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ELECTRON MICROSCOPY IN THE NON-HODGKIN'S LYMPHOMATA

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Summary.—The component cells of peripheral lymphoid tissue have been divided into the lymphocyte and plasma cell lines, mononuclear phagocytic cells, dendritic " reticular cells ", the reticular (supporting) cells and endothelial cells, and it is suggested that this system of cells should collectively be referred to as the lympho-reticular mononuclear phagocyte system or LRMPS. Seventeen tumours of the LRMPS (excluding Hodgkin's disease) have been studied at ultrastructural level. Of these 17 non-Hodgkin lymphomata 5 were follicular lymphomata and 12 diffuse. It is concluded that electron microscopy plays a valuable role in the diagnosis of this group of tumours. Not only does it allow rejection of a diagnosis of lymphoma in certain anaplastic tumours, but it also enables a more precise identification of the cellular components of a lymphoma as well as indicating the degree of differentiation of the cell line involved. Additional advantages are the visualization of subcellular structures useful as markers, and by means of specialized immunoelectron microscopic techniques the identification of antigens and antibody formation within a given tumour.

Two other results of this ultrastructural study are the indication that the dendritic cells of lymphoid follicles are derived from capillary endothelium, and the identification of certain anomalous formations derived from rough endoplasmic reticulum in the case of tumours showing plasmacytoid differentiation.

BEFORE discussing the role of electron microscopy in the diagnosis of the lymphomata, it is useful to consider briefly some aspects of current concepts and terminology of the lymphoreticular tissue and mononuclear phagocyte cell system (LRMPS), and the ultrastructural appearances of the component cells.

The component cells of peripheral lymphoreticular tissues (Table I) are lymphocytes and plasma cells (T and B cell lines) (Roitt *et al.*, 1969), mononuclear phagocytic cells, the dendritic " reticular " cells of the lymphoid follicles, the supportive reticular (fibre associated) cells and endothelial cells (Henry, 1972). The usage of the term " reticuloendothelium " has been avoided, the reason being that at a recent Symposium on mononuclear phagocytes Aschoff's original concept of the reticuloendothelial system was abandoned (Van Furth, 1970) and replaced by the mononuclear phagocyte system (MPS). Evidence was provided and accepted at the Symposium that within the bone marrow there is a stem cell which develops into an avidly phagocytic cell—the macrophage. Macrophages are widely distributed throughout the body and terminology differs according to the site in which they are found. The only phagocytic cells where proof of a monocytic origin still remains to be established are the fixed macrophages, including the sinusoidal lining cells of peripheral lymphoreticular tissue. However, since these cells are markedly phagocytic and share morphological features in common with cells of the MPS, they are included within this system. In lymphoreticular tissue macrophages have a characteristic appearance at electron microscopic level. They possess a ruffled plasma membrane due to the presence of numerous finger-like projections from the cell surface, a prominent Golgi apparatus and numerous

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TABLE I.—*Component Cells of the Peripheral Lymphoreticular Tissue and Mononuclear Phagocyte System (LRMPS)*

Lymphocyte and plasma cell series (T and B lymphocyte cell lines)
Mononuclear phagocytic cells
Dendritic " reticular " cells
Endothelial cells
Reticular fibre associated cells (fibroblasts)

mitochondria, and contain many electron dense residual bodies of lysosomal nature. Their nucleus usually is eccentrically located and contains a well defined nucleolus. The true reticular cells or supporting cells have a very different appearance. They tend to be elongated, show a dense fibrillary or felt-like material along their cytoplasmic borders and desmosomal attachments between these cells can also be demonstrated. In some instances they are indistinguishable from fibroblasts and, indeed, they are the cells responsible for the production of connective tissue fibres with the same periodicity of collagen (reticulin). They are found in close relationship to mononuclear phagocytic cells. The dendritic reticular cells first described by Maruyama and Masuda (1964) are not included in the mononuclear phagocyte cell system since they are not avidly phagocytic. They are the cells especially associated with lymphoid follicles. However, they are found elsewhere in peripheral lymphoid tissue and are thought to function by virtue of trapping antigens on to their surface (Nossall *et al.*, 1968). Dendritic cells also have a very distinctive appearance, with long cytoplasmic processes forming complex inter-digitations with neighbouring dendritic cells and lymphocytes (Fig. 1). A special feature, to which attention has been drawn only comparatively recently (Swarzendruber, 1965) is the presence of desmosomal attachments between one dendritic cell and another (Fig. 1, inset). Other common findings are fine filaments within the cytoplasmic processes and electron dense material lying between the cytoplasmic extensions (Fig. 2). Their origin has not been established but there is evidence at electron microscopic level that the follicular dendritic cells are derived from capillary endothelial cells, for it is possible to demonstrate continuity between endothelial cells and dendritic cell processes (Fig. 3), (Henry, 1973, unpublished). Also, endothelial cells, like dendritic cells, possess desmosomal attachments. It is particularly interesting in this context that Soderström, following a light microscopic study of the development of lymph nodes in mice, suggested that the secondary follicles (germinal centres) might develop from post-capillary venules due to a massive proliferation of the tall endothelial cells (Soderström, 1967).

The lymphoid cells found within germinal centres are lymphocytes with distinctive irregularly shaped nuclei, often with nuclear protrusions or blebs (Fig. 2). They are the germinocytes described by Mori and Lennert (1969) and the cleaved cells originally described as haematogones by Rosenthal (1954). Compared with paraffin embedded material or imprint preparations, the nuclei do not appear so clearly notched and there is also more cytoplasm. Other lymphoid cells present in germinal centres are larger, also contain an irregular nucleus with a prominent nucleolus, and contain an appreciable RNA content with formation of polyribosomes. These correspond to the germinoblasts (Mori and Lennart, 1969). Both types of lymphocytes inter-digitate with processes of the dendritic reticular cells. As one proceeds out of the germinal centre, communications between the cells become less prominent and one also encounters large rounded lymphoid cells or immunoblasts (Fig. 4). These cells are rich in polyribosomes and also show profiles of rough endoplasmic reticulum. They correspond to the pyroninophilic blast cells and are also found outside the lymphoid follicles.

One of the main disadvantages of electron microscopy in the lymphomata is that of sampling and this applies particularly to the follicular lymphomata. Because the tissue is cut into small pieces for

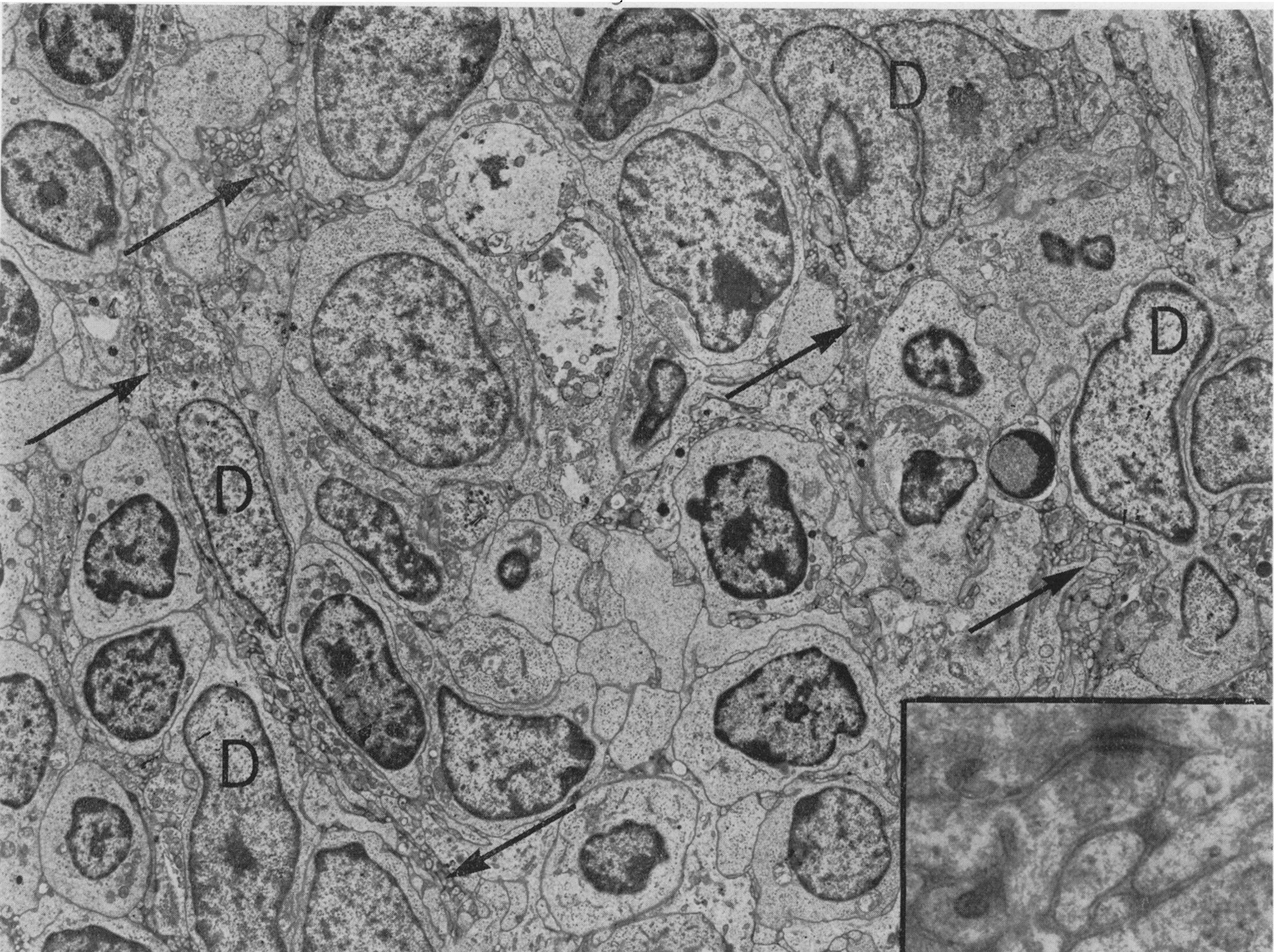

FIG. 1.—Germinal centre showing relationship of dendritic cells (D) to the lymphoid cells. Note the complex cytoplasmic interdigitations (arrows) between the dendritic and lymphoid cells. ×4960. Desmosomal connections (inset × 48,000) are present between the processes of the dendritic cells.

