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The fine structure of microfibrils and microtubules in macrophages and other lymphoreticular cells in relation to cytoplasmic movement

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INTRODUCTION

Most cells in the mammalian body do not show much active translatory movement. Conspicuous exceptions are the phagocytes, whose functions depend on cytoplasmic movements of one kind or another. The casual observation of an apparently elaborate system of microtubules in mouse lymph node macrophages led to a survey of a collection of micrographs of lymphoreticular tissues in search of evidence which might relate the process of cytoplasmic movement to some anatomical components of the cell.

MATERIAL AND METHODS

The material studied was as follows:

(*a*) Popliteal lymph nodes from C3H mice. Some of this material came from normal (control) mice, some from mice which had received 50 μg ferritin into the footpad 1–8 hours before killing, and some from mice whose lymph nodes had been ncubated *in vitro* in Hank's solution with or without ATP 1 mg/ml for one hour.

(*b*) Lymph nodes, spleen, liver and thymus from out-bred Swiss white mice.

(*c*) Human tissues obtained at surgical biopsy from patients; some of these tissues were histologically normal and others showed malignant lymphoma.

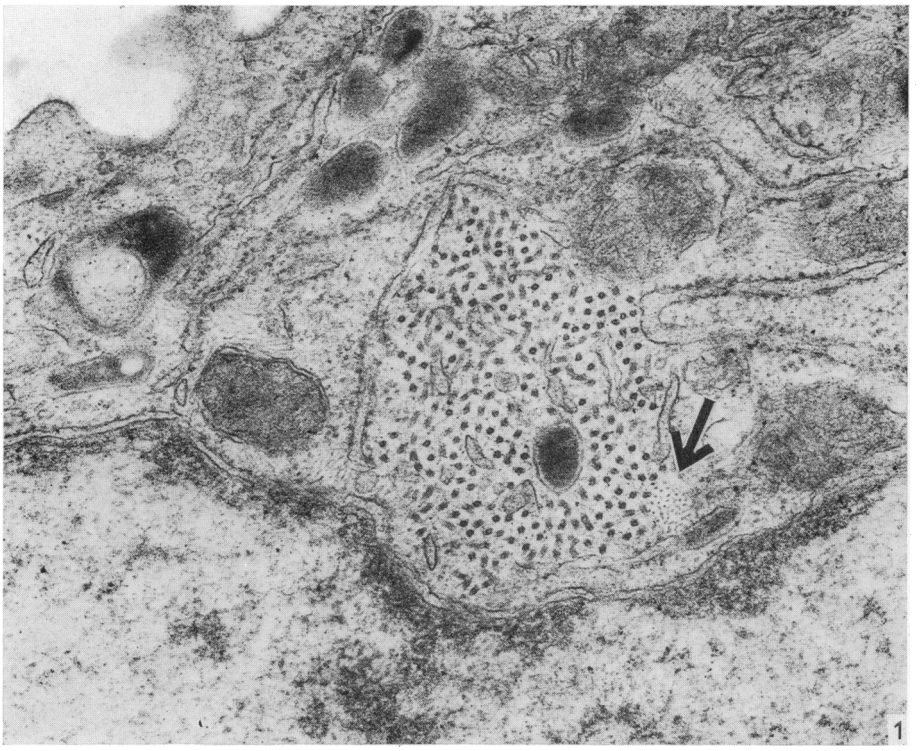
Material was fixed in 3% phosphate-buffered glutaraldehyde, washed in sucrose buffer and post-fixed in 2% osmium tetroxide. Blocks were stained with uranyl nitrate and embedded in Araldite. Sections cut on a Reichert ultramicrotome were stained with lead nitrate and examined in AEI 6B and 801 microscopes.

RESULTS

Popliteal lymph nodes

The experimental procedure described did not have any systematic effect on ultrastructure. The following account is based on normal and treated nodes regarded as one group.

