MICROTUBULES IN HAMSTER PLATELETS

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INTRODUCTION

The increasing frequency with which microtubules are observed in electron micrographs of many cell types has led to much speculation about the possible structural and functional significance of these cytoplasmic elements. Two basic interpretations have been made: that they serve as cytoskeletal elements has been suggested by the work of many investigators (3, 4, 5, 9, 10, 12, 16–18); that they perhaps function in the intracellular transport of various substances has also been suggested (3, 9, 10, 16–18).

Incidental to recent light and electron microscope studies of hamster tissue, we have observed characteristic arrays of parallel microtubules in the ultrastructure of individual and clumped platelets. These microtubules are of the same basic dimensions as those previously reported in the literature.

MATERIALS AND METHODS

The cheek-pouch of the golden hamster, *Cricetus auratus*, is everted and spread out on a specially designed light microscope stage for *in vivo* studies as previously described (7).

Selected hamster tissues are fixed in phosphate (15) or collidine (2), buffered 4 per cent glutaraldehyde for 2 hours at room temperature, and postfixed in 1 or 2 per cent buffered osmium tetroxide for 2 hours. The tissues are dehydrated in graded alcohols or acetone at room temperature and embedded in Epon 812 (11).

Thin sections are cut with a Porter-Blum MT-2 ultramicrotome, mounted on grids coated with Formvar and carbon and stained with lead hydroxide (13), uranyl acetate, or lead citrate (14). Samples of platelets are collected from fresh whole blood and studied as previously described (8). The preparations are studied in a Philips EM 200 electron microscope and photographed at a variety of magnifications.

OBSERVATIONS

In the living hamster cheek-pouch studied by optimal light microscopy, individual platelets in slow-flowing plasma within small vessels appear as ellipsoids approximately 1 micron thick and 4 microns in diameter. On occasion, platelets may be seen to clump and subsequently to disperse without significant alteration of their shape. Neither platelet processes nor stages of viscous metamorphosis have been observed within the vessels in the living cheek-pouch preparations.

By electron microscopy of thin sections, hamster platelets are seen to have a structure similar to that seen by light microscopy. The aggregation of small circular structures at the poles of the platelets shown in Fig. 1 represents a cross-sectional cut through an array of parallel microtubules. Along the margins of tangentially cut platelets, these structures are clearly tubular and have a diameter of approximately 250 A. We have not found a section of a platelet completely in the plane of the tubules; however, the arrangement of the tubules in tangential sections suggests that they extend around the entire rim of the platelet. Between 5 and 10 microtubules are found in each array. The parallel arrays of microtubules have been observed in all platelets found in the hamster tissues studied.

Microtubules organized in membrane-bounded bodies within endothelial cells as described by Weibel and Palade (19) have also been found.

When isolated from fresh whole blood, the hamster platelets have an appearance similar to that of human platelets previously described (8). Stages of viscous metamorphosis associated with fibrin formation are seen. The whole platelets, however, are too thick to give adequate resolution of cellular organelles in the electron microscope. It is not possible to identify any tubular structures in such preparations.

DISCUSSION

Microtubules with a bimodal diameter distribution of either 270 A or 120 to 200 A have frequently been reported in the literature as recently reviewed by Slautterback (18). Fawcett and Witebsky have recently reported that microtubules in erythrocytes and thrombocytes of fish, amphibians, reptiles, and birds possess elastic properties and thus provide the structural basis of their shape. The endoplasmic ring which they find in nucleated thrombocytes, however, is different from the microtubules we have found in