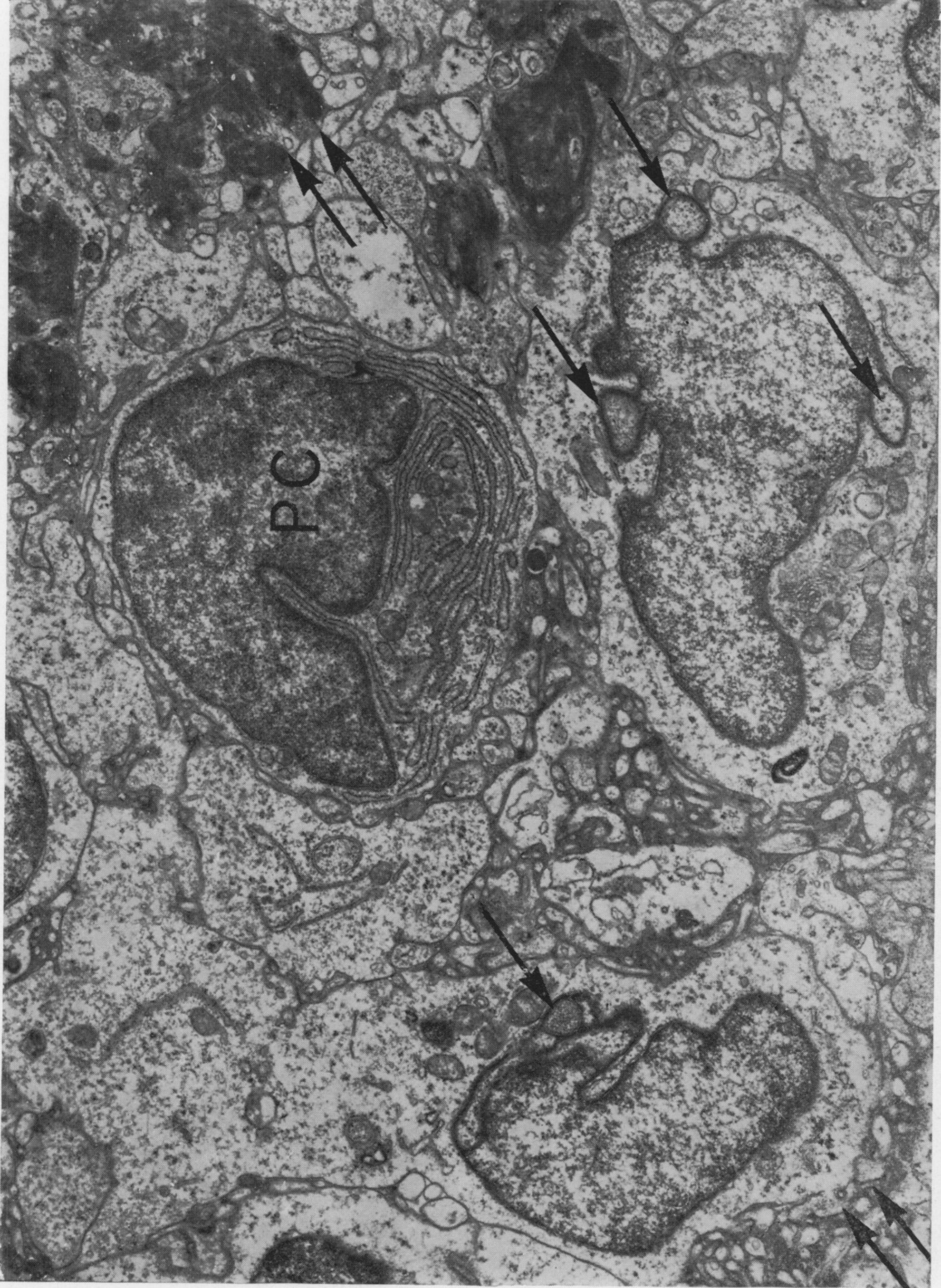

Fig. 2.—Characteristic lymphocytes (cleaved cells) within a germinal centre. Note the highly irregular nuclei with nuclear extrusions or blebs (arrows) and the presence of electron dense material (double arrows) between the cytoplasmic processes of lymphocytes and dendritic cells. A cell showing plasmacytoid features (PC) is also illustrated. × 8490.

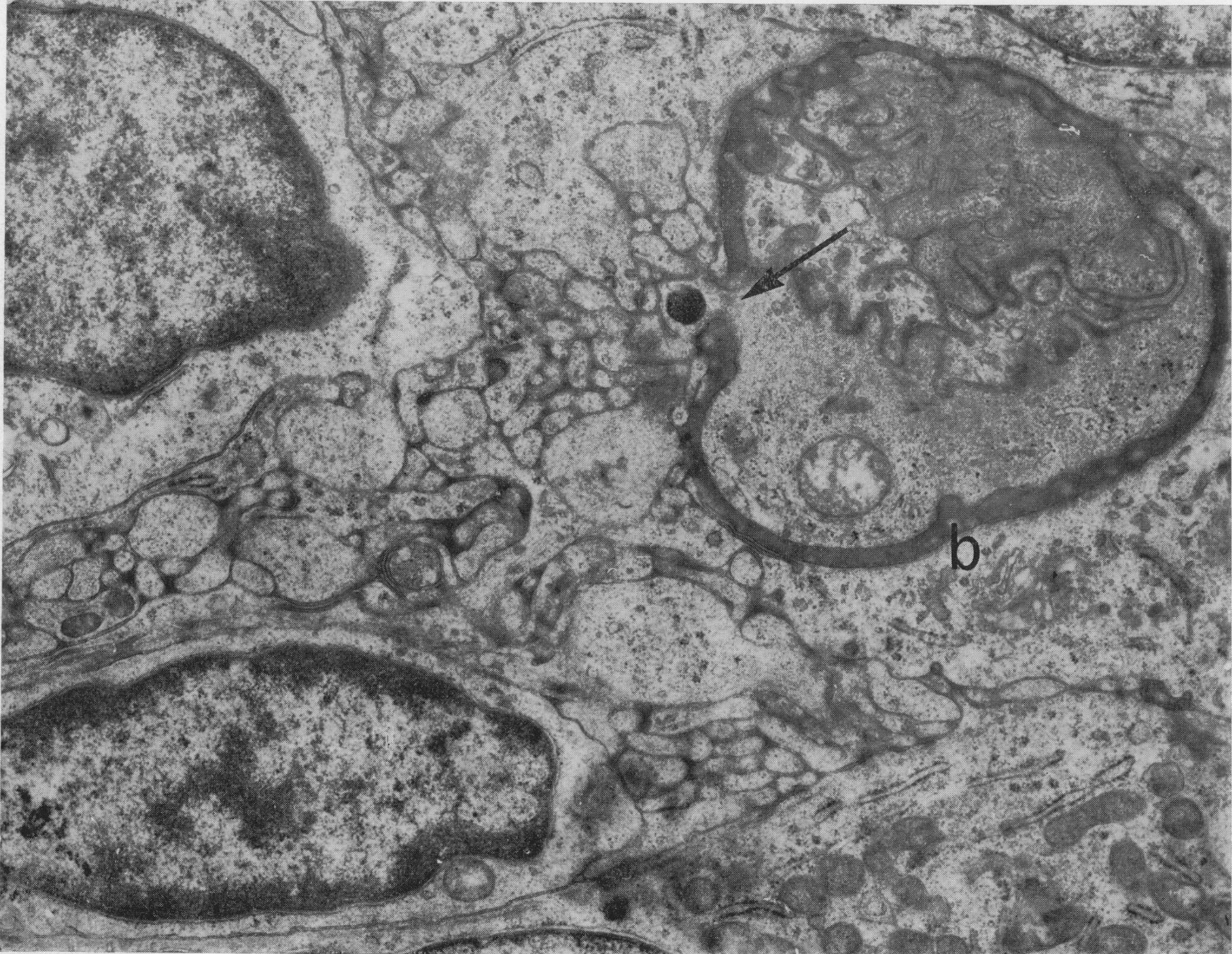

FIG. 3.—A capillary with well developed basal lamina (b) is present on the right side. Continuity between the cytoplasm of the endothelial cells (arrow) and the inter-digitating processes of dendritic reticular cells is present. ×12,000.