Occasional macrophages at various sites within the node contained aggregates of microtubules, which usually lay near the centre of the cell and measured overall some 4×1 μm. The component microtubules measured 20–25 nm in diameter and had an electron-dense periphery and a less dense core. Individual tubules appeared to



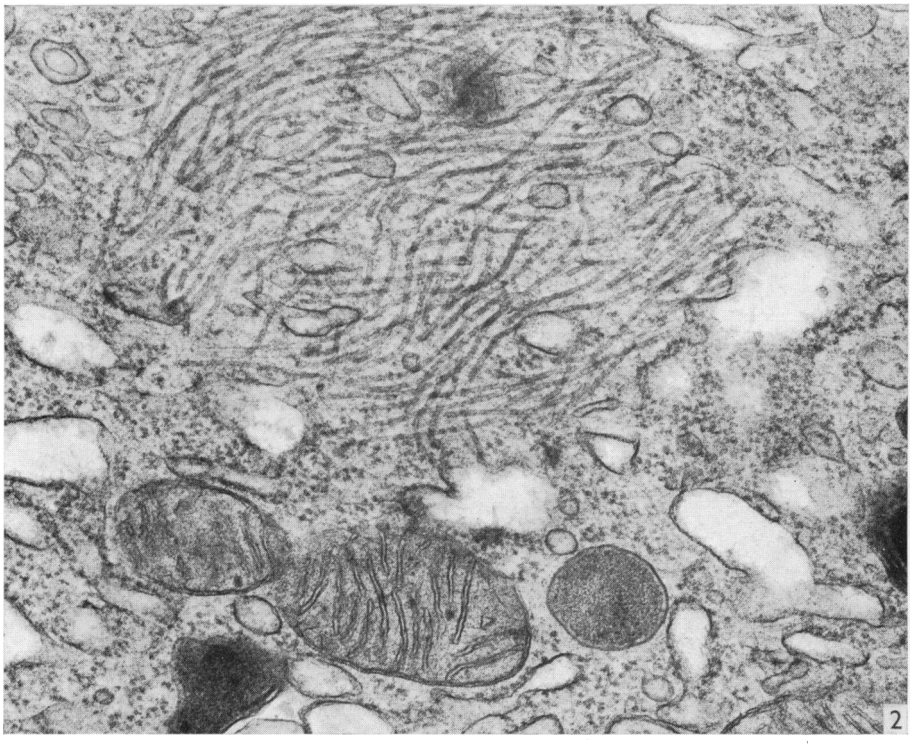


Fig. 1. ×36000. Lymph node macrophage (mouse) showing microtubular complex cut in cross-section: microfibrils are present in a group at the edge of the complex (arrow).

Fig. 2. ×26000. As above. Microtubular complex cut along its longitudinal axis showing branching and intersecting tubules. A few microfibrils are present.

branch and intersect, being more dense at the point of junction. The aggregates are illustrated in cross-section in Fig. 1 and longitudinal section in Fig. 2.

Related to these microtubular aggregates were fine microfibrils some 4–7 nm in diameter (mean 6 nm), devoid of obvious periodic banding. These were clearly seen at the immediate periphery of the aggregate but were also evident within it. Microfibrils and microtubules were sometimes in very close relationship but there was no unambiguous evidence of actual contact (Figs. 1, 2).

Within the cytoplasm microfibrils were found isolated or in groups of up to 50 in a single section. Fibrils appeared to branch, and commonly ran in groups with unidirectional orientation, notably in elongated macrophages. The areas of cytoplasm occupied by microfibrils contained no other organelles (Figs. 3, 4).

The organelle-free areas of ectoplasm at the edge of the cell occasionally contained a few fibrils. These became more prominent in incubated cells; probably in such cells cytoplasmic sap was washed out. Groups of fibrils in places ran tangential to or at right angles to the cell surface and approached it very closely (Fig. 5), though unambiguous evidence of attachment to the cell membrane was not obtained. Microfibrils similarly ran very close to the surface membrane of mitochondria (Fig. 6).

A prominent specialization of lymph node macrophages was the presence of cytoplasmic flaps within the subcapsular sinus, derived either from macrophages lining the inner surface of the sinus or from macrophages which lay in the pulp of the lymph node just deep to the subcapsular sinus. These flaps measured some 0·5–2 μm in greater diameter and contained few organelles. Their major component was a meshwork of ill-defined fibrils within the same size range as the other fibrils, but at the bottom end of the range (4 nm) these fibrils were rarely closely related to the cell membrane.

