Br. J. exp. Path. (1970) **51**, 114.

EFFECT OF ENDOTOXIN ON THE ULTRASTRUCTURE OF LIVER AND BLOOD CELLS OF HAMSTERS

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Received for publication October 17, 1969

SUMMARY.—The liver has been identified as one of the main target organs in fatal endotoxic shock (Fine, 1967). The present study was undertaken to study the ultrastructural changes induced in the liver and formed elements of the blood contained in liver sinusoids 30 min. and 24 hr after intracardial injection of an LD_{50} dose of *Escherichia coli* endotoxin. No changes were detectable after 30 min., but after 24 hr: (1) the hepatic cells were extensively vesiculated; (2) the arrangement of the lining cells was grossly disturbed leaving wide gaps so that the lumen communicated freely with the space of Disse; and (3) the formed elements were all altered. Platelets were no more than " balloons " containing an abnormally light cytoplasm and a few vesicles. White cells had wide perinuclear zones, and red cells were aggregated. Such severe and extensive damage could be expected to result in liver disfunction, and likely inability of the cells to recover and regenerate. The damage could have resulted from direct action of endotoxin, from release of 5-HT, lysosomal enzymes and possibly other substances from platelets and other cells, or from ischaemia. Possibly all three were involved.

THE liver has been identified as one of the major target organs in endotoxic shock, and as a major site of detoxification of lipopolysaccharide from Gram negative bacteria. Death from endotoxic shock has been attributed to failure of liver function caused by ischaemia (Fine, 1967). The ultrastructural damage to liver has been investigated by McKay, Margaretten and Csavossy (1966) who found that in the rat, injection of small doses of endotoxin caused swelling of Kupffer cells which was visible at 1 hr and continued for the 4 hr of the study. The damage to the Kupffer cells was attributed to a secondary response produced by the fibrin and platelet masses.

The present study was undertaken to investigate ultrastructural damage produced in the hamster liver by large doses (LD_{50}) of endotoxin 30 min. and 24 hr after injection.

MATERIALS AND METHODS

Hamsters were anaesthetized with nembutal for all procedures. Endotoxaemia was produced by injection intracardially of 0·3 mg. of endotoxin (*Esch. coli* 0111: B_4, Difco) per 100 g. body weight. This was an LD_{50} dose of endotoxin for the particular lot (Masucci and Berman, 1965). Samples of tissue were taken from mid-portions of livers of different animals 30 min. and 24 hr after endotoxin injection. Control specimens were similarly taken from untreated hamsters. Specimens from control and endotoxin-injected animals were fixed in 3 per cent glutaraldehyde in Millonig's buffer at pH 7·4 for 1 hr, washed overnight in buffer and fixed for 25 min. in buffered 1 per cent osmium tetroxide or else fixed in 1 per cent osmium tetroxide in Millonig's buffer for 30–60 min. The specimens were dehydrated with ethyl

alcohol and embedded in Epon 812. Sections were stained with uranyl acetate followed by lead citrate.

RESULTS

Specimens of liver removed from control and 30 min. post-injection hamsters exhibited typical parenchymal and lining cells (Figs. 1–4). The formed elements were all typical. However, specimens removed from 24 hr post-injection hamsters exhibited severe alteration of parenchymal and lining cells, and all 3 types of formed elements (Figs. 5–7).

The liver sinusoids were lined with a sheet of endothelial and Kupffer cells which overlapped in some places but floated free in others. Gaps between the cells were common. The basement membrane was discontinuous. The cells contained the usual complement of internal organelles including nucleus, mitochondria, golgi, vesicles, and glycogen. Mitochondria and glycogen were especially prominent in the parenchymal cells.

The appearance of the liver had not changed appreciably 30 min. after endotoxin injection. However, 24 hr after injection, dramatic changes had occurred. The lining cells contained little recognizable subcellular structure, and had wide gaps between them. A large number of short, thick, pseudopod-like structures suggested that some lining cells were missing, putting the lumen in direct contact with the space of Disse. The cytoplasm of parenchymal cells was extremely vesiculated, the mitochondria distorted, and the glycogen depleted.

The formed elements of the blood also exhibited severe damage. The platelets were no more than " balloons " containing a few vesicles. The white cells exhibited greatly exaggerated perinuclear zones and loss of internal mitochondrial structure. The red cells were almost always in aggregates.

The plasma contained a large number of free vesicles of various sizes and shapes and occasional free mitochondria.