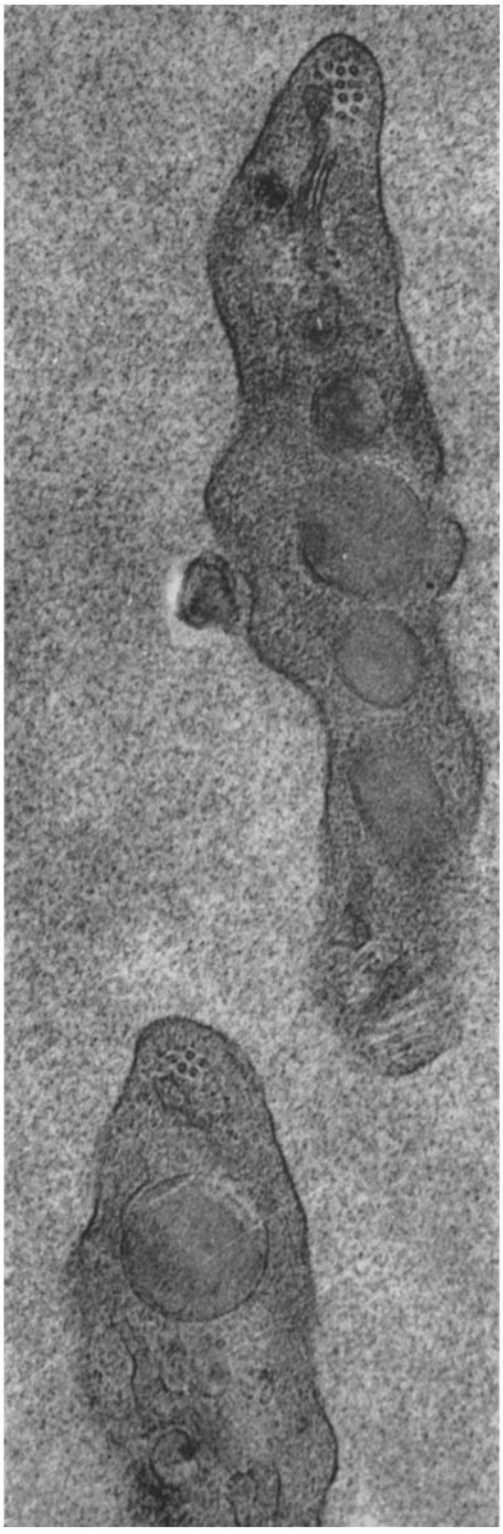

FIGURE 1 Two hamster platelets in a liver sinus are shown. The microtubules appear in cross-section at one pole and in slightly tangential section at the other. × 51,000.

hamster platelets. The latter more nearly resemble the marginal bands they describe in nucleated erythrocytes.

The microtubules recently reported by Sandborn *et al.* (16) and by Behnke (1) represent an arrangement very different from that of microtubules previously reported. Instead of occupying a specific intracellular location and being arranged in an orderly manner, they are of ubiquitous occurrence and are arranged individually or in complex networks throughout the cell. Behnke reports these microtubules in rat eosinophils and lymphocytes but not in platelets.

Similar in arrangement and distribution to the microtubules in the hamster platelets, the microtubules reported by Ledbetter and Porter (9, 10) occur in "parallel arrays" immediately under the plasma membrane and are separated by a space which is devoid of major particulate and membranous elements of the cytoplasm.

The small cylindrical tubules recently reported by Weibel and Palade (19) in the cytoplasm of endothelial cells are also quite different from those of previous reports. The individual tubules are of similar basic dimensions, but their situation is unique in that bunches of tubules are grouped together within a membrane-bounded body.

It is of interest that only recently have so many cytoplasmic microtubules been demonstrated in electron micrographs and that they are now being found in so many different cell types. It is suggested by Sandborn *et al.* that this is due primarily to improved methods of fixation and, in their experiments, specifically to an increased rate of penetration of fixative due to the combined action of acrolein and glutaraldehyde. In the past, however, many studies have been carried out in which either very small pieces of tissue or small organisms have been rapidly fixed by many methods including aldehyde fixation. Sections taken from the outermost region of such tissue blocks and examined in the electron microscope have often not revealed the presence of a network of microtubules.

In our hamster cheek-pouch tissue, penetration of any fixative is much slower than that in most other tissues, due to the fact that the cheek-pouch is composed almost solely of connective tissue; yet microtubules are seen in platelets in deeply buried capillaries and in large platelet clumps in some of the larger vessels. These observations suggest that the increased rate of penetration of the fixing solution is not solely, if even at all, responsible for the appearance of microtubules.

It is becoming increasingly evident that fixation in glutaraldehyde followed by postfixation in osmium tetroxide preserves cytoplasmic structures better than fixation in osmium tetroxide alone (1, 5, 9, 15, 16, 19). Why this should be so is not understood. Behnke points out that at the present time most reports concern the presence of microtubules in lower animals where they are preserved by fixation in OsO_4 alone.

French *et al.* (6), in a recent study of artificially induced platelet thrombi in hamster cheek-pouch tissue, have published several electron micrographs of hamster platelets in which no microtubules are discernible. Their use of Veronal-acetate–buffered 2 per cent osmium tetroxide fixation alone for their preparations might preclude the demonstration of these microtubules.

It seems reasonable that improvements not only in fixation but also in embedding, staining, and other techniques which contribute to the production of better electron micrographs are responsible, at least in part, for the increased observation of microtubules.

The most obvious function that such microtubules might serve is structural. Meves (12), in 1911, and more recently Fawcett and Witebsky (5) have suggested that the marginal bands of nucleated erythrocytes are probably important in maintaining the characteristic ellipsoid shape of these cells.

In our findings, the hamster platelets circulating *in vivo* have a characteristic shape which is consistent with various planes of sectioned platelets seen in the electron microscope. The marginal location and the long, unconnected nature of these tubules strongly suggest that they function as a cytoskeleton. Cytoskeletons have been postulated for many years by embryologists and anatomists, but no cytoplasmic inclusion has yet been assigned such a function.

The work of Ledbetter and Porter (9) and of Cronshaw and Bouck (3) suggests that microtubules in plants may be involved in the organization and orientation of cellulose in the cell wall.