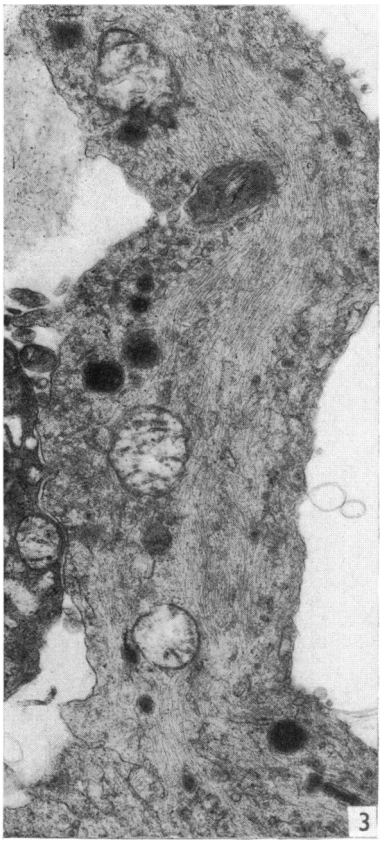
Other lymphoreticular tissues

In the other mouse lymphoreticular tissues examined, microfibrils occurred with regularity, though rarely in the prominent aggregates found in lymph node macrophages. In the finger-like cytoplasmic processes of peritoneal macrophages isolated fibrils were occasionally identified, notably in stimulated cells.

Human tissues

In the phagocytic endothelial cells of the sinusoids of spleen numerous microfibrils were seen, again about 6 nm in diameter, running in places into electron-dense bodies apparently composed largely of similar fibrils. These bodies did not have a microtubular component and resembled the dark bodies of smooth muscle. These structures appeared prominent in segments of sinus wall across which white and red cells were obviously passing, where presumably active contraction of endothelial cells was occurring (Fig. 7).

Microfibrils occurred with regularity in human lymph nodes at different sites. The best examples of fibrils within cytoplasmic processes were seen, however, in the numerous cytoplasmic processes projecting from the surface of neoplastic cells in a lymph node from a patient with Hodgkin's disease. Evidence will be presented elsewhere that such cells are of macrophage lineage. The processes, which were some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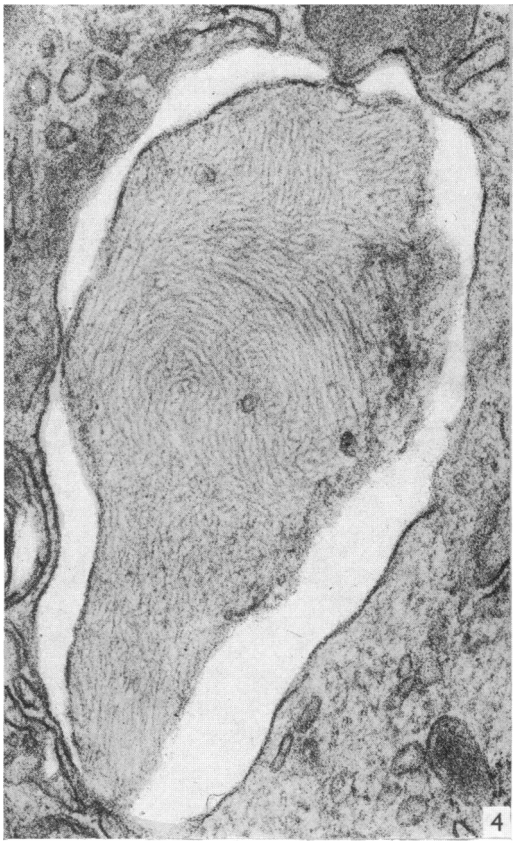
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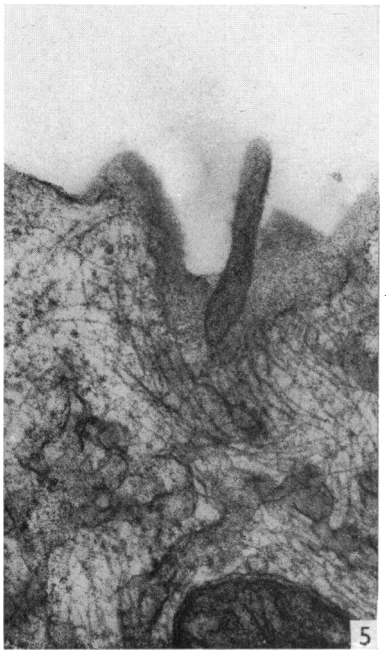
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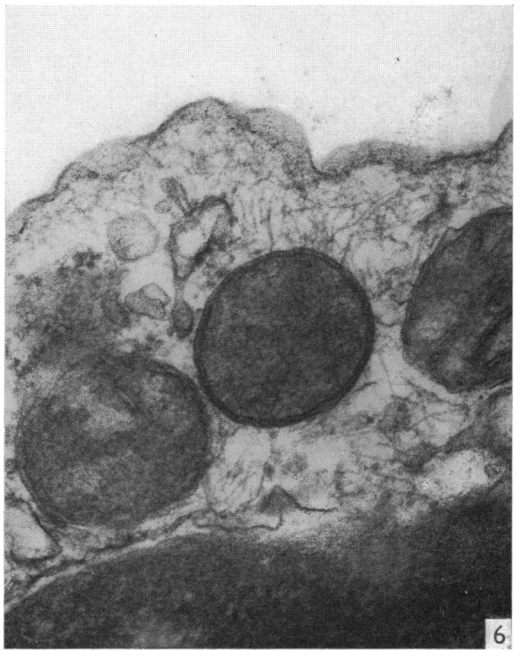
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0·2–0·8 μm long as seen in sections, contained 1–10 microfibrils, which often branched (Figs. 8, 9).