DISCUSSION

The devastating ultrastructural damage observed in this study could have been produced by hypoxia. However, it is also possible that 5-HT, a permeability factor, and hydrolytic enzymes released from platelets and leucocytes, contributed to the observed ultrastructural damage. These substances are released by the interaction of endotoxin with platelets (DesPrez and Bryant, 1966), and neutrophils (Thomas, 1965; Movat, Uriuhara and Macmorine, 1964; and DesPrez and Bryant, 1966). Platelets phagocytose a variety of particles, including antigen–antibody complexes, with the dissolution of granules and the release of ADP, 5-HT, thromboplastic material, and a permeability factor (for review see Mustard and Packham, 1968). Exposure of platelets to endotoxin *in vitro* causes similar reactions (DesPrez, Horowitz and Hook, 1961; DesPrez, 1964; and DesPrez and Bryant, 1966).

Separation of endothelial cells along their junctions is known to be caused by 5-HT, allowing escape of fluid and particulate matter (Majno and Palade, 1961). If a similar process occurs in sinusoidal linings, it could be expected that blood cells, especially polymorphonuclear leucocytes, migrate into the tissue. The specific granules of leucocytes appear to be synonymous with lysosomes and are strongly implicated in the necrotic response of tissues in local and generalized Shwartzman-like reactions (Thomas, 1965). Similarly, the specific granules of leucocytes which have migrated into the tissues around the sinusoids may dis-

integrate, releasing substances which cause damage to adjacent hepatic cells. There is also the possibility that the vascular endothelium may be capable of phagocytosing endotoxin under some conditions (Grant, 1965; Benacerraf, McCluskey and Patras, 1959; and Jennings, Marchesi and Florey, 1962), with the release of damaging substances from their storage sites.

The severe ultrastructural alteration exhibited by parenchymal and lining cells and formed elements would doubtless greatly reduce or destroy the functional capacity of these cells resulting in distress due to liver failure, thrombocytopenia, leucopenia, and anaemia. The products of cellular damage doubtless contribute to the commonly observed intravascular coagulation.

This research was supported by grant No. HE-09447 from the National Heart Institute.

REFERENCES

BENACERRAF, B , MCCLUSKEY, R. T. AND PATRAS, D.—(1959) *Am. J. Path.*, **35**, 75.
DESPREZ, R. M.—(1964) *J. exp. Med.*, **120**, 305.
DESPREZ, R. M. AND BRYANT, R. E.—(1966) *J. exp. Med.*, **124**, 971.
DESPREZ, R. M., HOROWITZ, H. I. AND HOOK, E. W.—(1961) *J. exp. Med.*, **114**, 857.
FINE, J.—(1967) *Gastroenterology*, **52**, 454.
GRANT, L.—(1965) *in* ' The Inflammatory Process '. New York (Academic Press).

EXPLANATION OF PLATES

FIGS 1–3.—Liver sinusoids from control hamsters. Fig. 1 shows a cross section of a sinusoid and surrounding parenchymal cells (P). The lumen (L) is lined with a single layer of endothelial (E) and Kupffer (K) cells which separate it from the space of Disse (SD). The lining cells are thin except in nuclear areas (N). Fig. 2 shows a higher magnification of a portion of a sinusoid in which extremely thin, adjacent endothelial cells interdigitate (arrow), fail to meet, leaving a gap (double arrows), and overlap (triple arrows). Some parenchymal cells stain darker (PD) than others (PL). Parenchymal cells are rich in mitochondria (M) and glycogen (G). Fig. 3 shows a section of a sinusoid containing a white cell (WC) in the lumen. The endothelial cells lining the lower side are extremely thin with a gap through which a pseudopod (P) projects. A hepatic cell (H) from another plane appears to be interposed between the endothelial lining and the space of Disse. The upper side is lined by a much thicker cell which contains a nucleus, ribosomes, vacuoles and glycogen. Caveolae are evident on the luminal and parenchymal sides (arrows). Mitochondria (M) and glycogen (G) are abundant in parenchymal cells.

Fig. 1, × 14,000; Fig. 2, × 12,000; Fig. 3, × 12,000.

FIG. 4.—Liver sinusoid from a hamster 30 min. after intracardial injection of an LD_{50} dose of endotoxin. The lining and parenchymal cells appear normal. The lumen contains an RBC which is distorted, but this is frequent in normal tissue also. The platelets vary from near normal (P_1 and P_2) to extensively altered (P_3). × 12,000.