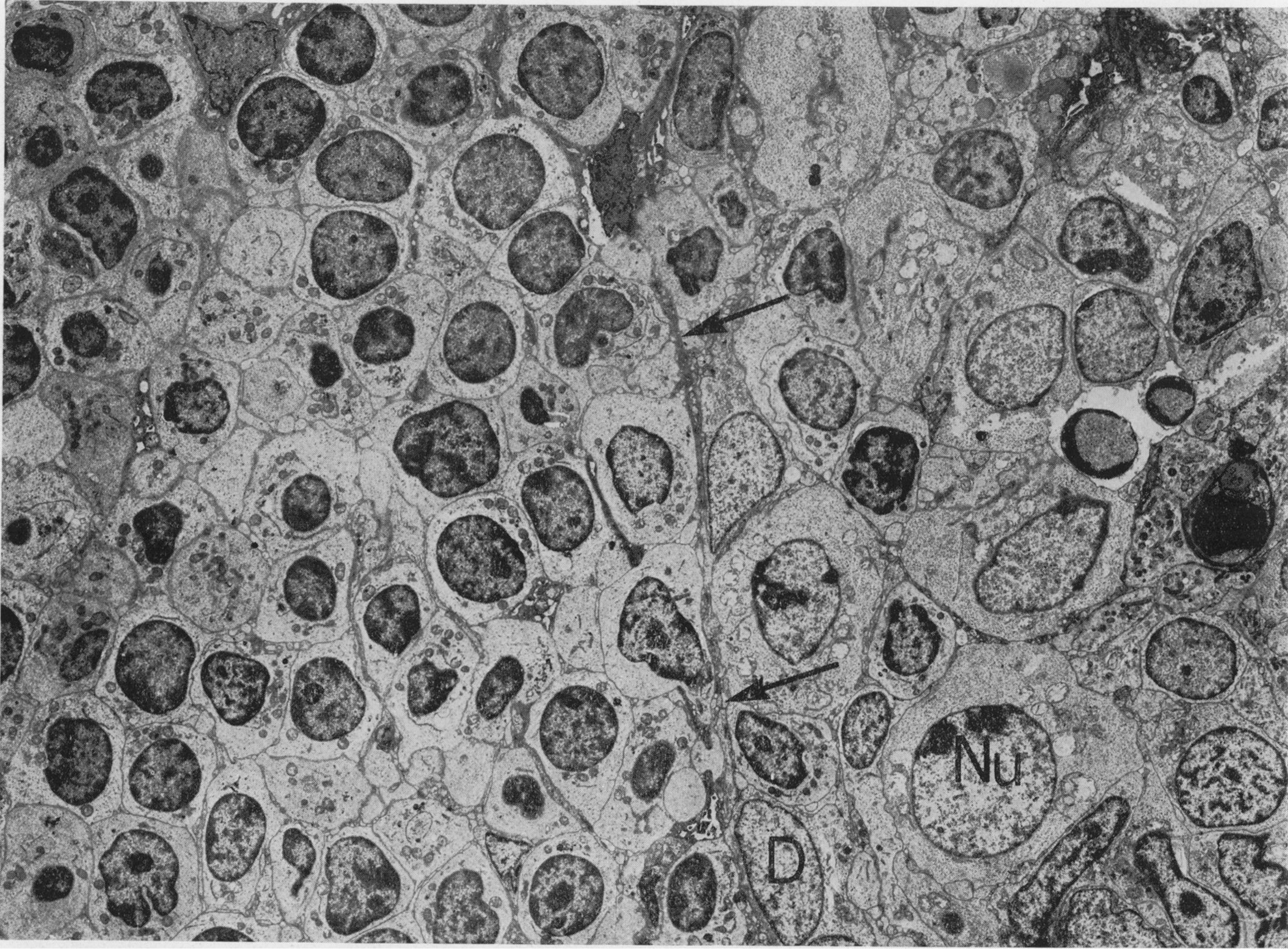

FIG. 4.—Junctional zone of germinal centre with the surrounding cuff of small lymphocytes. Note the demarcation of large lymphoid cells (immunoblasts) with prominent nucleoli (Nu) from the lymphocytic mantle by processes (arrows) of dendritic cells (D). Note also the rounded regular nuclei of the surrounding small lymphocytes. × 2770.

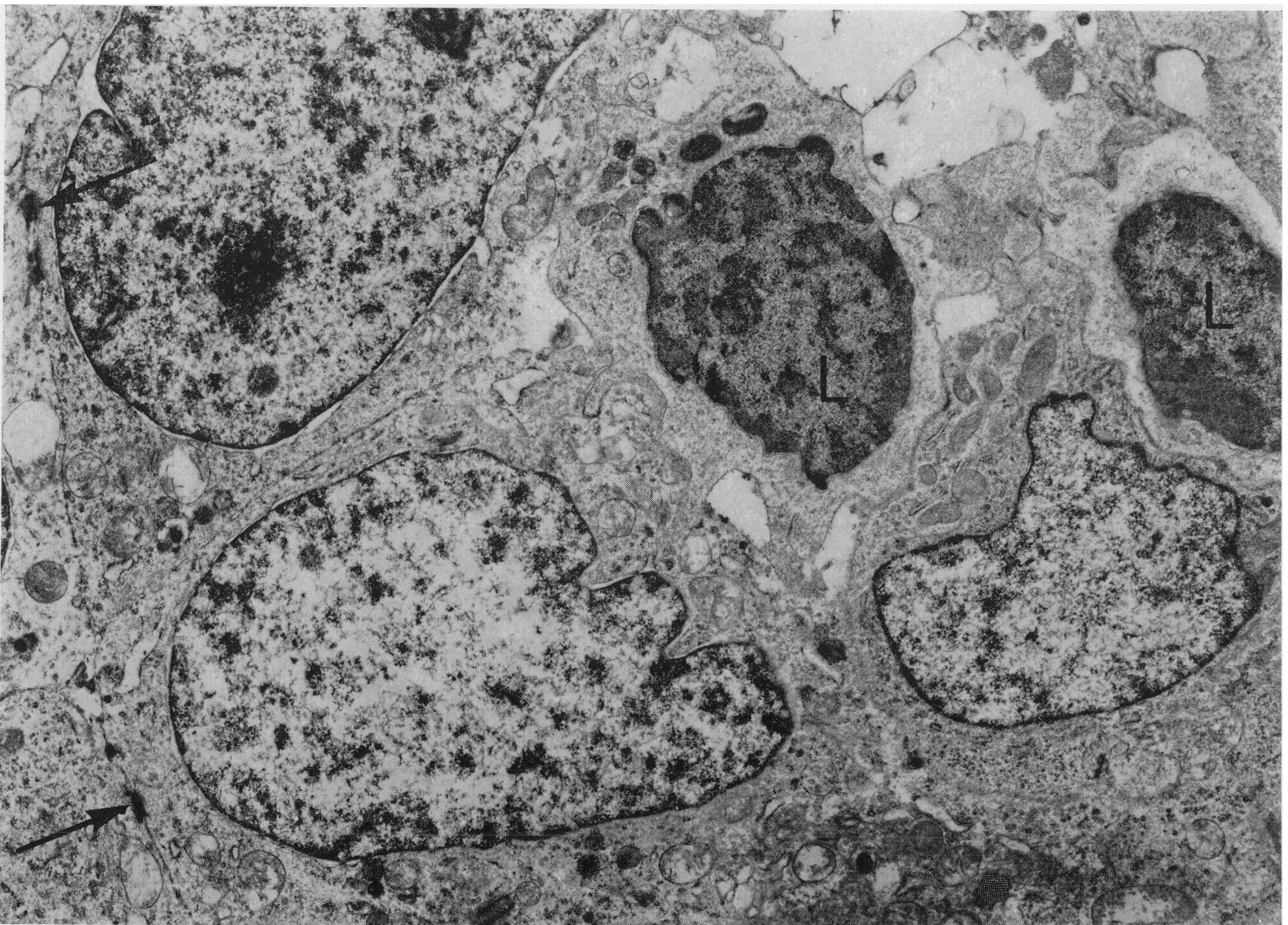

FIG. 5.—Lymph node metastases of an anaplastic tumour which ultrastructurally shows the features of epithelial cells. Numerous desmosomes (arrows) can be seen. Normal lymphocytes (L) are present between the tumour cells. × 9800.