DISCUSSION

These micrographs illustrate an unusual microtubular complex and establish the ubiquity of microfibrils and microtubules in reticuloendothelial cells. The presence of the former in guinea-pig monocytes was clearly established by de Petris, Karlsbad & Pernis (1962) and the presence of microtubules in macrophages by Carr (1968). Allison, Davies & de Petris (1971) have recently suggested a contractile role for microfibrils and a skeletal role for microtubules in concanavalin-treated cells.

Two postulates are appropriate in considering the role of microfibrils in macrophages.

(1) That the contractile mechanism involves elongated structures similar to those seen in other motile cells.

(2) That the same elongated structure is likely to be involved in all the types of cytoplasmic movement which occur in macrophages; that is, in translational movement, in movement of a mass of cytoplasm without translational movement, in the movement of cytoplasmic processes, and in organelle movement.

There is now little reasonable doubt that a 6 nm microfibril, probably composed of actin, is the contractile element in simple organisms such as slime moulds (Wolpert, Thompson & O'Neil, 1964) and in smooth muscle (Needham & Schoenberg, 1964). In both rat and human cells in tissue culture cytoplasmic movement on phase contrast microscopy has been associated with the presence of 6 nm microfibrils in appropriate areas of cytoplasm, usually ectoplasm (Buckley & Porter, 1967; Franks, Riddle & Seal, 1969). Similar fibrils have been associated with movement in cultured macrophages (Allison *et al.* 1971).

In contrast with the available evidence relating to fibrils in cells of various kinds in tissue culture, there is little information on their role in tissue macrophages *in vivo*.

The structure of the endothelial cells of splenic sinuses has been reviewed elsewhere (Carr, 1970). The aggregates of microfibrils described in the present report in splenic endothelial cells are presumably the same as the bodies staining with uranyl nitrate described by Weiss (1957).

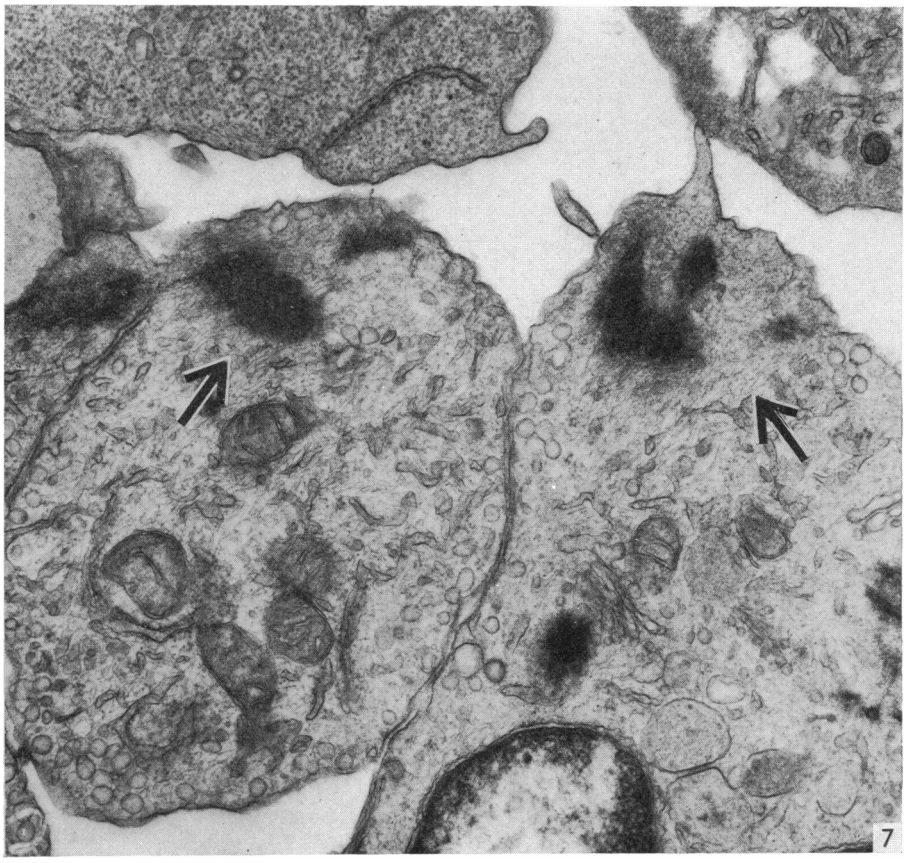
The 6 nm microfibrils presently described are in a position which suggests that they may be responsible for most forms of movement in macrophages, though not quite enough are seen around pinocytic vesicles to account for their movement. Micropinocytic vesicles do not appear to have 6 nm microfibrils related to them; possibly because their movements do not appear to be energy-dependent.