FIGS. 5–7.—Liver sinusoids from a hamster 24 hr after intracardial injection of an LD_{50} dose of endotoxin. The 3 types of cells observed in these preparations all show extensive damage.

In Figs 5, 6 and 7 the cytoplasm of the parenchymal cells is vesiculated.

The lining cells contain little recognizable subcellular structure, and appear to have wide gaps between them (arrows, Figs 5, 6 and 7). The large number of short, thick, pseudopod-like structures along the right side of the lumen in Fig. 6 suggests that the lining cells may be missing, and the lumen in direct contact with the space of Disse. The plasma contains a large number of free vesicles of various sizes and shapes (Figs 6 and 7).

One clearly recognizable, free mitochondrion (M) may be seen in the lumen near the bottom of Fig. 6.

The formed elements of the blood also show damage. The platelets (P) are no more than " balloons " containing a few vesicles (Figs 5 and 6). The white cell (WC) in Fig. 6 shows a greatly exaggerated perinuclear zone (PZ) and loss of internal mitochondria (M). The RBC were almost always in aggregates.

Fig. 5, × 15,500; Fig. 6, × 16,000; Fig. 7, × 17,000.

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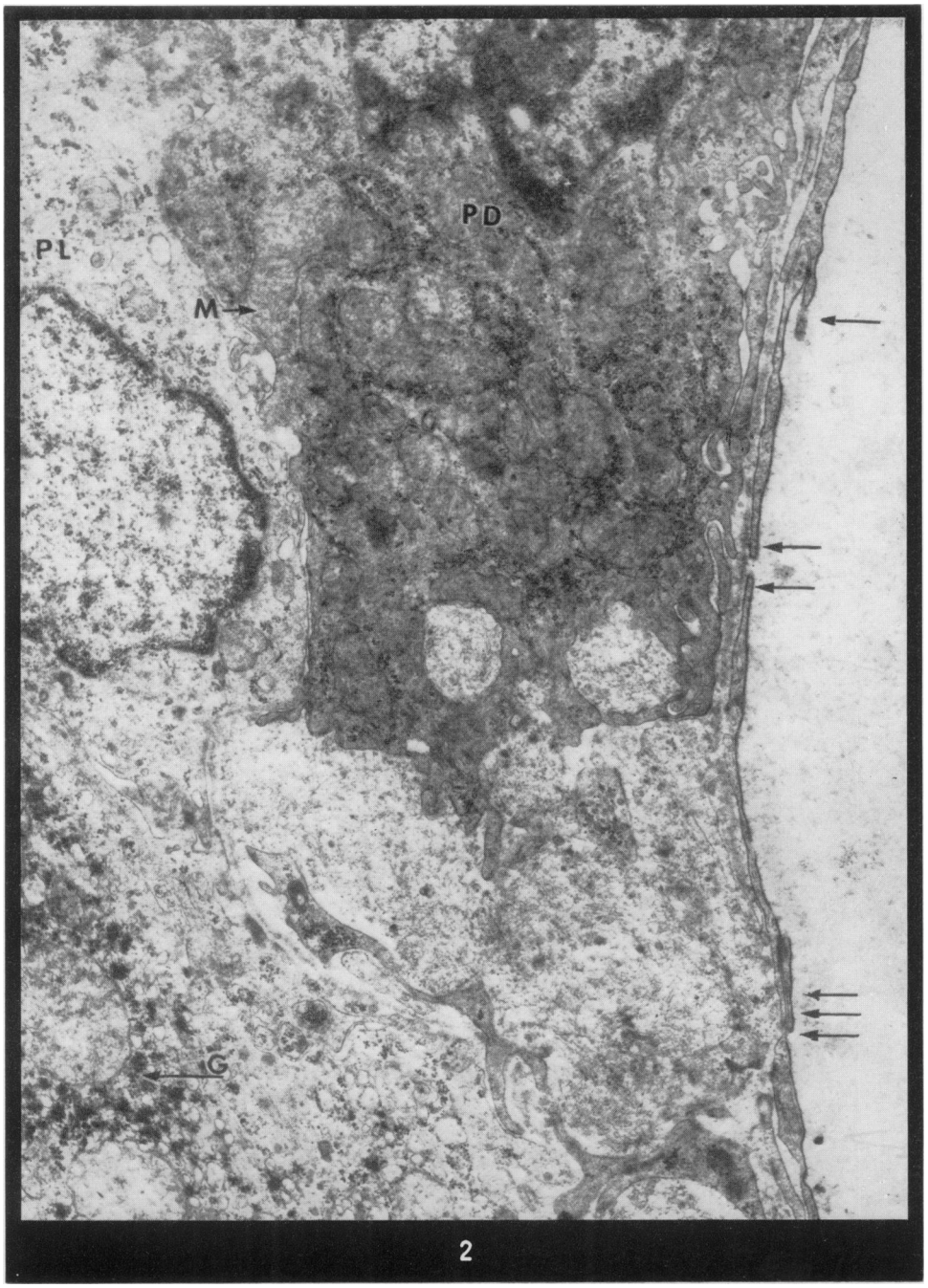