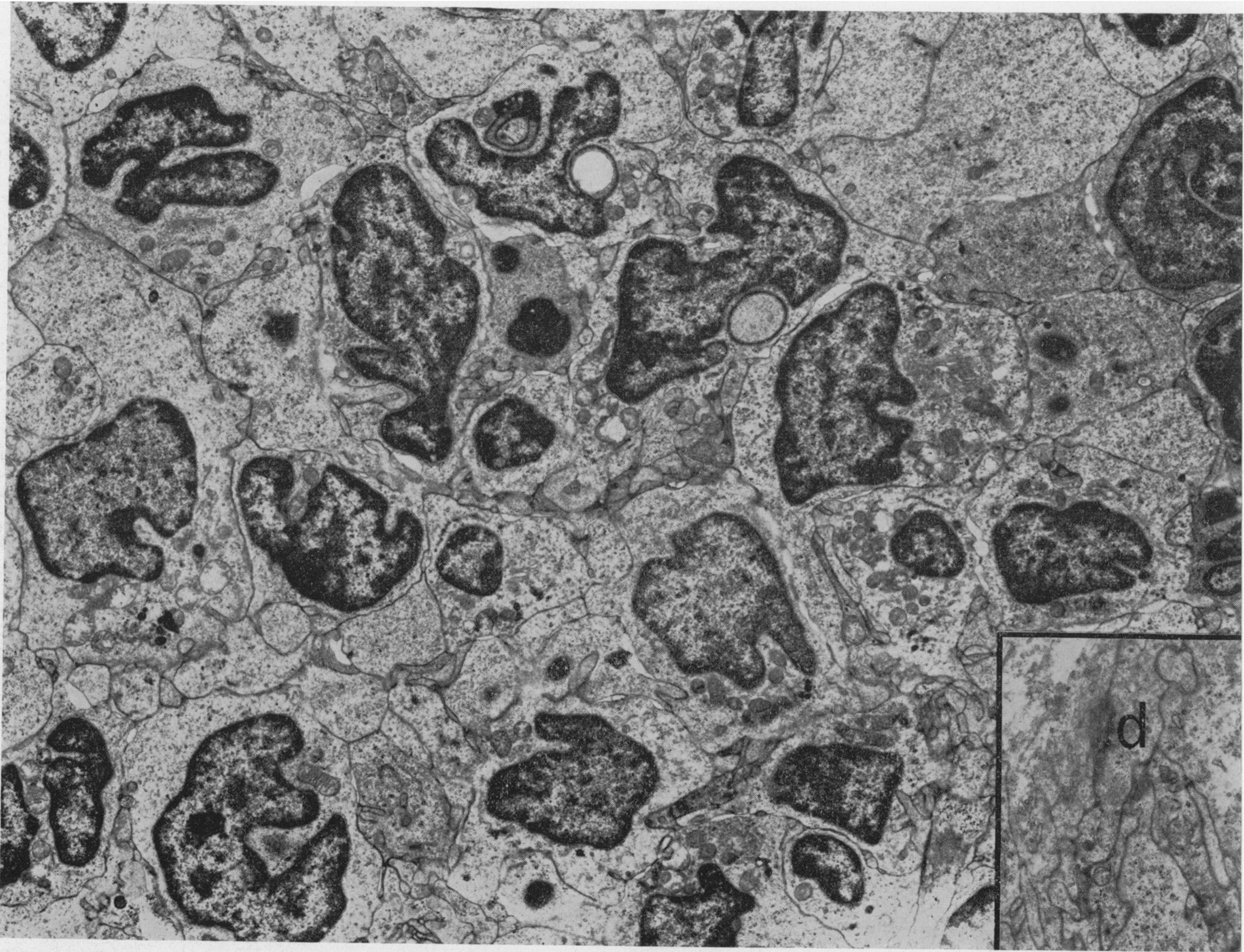

FIG. 6.—Electron micrograph of a follicular lymphoma composed predominantly of small " cleaved " lymphocytes or germinocytes. The nuclei are very irregular and there is more cytoplasm than in small mature lymphocytes. × 4860. Desmosomes (inset d) can be visualized. × 12,200.

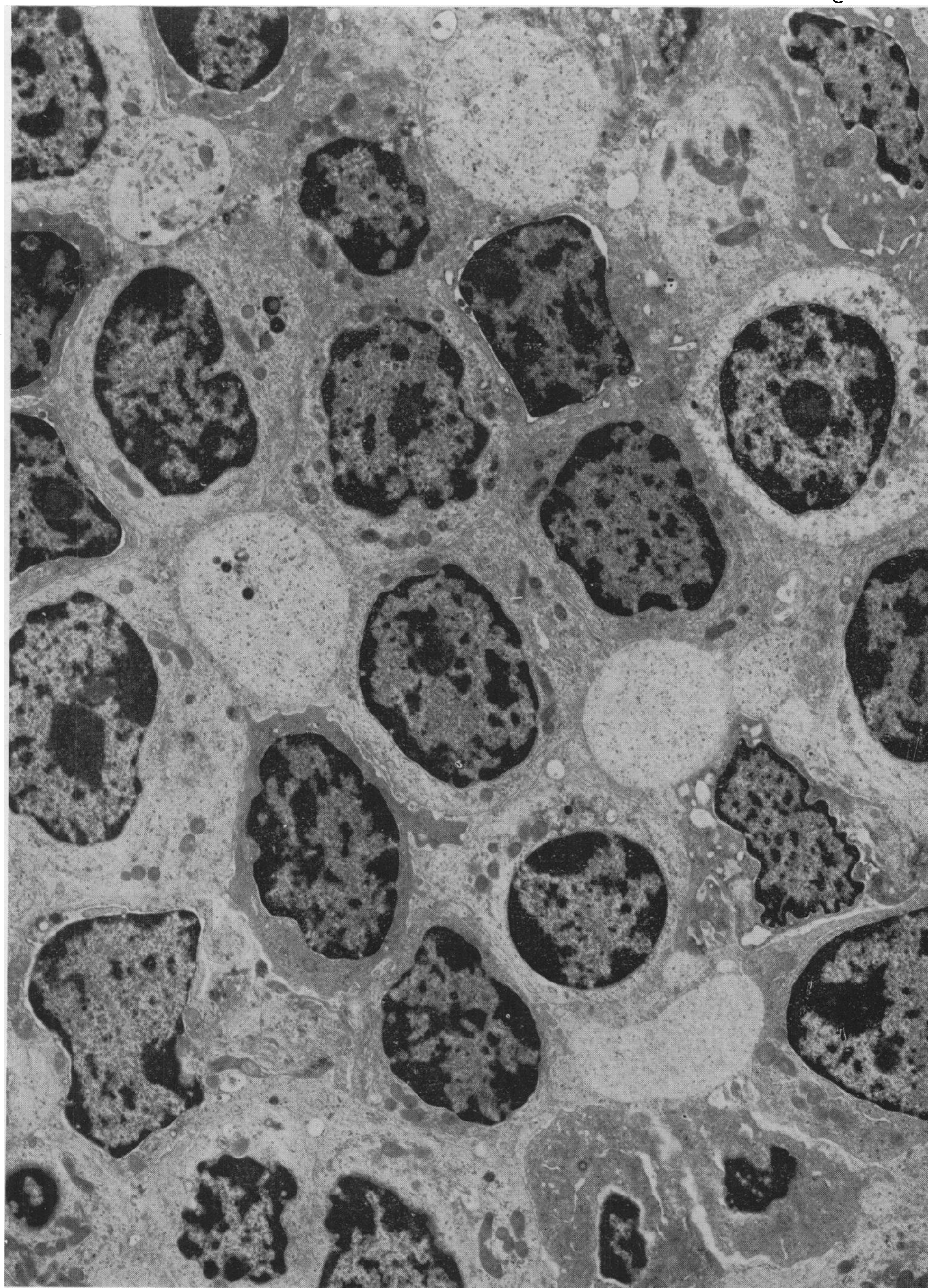

FIG. 7.—Electron micrograph of the cells of chronic lymphocytic leukaemia for contrast with Fig. 6. × 4800.

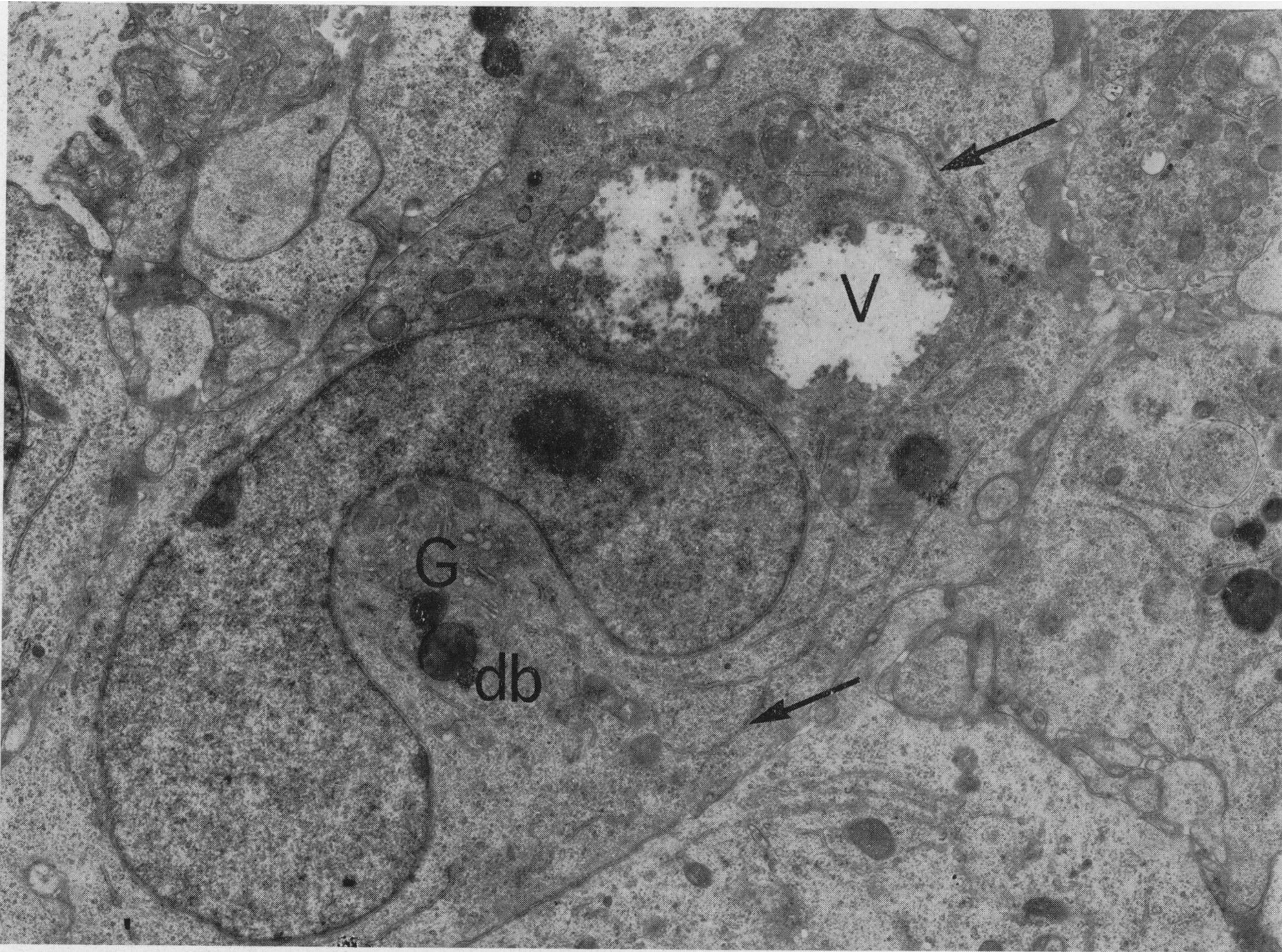

FIG. 8.—Malignant lymphoma composed of cells showing fine structural features resembling the monocyte series. The nucleus is reniform and there is a prominent Golgi apparatus (G). The cytoplasm contains several electron dense residual bodies (db) and numerous ribosomes. There is a modicum of rough endoplasmic reticulum (arrows) and large vesicular structures (V). × 8650.

