Brief Report

Cytoplasmic Microtubules in Blood Platelets

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THE BLOOD platelets or thrombocytes, in animals perfused with an
aldehyde solution,1 are often preserved in the sinusoids of the liver. These
rapidly fixed platelets exhibit a regular arrangement of cytoplasmic micro-
tubules when examined with the electron microscope. Previous investiga-
tions of the ultrastructure of this cytoplasmic unit have not included the micro-
tubule as one of its inclusions.

METHODS

Young adult rats were perfused with a solution containing 6.25 per cent glutaraldehyde
and 2 per cent acrolein in a phosphate buffer following replacement of the circulating
blood by a balanced salt solution.2 Blocks of liver tissue were given 30 minutes of addi-
tional fixation in the aldehyde solution before being washed in buffer, and were postfixed
in buffered 2 per cent osmium tetroxide solution. The blocks were dehydrated in graded
ethanol and embedded in epon as described by Luft.3 Sections cut on a Porter Blum
microtome were triple stained with lead hydroxide,4 0.5 per cent aqueous uranyl acetate,5
and lead hydroxide.4

OBSERVATIONS

The blood returning from the abdominal viscera was not removed entirely
from the liver sinusoids by the perfusion. However, the liver became fixed
rapidly in most instances. Blood platelets, well preserved and apparently
undamaged, were found frequently in the sinusoids. Seven to eight small
uniform circles at each end of the elongated platelets were observed (figs.
1 and 2). Each of the circles had a dense periphery and a light central zone.
They appeared as elongated structures with parallel, dark staining walls and
a light central zone in other planes of sections (figs. 1, 3 and 4). Many were
cut obliquely. At high power in the surface view (fig. 4), longitudinally
oriented filaments were seen between the outer walls of this structure. These
filaments had dense outer layers and a lighter central zone giving them a
three-layered appearance. When the outer membrane of the platelet was sec-
tioned transversely, regularly spaced particles could be seen protruding from
the surface, giving it a fuzzy appearance (figs. 1, 2 and 3). These particles

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Fig. 1.—Two of the platelets in a liver sinusoid. Note the presence of small circular structures at their “poles” (arrows). One platelet has a greater width than the others due to the oblique direction of the section. In this platelet, the structures at the poles are seen to be elongated. These are typical cytoplasmic microtubules as seen in many plant and animal cells. (× 28,000)

at higher power (fig. 4) had a stem and a head which was larger than the stem. A light central zone was seen in both the stem and the head. The measurement of the stem was 50 Å and the head was 80 Å with considerable variations.

Discussion

It is difficult to determine the normal ultrastructure of the blood platelet in the circulating blood because of the rapid changes occurring in this cytoplasmic unit when subjected to abnormal influences. Fixation by perfusion of the animal could be expected to arrest the changes more rapidly than other methods, provided that the fixative does not in itself create distortion of the ultrastructure. The improved retention of cellular content by the use of aldehydes preceding osmium tetroxide fixation was illustrated by Sabatini, Bensch and Barnett. By combining the advantages of two of these aldehydes, we found that better preservation of cellular fine structure could be obtained than when we employed one alone.

The organelles preserved in these platelets are elongated tubular structures and appear similar to the cytoplasmic microtubules described in Hydra by
Fig. 2.—A higher magnification of a part of another platelet. Transverse section of the microtubules (arrow), with their dense wall and a clear central region. Some seem to have filamentous interconnections between adjacent microtubules. At the opposite border of the platelet these inclusions are seen as parallel walls separated by a clear central region (arrow). The diameters of these microtubules are from 190–220 Å. (x 98,000)

Fig. 3.—Cytoplasmic microtubules in longitudinal section. They are seen near the border of the platelet sectioned in an opportune plane. At the opposite border evidence of breakdown of the platelet is beginning to appear. (x 65,000)
Fig. 4.—A higher magnification of the border of the platelet. Note particles projecting from the surface membrane (arrow). The particles have a stem and head, each with a dark periphery and a lighter central zone. In the microtubules, longitudinally oriented lines are seen when the surface view is obtained and three layers appear in the wall occasionally (arrows). (× 175,000)

Slatterback.7 The microtubules in the platelets were of the same order as those in the cytoplasm of many cell types, both plant and animal.1,7,8 They appeared to be structurally indistinguishable from those found in the spindle of dividing cells8,9 and in association with the centriole in interphase cells as well.1,10,11 Although microtubules have not been reported in the platelet, there is some evidence of their presence in human thrombocytes in figure 3 of the report by Firkin, O’Neill, Dunstan and Oldfield.12

The presence of these structures within the platelet suggests that they are possibly an inclusion to be found in all cytoplasm. The critical factor in their visualization appears to be the utilization of methods of tissue preservation which prevent the disruption of the continuity of the microtubules. The disruption must be accompanied by a change to a form not recognizable as the same organelle. This could be the breakdown to the basic filaments of which they appear to be formed11 or an enlargement to a vesicular form. The function of the microtubule can only be surmised. Indirect evidence from isolated metaphase spindle cells in mitosis13 suggests that the similar structures therein contain contractile proteins. If an increase in diameter of the microtubule should accompany contraction in its length, then additional functions could be anticipated. This possibility has become more apparent since we have found in the neurone, the liver and other cells that the microtubular wall has a three-layered structure in longitudinal section and is globular in transverse section.1,11 The globular substructure in transverse sections was also illustrated in plant cells by Ledbetter and Porter.14 This is in keeping with the
findings of Sjöstrand in the membranes of other cytoplasmic organelles. Whether the granules on the surface membrane of the platelet bear any relationship to the mitochondrial elementary particles of Fernandez-Moran is unknown. Surface particles may be the result of an instability of the structure of some membranes. Sjöstrand has attributed the appearance of the surface particles on mitochondrial membranes to the breakdown of their structure. The appearance of particles on the surface of membranes of the platelet might similarly indicate some degree of disruption of its structure.

Summary

Regularly arranged cytoplasmic microtubules are present in blood platelet fixed in liver sinusoids during aldehyde perfusion of the animal. This finding in addition to other recent reports on the cytoplasmic microtubule suggests that these organelles might be found in all cytoplasm if the ultrastructure is preserved adequately.

Note: Subsequent to the submission of this article a description of filaments and microtubules in spread platelets by Bessis and Breton-Gorius appeared. The greater number of filaments in their illustrations suggests a breakdown of some of the microtubules to the smaller filaments. These authors speculate on the possibility of a contractile function for microtubules.

Summario in Interlingua

Microtubulos cytoplasmatic de disposition regular es presente in plachettas sanguinee fixate in sinusoides hepatic durante le perfusion del animal con aldehydo. Iste constatation, addite a altere recente reportos relative al microtubulo cytoplasmatic, suggere que iste organellas se trova possibilemente in omne cytoplasma si su ultrastructura es preservate adequatemente.

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REFERENCES

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