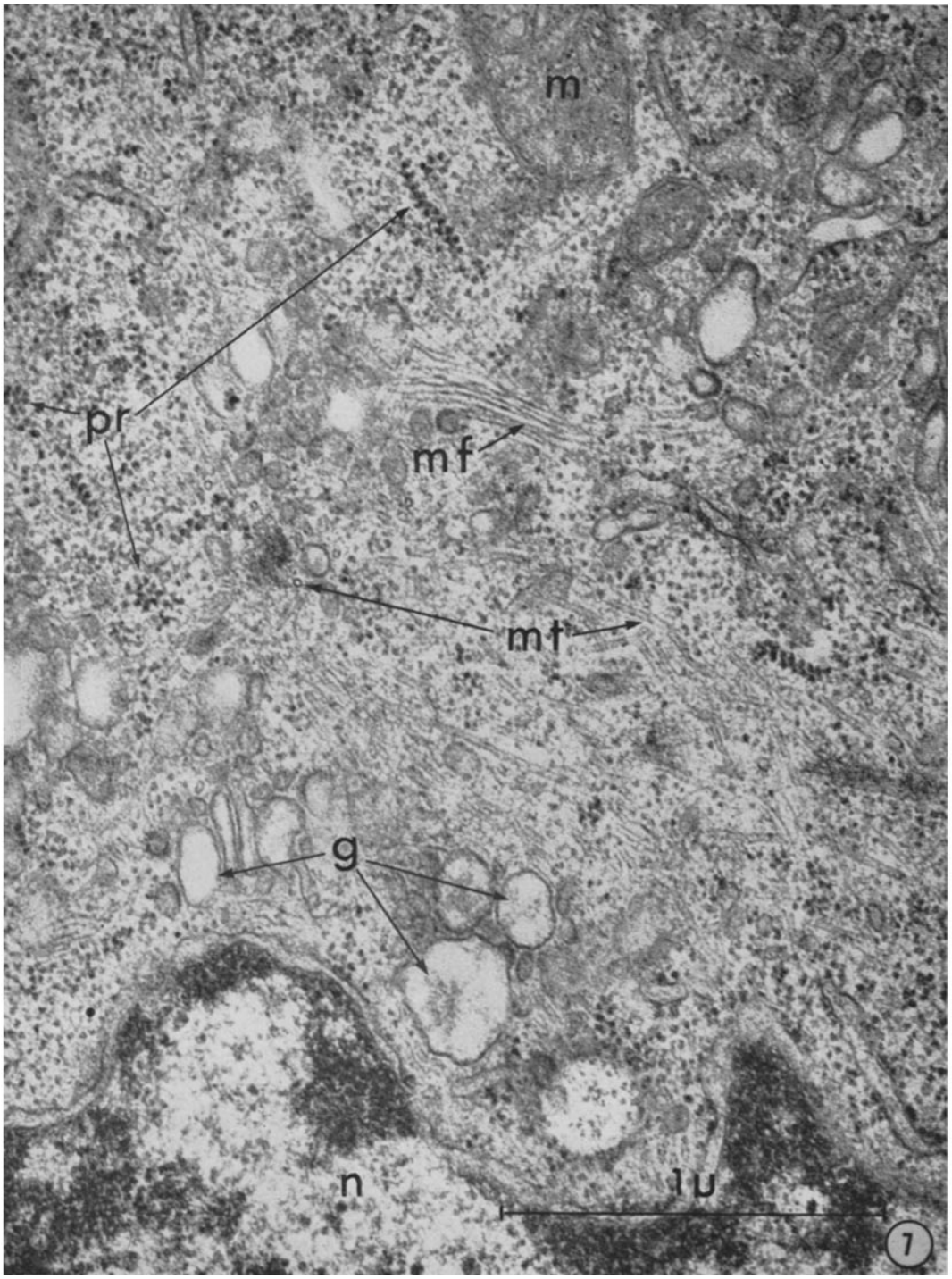
FIG. 6. A cell showing some concentric development of endoplasmic reticulum. The cytoplasm contains many polyribosomes. Nucleus (*n*), endoplasmic reticulum (*er*), and mitochondrion (*m*). × 28,000.