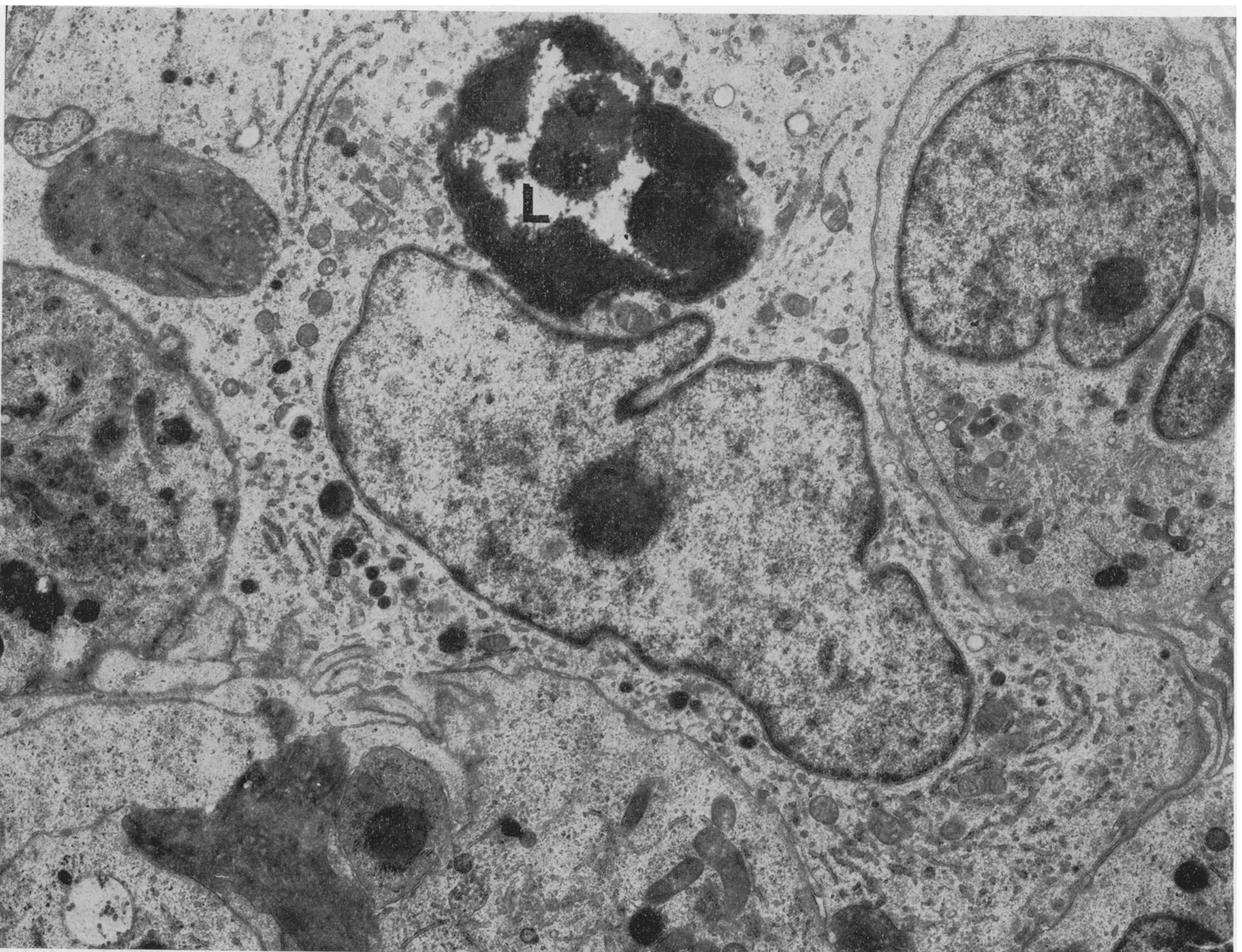

FIG. 9.—Same tumour as Fig. 8. The central cell has a voluminous cytoplasm with the fine structure of the macrophage series (histiocytes). Note the phagocytic activity as evidenced by the engulfed disintegrating lymphocyte (L). × 8900.

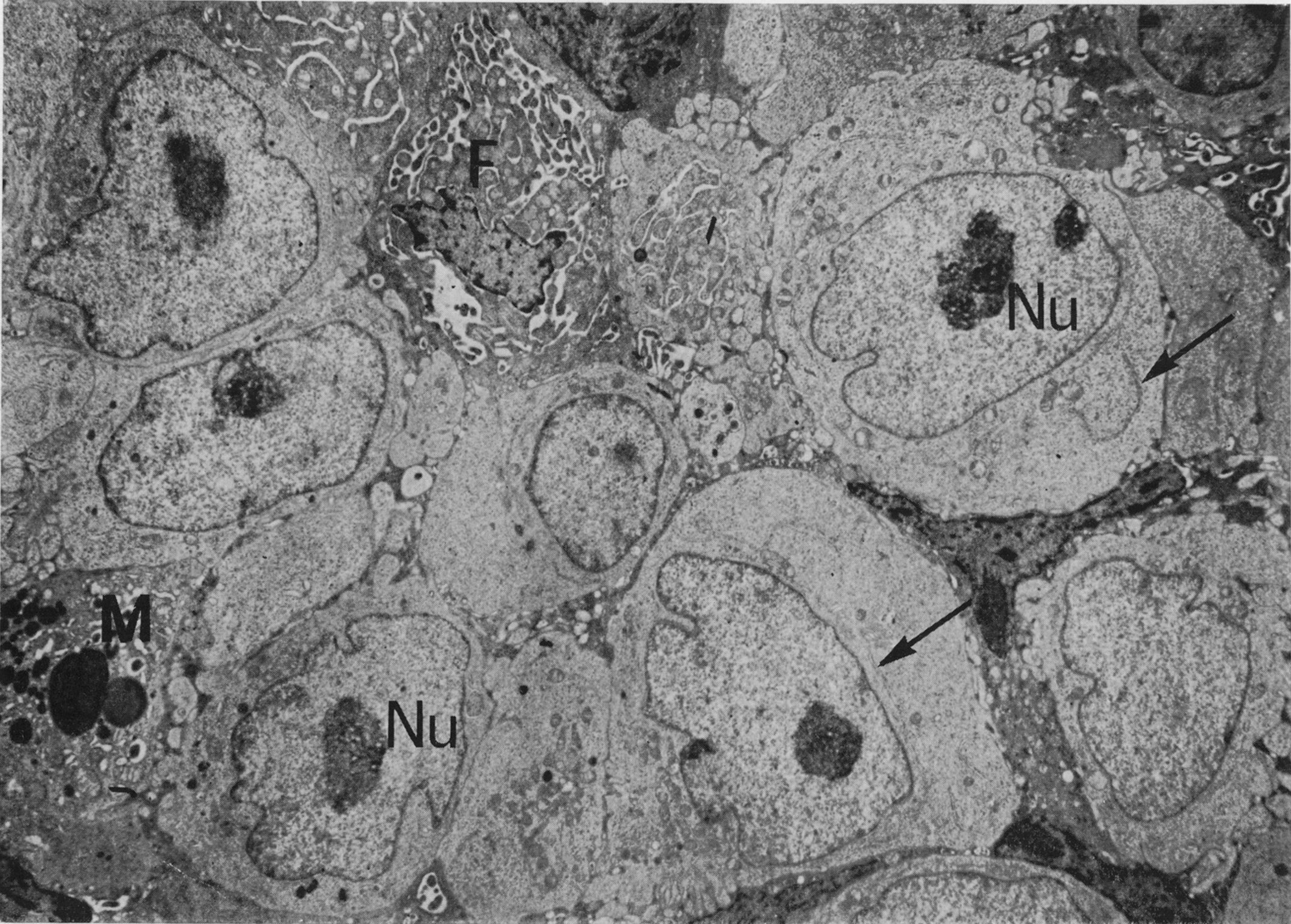

Fig. 10.—Tumour composed of large lymphoid cells or immunoblasts. There are prominent nucleoli (Nu), the cytoplasm is rich in polyribosomes and there is formation of rough endoplasmic reticulum (arrows). A fibroblast (F) and a macrophage (M) are included in the field. × 7440.