Slautterback (18) suggests that, because of the proteolipid nature of the outer surface of the microtubules, they should, like other complex phospholipid-protein membranes, be able to concentrate ions on their surface, and therefore they might function in intracellular transport.

Weibel and Palade (19) are not able to suggest a particular function for their membrane-bounded group of microtubules other than a possible connection with vascular or blood physiology due to their location.

Sandborn *et al.* suggest that microtubules serve to transport fluids and suspended solids. They suggest that the complex network forms a continuous membrane system of connecting channels between the plasma membrane and cytoplasmic organelles, thus forming an essentially extracellular microcirculatory system. It is, however, not yet clear exactly what substances are being transported to what intracellular location and for what purpose.

That the suggested functions of transport of materials and the maintenance of structure are not mutually exclusive raises the possibility that the microtubules perform both functions simultaneously. A network of a parallel array of microtubules could serve as a cytoskeleton and at the same time connect various regions of the cytoplasm with each other or with the extracellular environment.

In the present study, we are not able to find any evidence to suggest that the microtubules described are involved in intracellular transport. The appearance of the hamster platelet microtubules is most consistent with the interpretation that they provide a structural basis for the characteristic shape of these cells as seen *in vivo*.

This investigation was supported in part by Public Health Service Research Grant HE06861 from the National Heart Institute.

Received for publication, January 19, 1965

REFERENCES

1. BEHNKE, O., A preliminary report on "microtubules" in undifferentiated and differentiated vertebrate cells, *J. Ultrastruct. Research*, 1964, **11**, 139.
2. BENNETT, H. S., and LUFT, J. H., *s*-Collidine as a basis for buffering fixatives, *J. Biophysic. and Biochem. Cytol.*, 1959, **6**, 113.
3. CRONSHAW, J., and BOUCK, G. B., The fine structure of differentiating xylem elements, *J. Cell Biol.*, 1965, **24**, 415.
4. FAWCETT, D. W., Physiologically significant specializations of the cell surface, in symposium on the plasma membrane, *Circulation*, 1962, **26**, 1105.
5. FAWCETT, D. W., and WITEBSKY, F., Observations on the ultrastructure of nucleated erythrocytes and thrombocytes, with particular reference to the structural basis of their discoidal shape, *Z. Zellforsch. u. Mikr. Anat.*, 1964, **62**, 785.
6. FRENCH, J. E., MACFARLANE, R. G., and SANDERS A. G., Structure of haemostatic plugs and experimental thrombi in small arteries, *Brit. J. Exp. Path*, 1964, **45**, 467.
7. HAYDON, G. B., Untersuchungen zur Physiologie und Pathologie des peripheren Kreislaufs, *Beitr. path. Anat. u. allg. Path.*, 1962, **126**, 127.
8. HAYDON, G. B., and COREY, D. L., Platelets in the thromboplastin generation test, *Arch. Path.*, 1961, **71**, 615.
9. LEDBETTER, M. C., and PORTER, K. R., A "microtubule" in plant cell fine structure, *J. Cell Biol.*, 1963, **19**, 239.
10. LEDBETTER, M. C., and PORTER, K. R., Morphology of microtubules of plant cells, *Science*, 1964, **144**, 872.
11. LUFT, J. H., Improvements in epoxy resin embedding methods, *J. Biophysic. and Biochem. Cytol.*, 1961, **9**, 409.
12. MEVES, F., Gesammelte Studien an der roten Blutkorperchen der Amphibien, *Arch. mikr. Anat.*, 1911, **77**, 465.
13. MILLONIG, G., A modified procedure for lead staining of thin sections, *J. Biophysic. and Biochem. Cytol.*, 1961, **11**, 736.
14. REYNOLDS, E. S., The use of lead citrate at high pH as an electron-opaque stain in electron microscopy, *J. Cell Biol.*, 1963, **17**, 208.
15. SABATINI, D. D., BENSCH, K., and BARRNETT, R. J., Cytochemistry and electron microscopy. The preservation of cellular ultrastructure and enzyme activity by aldehyde fixation, *J. Cell Biol.*, 1963, **17**, 19.
16. SANDBORN, E., KOEN, P. F., MCNABB, J. D., and MOORE, G., Cytoplasmic microtubules in mammalian cells, *J. Ulstruct. Research*, 1964, **11**, 123.
17. SATIR, P., and STUART, A. M., A new apical microtubule-associated organelle in the sternal gland of *Zootermopsis nevadensis* (Hagen), Isoptera, *J. Cell Biol.*, 1965, **24**, 277.
18. SLAUTTERBACK, D. B., Cytoplasmic microtubules, I. Hydra, *J. Cell Biol.*, 1963, **18**, 367.
19. WEIBEL, E. R., and PALADE, G. E., New cytoplasmic components in arterial endothelia, *J. Cell Biol.*, 1964, **23**, 101.