(Hall et al.: Ultrastructure and function of lymph cells)

PLATE 12

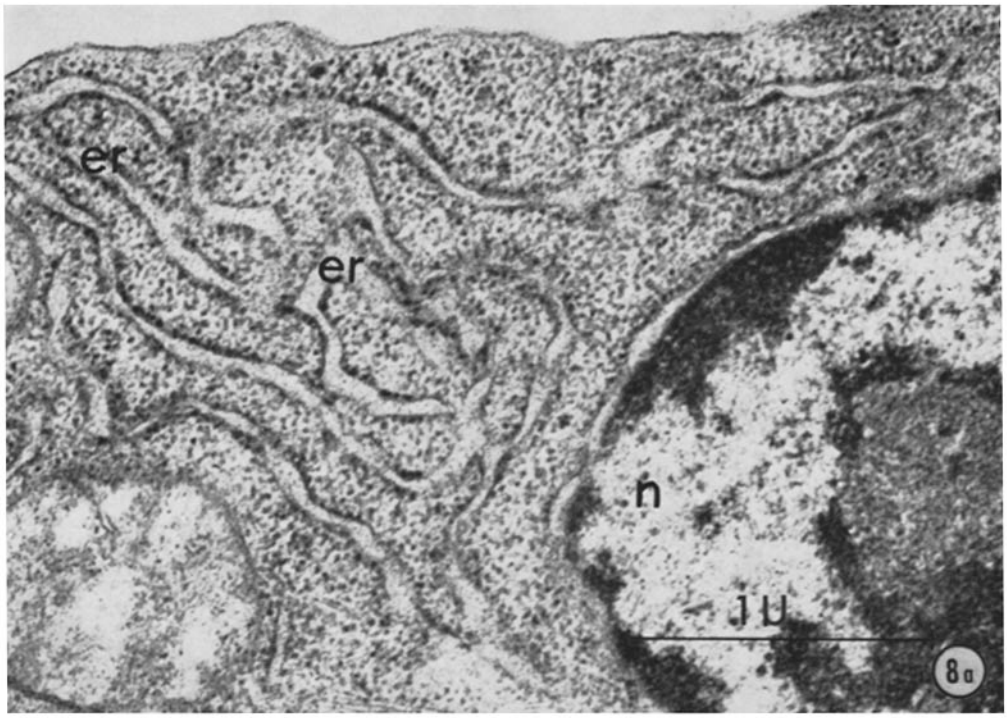
FIG. 7. A high power view of the centrosomal region of a large lymphoid blast cell showing short spirals and rosettes of ribosomes (arrows). Nucleus (*n*), polyribosomes (*pr*), mitochondrion (*m*), golgi bodies (*g*), microfibrils (*mf*), and microtubules (*mt*). × 57,000.

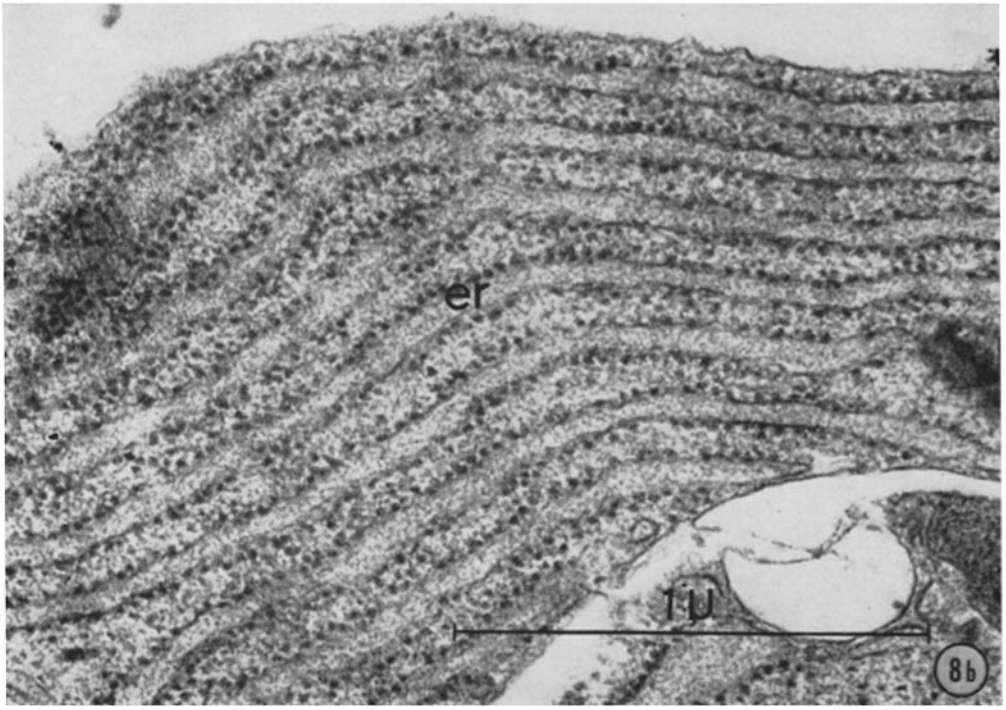


(Hall et al.: Ultrastructure and function of lymph cells)

PLATE 13

FIGS. 8 *a* and 8 *b*. The arrangement of the endoplasmic reticulum in a basophilic cell found in efferent lymph (Fig. 8 *a*) and in a typical plasma cell found in the lymph node (Fig. 8 *b*). Nucleus (*n*), and endoplasmic reticulum (*er*). 8 *a* × 40,000. 8 *b* × 63,000.





(Hall et al.: Ultrastructure and function of lymph cells)