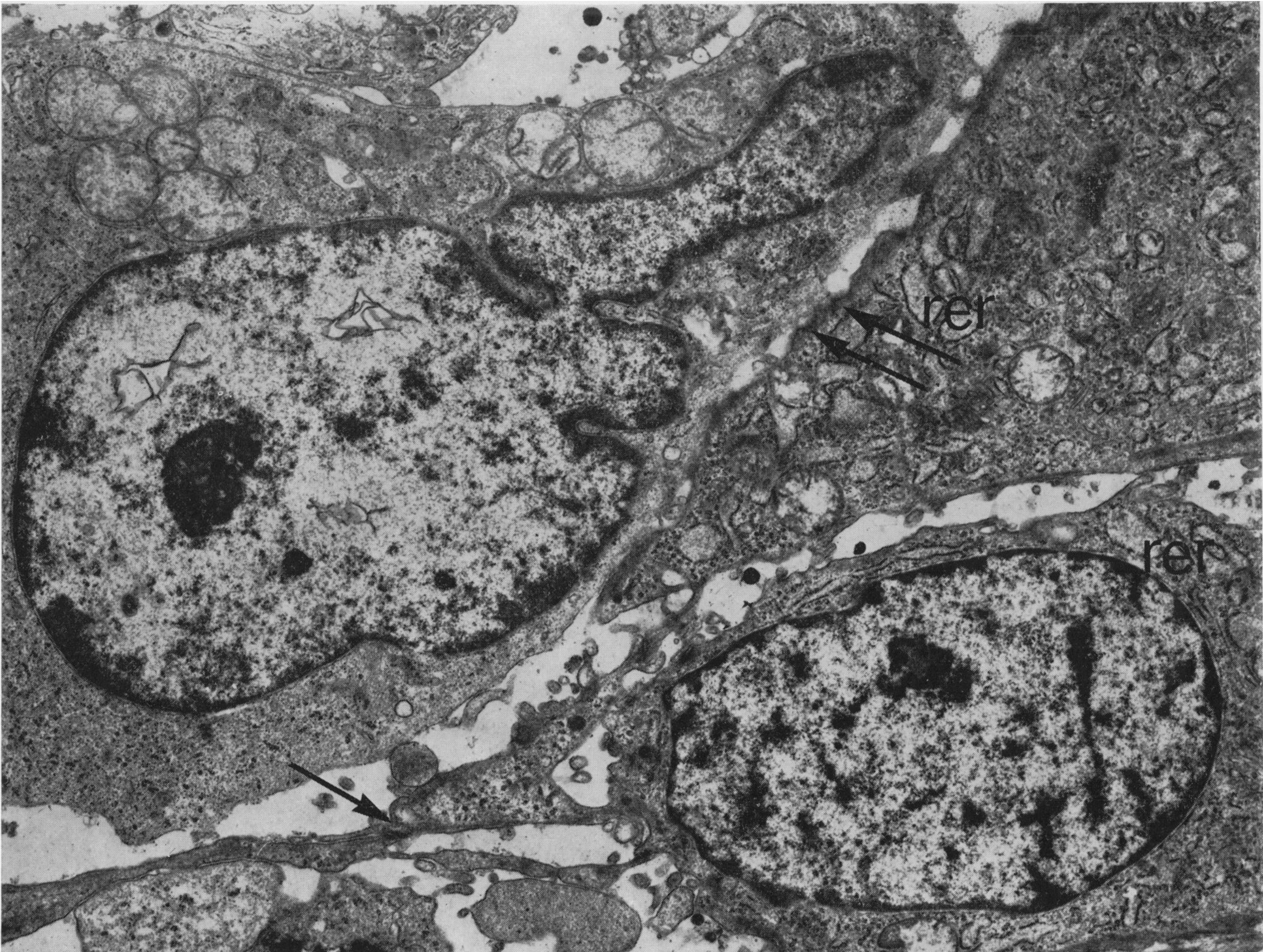

Fig. 11.—A tumour composed of elongated cells, the processes of which are connected by desmosomes (arrow) and which show dense fibrillary material (double arrow) along their cytoplasmic borders. Rough endoplasmic reticulum (rer) is moderately well developed and shows dilated cisternae. These cells resemble the reticular fibre forming cells of lymphoid tissue. × 11,600.

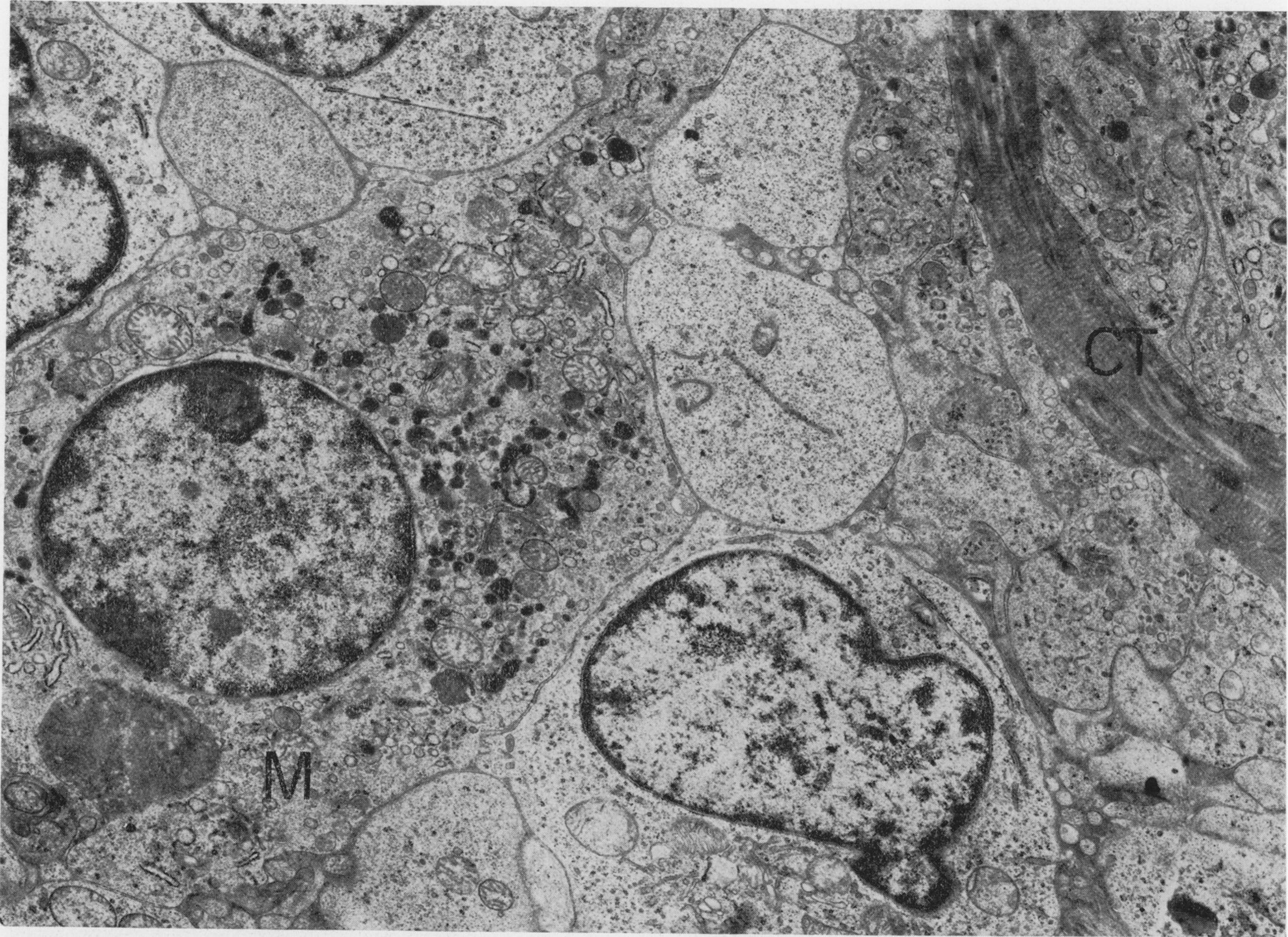

Fig. 12.—A diffuse lymphoma composed of large lymphocytes with numerous polyribosomes in which there were many reactive macrophages (M). Note the extracellular connective tissue (CT) with prominent periodicity of longitudinal fibres. × 8650.