Fig. 3. × 16000. Lymph node macrophage (mouse). An elongated process of macrophage cytoplasm showing bundles of microfibrils running in its long axis.

Fig. 4. × 64000. As above. Similar process cut where it invaginates another cell, showing whorled microfibrils in places closely related to cell membrane.

Fig. 5. × 64000. As above. Microfibrils running perpendicular to cell surface.

Fig. 6. × 64000. As above. Microfibrils related to the surface of a mitochondrion.



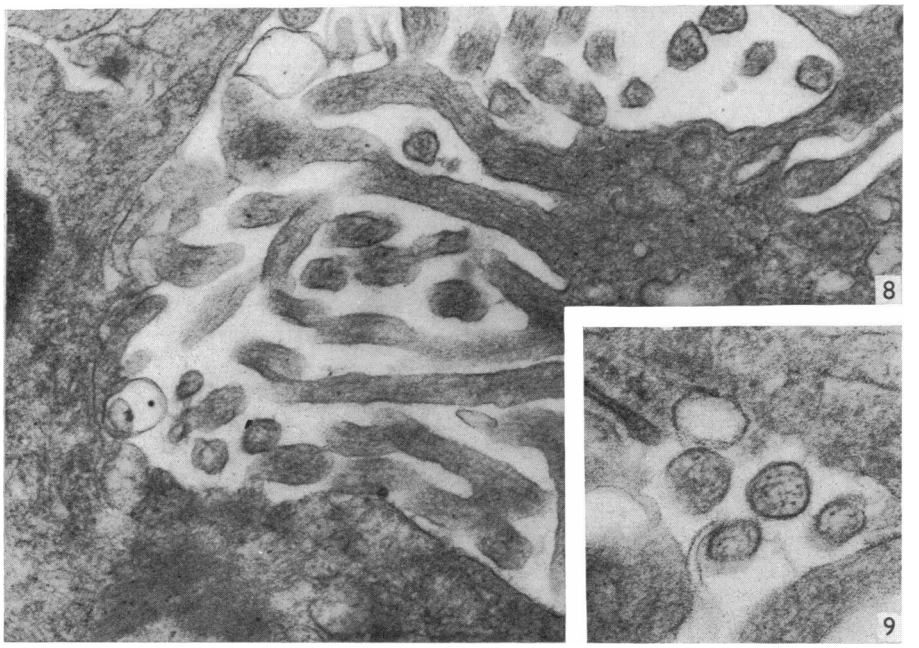


Fig. 7. ×45000. Human spleen. Endothelial cells of sinusoid showing numerous microfibrils in places running into dark bodies (arrows).

Fig. 8. ×45000. Fig. 9. ×86000. Human lymph node (Hodgkin's disease). Cell processes of abnormal cells of macrophage lineage showing microfibrils in the core of the processes cut in longitudinal and transverse section.

The function of microtubules, which are, of course, common in cells, is less clear. The present micrographs illustrate an unusual if not unique complex of these structures. If it be assumed that the lymph node macrophages studied are static but have moving processes, then a firm cytoskeleton would be required. The microtubular complex is in the right position for this. On this hypothesis such freely moving cells as peritoneal cells would not need a developed microtubular apparatus.

SUMMARY

1. The complex microtubular and microfibrillar apparatus of lymph node macrophages is described.

2. The universal occurrence of 6 nm microfibrils in reticuloendothelial cells is illustrated.

3. The appearances fit the view that microfibrils are contractile and microtubules skeletal in function.

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