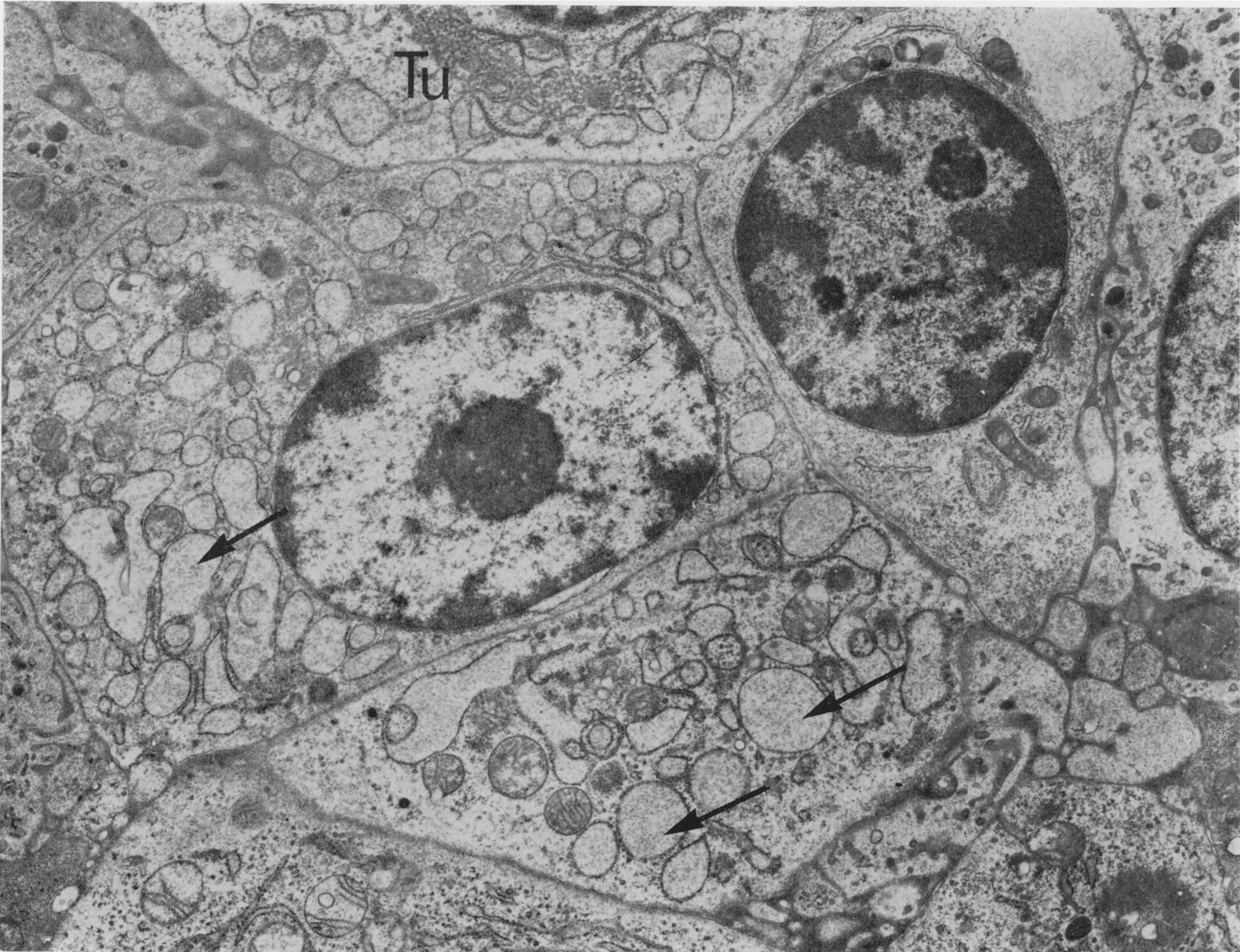

FIG. 13.—Splenic tumour composed of cells showing plasmacytoid features. The cytoplasm is filled with dilated, rough endoplasmic reticulum containing granular moderately electron dense material (arrows). Note the tubular profile in the upper cell (Tu). × 9650.

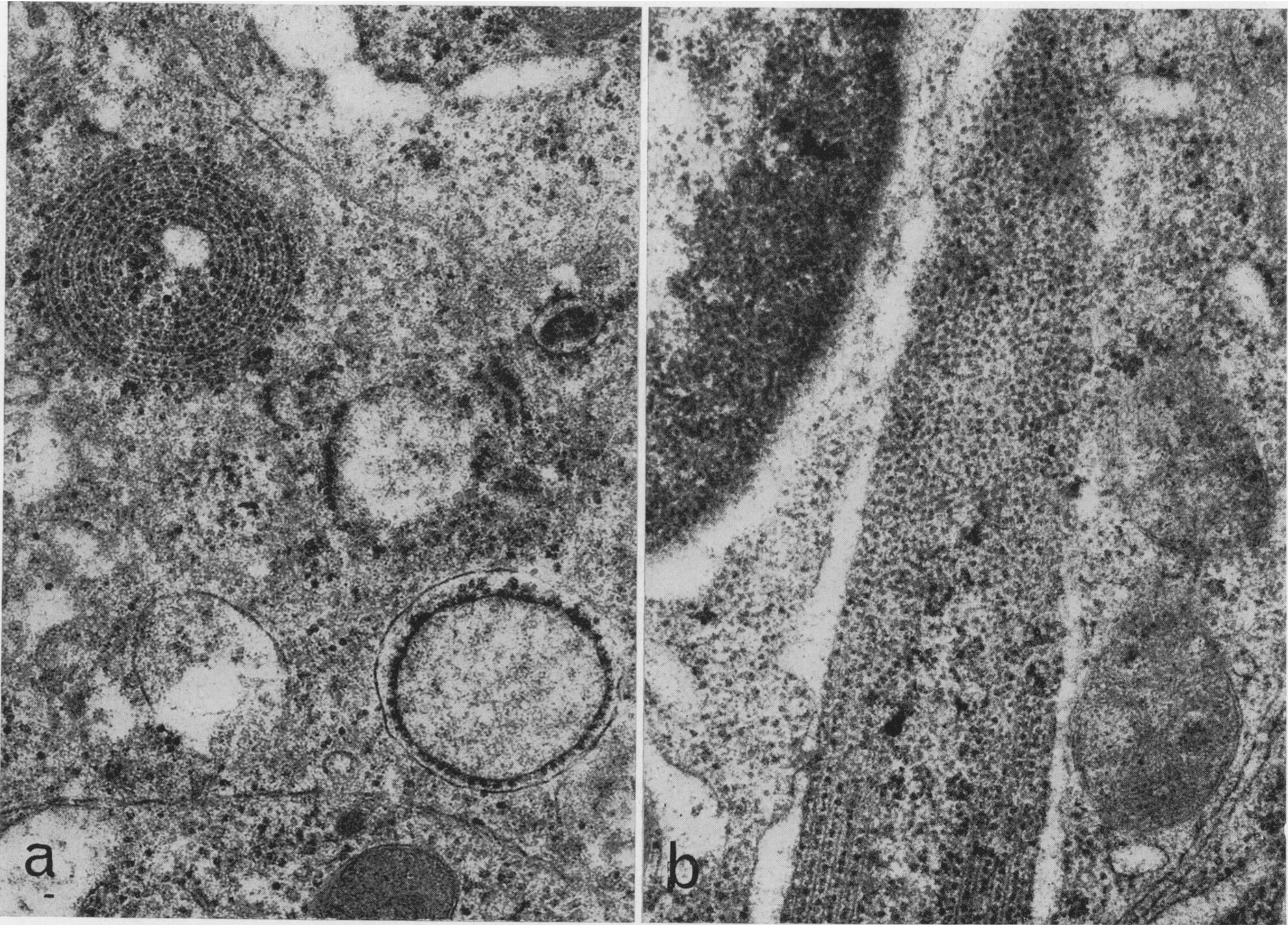

FIG. 14.—Cytoplasmic detail of structures derived from rough endoplasmic reticulum, (a) transversally sectioned, (b) longitudinally sectioned. Note the regular arrangement of the ribosomes. × 40,000.

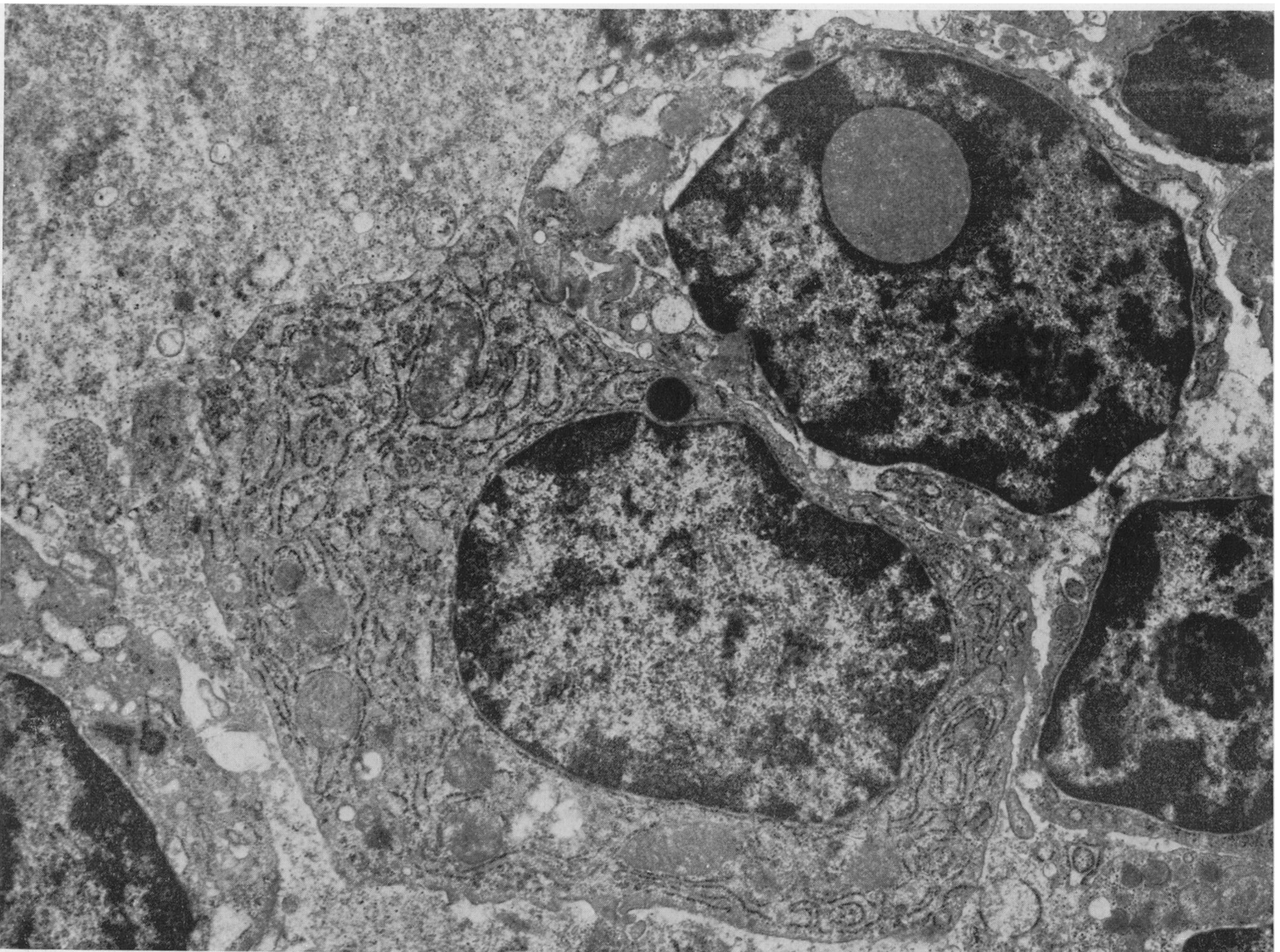

FIG. 15.—Component cells of a lymph node in macroglobulinaemia. The cell on the left shows the cytoplasmic configuration of a plasma cell, and the nucleus of the cell in the upper right hand corner contains a cytoplasmic inclusion. × 12,000.

the most satisfactory fixation, one may have to cut through several blocks before encountering a neoplastic follicle. Furthermore, the cellular components of the neoplastic follicles may vary as to whether the line of section passes through the centre or periphery of a follicle. Another limitation of electron microscopy is the considerable time expended in the processing and viewing of specimens. Bearing these problems in mind, what contribution can electron microscopy make to the investigation and diagnosis of lymphomata? Some of the advantages of the technique are: (a) the confirmation (or exclusion) of the diagnosis of lymphoma; (b) the identification of the cell type(s) involved in the neoplastic process; (c) the indication of the degree of " differentiation " of the component cells; (d) the demonstration of various inclusions, organelles and cell to cell contact which may prove useful as markers; and (e) the utilization of histochemical and immunoelectron microscopic techniques helpful in the identification of the different cell types.

The first advantage, therefore, is that one can establish or refute the diagnosis of a lymphoma. This may seem surprising to clinicians but all pathologists are familiar with the difficulty experienced in making a decision as to whether a given tumour is a lymphoma or an anaplastic carcinoma, as shown in this example of a tumour labelled as a reticulum cell sarcoma (Fig. 5). However, at electron microscopic level it proved to be a metastatic carcinoma, with the typical nuclear appearance of epithelial cells, together with cytoplasmic condensations of tonofilaments and desmosomal attachments.

The second advantage is that one can identify or, in many cases, confirm the cell type involved in the neoplastic process much more precisely than in conventional paraffin embedded material. This statement is based on the results of a study during the past 3 years of a range of peripheral neoplasms of the LRMPS listed in Table II.

TABLE II.—*Initial Histological Diagnoses of Tumours of the Lymphoreticular and Mononuclear Phagocyte System (LRMPS) Studied at Ultrastructural Level*

A. Non-Hodgkin's lymphomata	17
Follicular lymphomata (5)	
Diffuse lymphomata (12*)	
B. Hodgkin's disease	18
C. Others	12
Macroglobulinaemia (4)	
Extramedullary plasmacytoma (3)	
Heavy chain disease (alpha) (3)	
Hairy cell leukaemia (spleens) (2)	
Total	47

* Includes one case of CLL.

This communication, however, is concerned only with the non-Hodgkin's lymphomata, of which 17 have been studied—5 follicular and 12 diffuse. With regard to the follicular lymphomata, sampling may be a real problem. Nevertheless, having succeeded in isolating follicles, one can indicate the predominant cell type, as in the follicular lymphoma illustrated which is composed predominantly of " cleaved " cells (Rosenthal, 1954; Galton, 1972) or germinocytes (Fig. 6).

It can be seen that the neoplastic lymphocytes are irregular both in nuclear and cytoplasmic configuration, resulting in complex cytoplasmic inter-digitations between one another and with dendritic cell processes which can be identified by the presence of desmosomes (Fig. 6 inset) as previously described (Kojima, 1969; Lennert and Niedorf, 1969). This appearance can be contrasted with the well differentiated lymphocytes found in chronic lymphocytic leukaemia (Fig. 7).

With regard to the presence of desmosomes between cell processes in diffuse lymphomata, caution should be exercised in attributing these to dendritic cells since desmosomes are also found between fibre forming reticular cells as well as endothelium.

It is in the field of the diffuse lymphomata, particularly those composed of large " undifferentiated " cells or " histiocytes ", that electron microscopy proves the most rewarding in the identifi-

cation of cell types. This is illustrated by the following 4 examples. The first example (Fig. 8, 9) is a tumour originally classified at light microscopic level as a malignant lymphoma, with differentiation of the lymphocytes towards the plasma cell series. However, the fine structure of the majority of the tumour cells showed the typical configuration of mononuclear phagocytic cells with bean-shaped nuclei, active Golgi apparatus and marked phagocytic activity, as evidenced by ingested material (Fig. 9) and electron dense residual bodies. The reason this tumour was originally considered to show plasmacytic differentiation was the intense pyroninophilia of the cytoplasm and the presence of PAS positive inclusions within the cytoplasm. From this one example alone there are two lessons to be learned. First that pyroninophilia does not necessarily indicate the plasma cell or immunoblast series, for other cells exhibiting pyroninophilia are activated macrophages, promonoblasts and fibroblasts; secondly, that the PAS positive cytoplasmic inclusions are vesicular structures probably derived from the Golgi apparatus. Thus, special stains can be misleading unless aided by ultrastructural studies. The second example of cell identification is a tumour composed of large cells designated as malignant lymphoma, histiocytic type, but which on electron microscopy proved to be composed of immunoblasts (Fig. 10), cells which are not related to the mononuclear phagocyte system. The third example is a lymphoma diagnosed as a poorly differentiated " histiocytic " lymphoma. It was composed of spindle-shaped cells exhibiting marked mitotic activity and associated with much reticulin formation. The fine structure of this lymphoma showed the features of the true reticular or fibre forming cells (Fig. 11), with innumerable transitions between fibroblasts and reticular cells, even to the extent of desmosomal attachments. The fourth and final example of identification of the component cell type is a tumour originally designated a malignant lymphoma, mixed lymphocytic and " histiocytic ". The reason for this was partially based upon the esterase-positive cells. However, at ultrastructural level the esterase positive cells proved to be simple reactive macrophages and the neoplastic elements of the tumour were large " undifferentiated " lymphoid cells (Fig. 12).

In addition to identifying the cell type of a lymphoma, electron microscopy can indicate the degree of " differentiation " of the tumour cells. The splenic tumour shown here (Fig. 13) was interpreted correctly as one derived from primitive lymphoid cells. By ultrastructural study, however, it was possible to show that the lymphoid cells belonged to the plasma cell series. The nuclear configuration was that of immature plasma cells and there was formation of moderate amounts of rough endoplasmic reticulum, the cisternae of which contained moderately electron dense material. The identification of plasmacytoid features is of more than academic importance since, as in this present example, these tumours may be associated with abnormal immunoglobulin production.

Electron microscopy may prove of value in the lymphomata in the visualization of certain specialized structures and organelles, and cell-to-cell contacts which may prove useful as markers. One such marker, the desmosome, has already been mentioned but it is worth re-emphasizing that it is common to at least 3 cell types, namely dendritic cells, endothelium and the fibre forming reticular cells. Other markers are abnormalities of the Golgi apparatus and abnormalities of the rough endoplasmic reticulum present in those tumours showing plasmacytoid features. These abnormalities include tubular profiles derived from hyperplastic rough endoplasmic reticulum and certain curious arrangements of rough endoplasmic reticulum (Fig. 14a, b), as in the cells from patients with macroglobulinaemia (Henry, 1973, unpublished), and which, unlike the nuclear PAS positive nuclear inclusions or

Dutcher bodies (Fig. 15) (Dutcher and Fahey, 1959), are not visible at light microscopic level.

Is there a place then for electron microscopy in the diagnosis of lymphomata? Table III relates the initial diagnosis made on the paraffin embedded sections of diffuse tumours with that subsequently made on ultrastructural investigation, and it is apparent that in a number of cases the diagnosis has been altered or at least modified. However, in all fairness, a diagnosis based on the light microscopic features of the Epon embedded material does not differ very greatly from that derived from the ultrastructural study. The reasons for this are better fixation during electron microscopic processing, less crush artefact of the cells and thinner sections cut on the ultramicrotome enabling clearer visualization of cells.

TABLE III.—*Diffuse Non-Hodgkin's Lymphomata*

Traditional classification	Classification of Bennett, Farrer-Brown and Henry (1973, 1974)	LM	EM
Lymphocytic lymphosarcoma	Lymphocytic well differentiated (small round lymphocyte)	2	1+1*
	Lymphocytic intermediate type (small follicle cell)	—	—
Lymphoblastic lymphosarcoma	Lymphocytic poorly differentiated	2	1
Reticulum cell sarcoma	Mixed small lymphoid and undifferentiated large cell	2	1+1
	Undifferentiated large cell	6	2+2*
	Histiocytic (true)		2+1†

* Tumours showing differentiation towards plasma cells.
† Ultrastructural appearances suggesting an origin from fibre-associated reticular cells.

Why then simply not rely on light microscopic examination of Epon embedded material? One very good reason is that while Epon embedded material provides a reasonably accurate diagnosis, it does not provide sufficient insight into the fine structural detail of the tumour cells or give more than a rough indication of the line of differentiation of a particular tumour.

Finally, we are exploring techniques utilizing conjugation of immunoproteins with the enzyme peroxidase which enable reaction products such as antigens and antibodies to be visualized at subcellular level (Avromeis, 1969).

In conclusion, ultrastructure has a role to play in the investigation of the lymphomata, paying due regard to its limitations and emphasizing that any investigator must be thoroughly conversant with the range of appearances of the normal component cells of the tissue investigated before embarking on pathological aspects.

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