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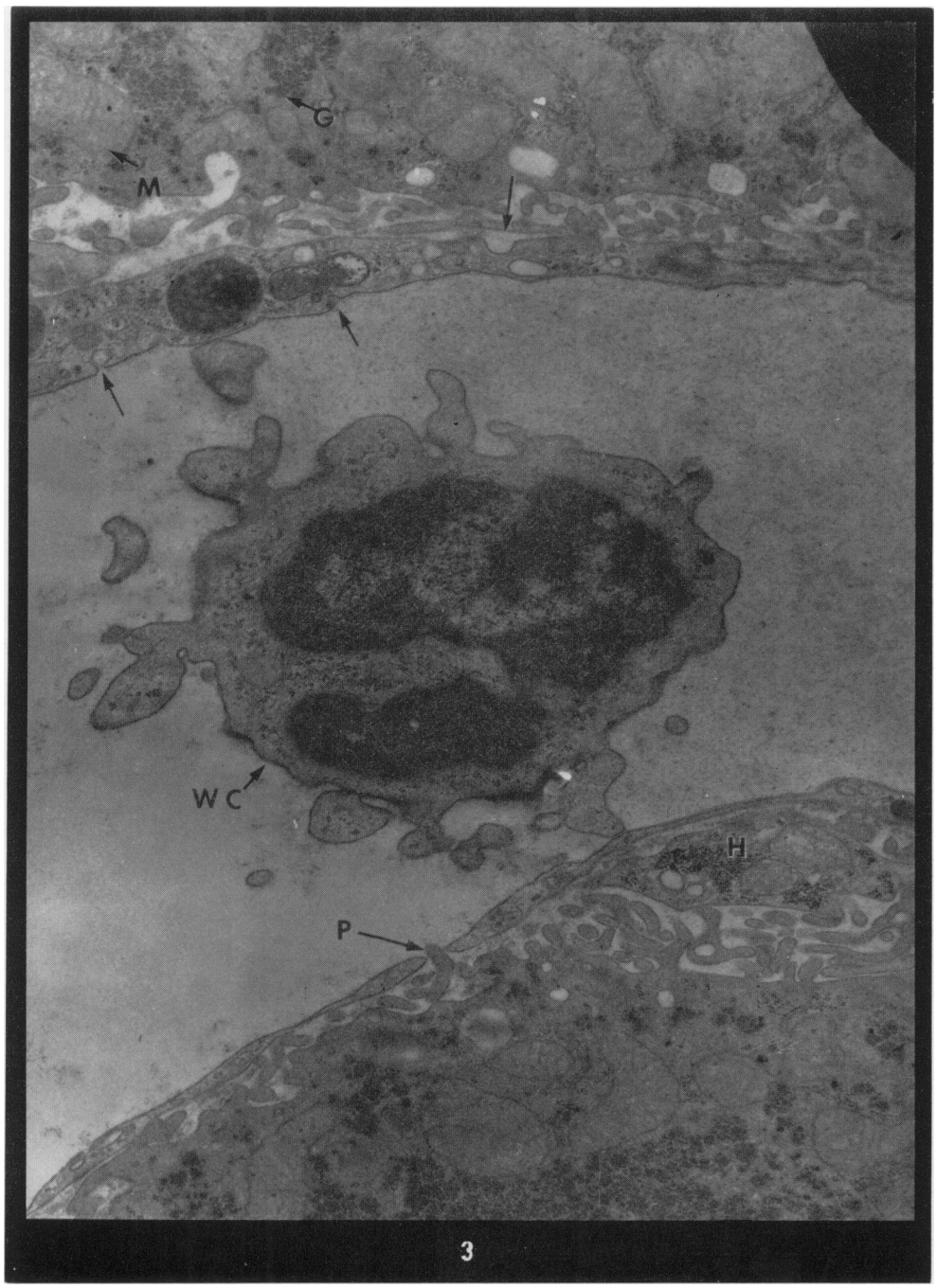

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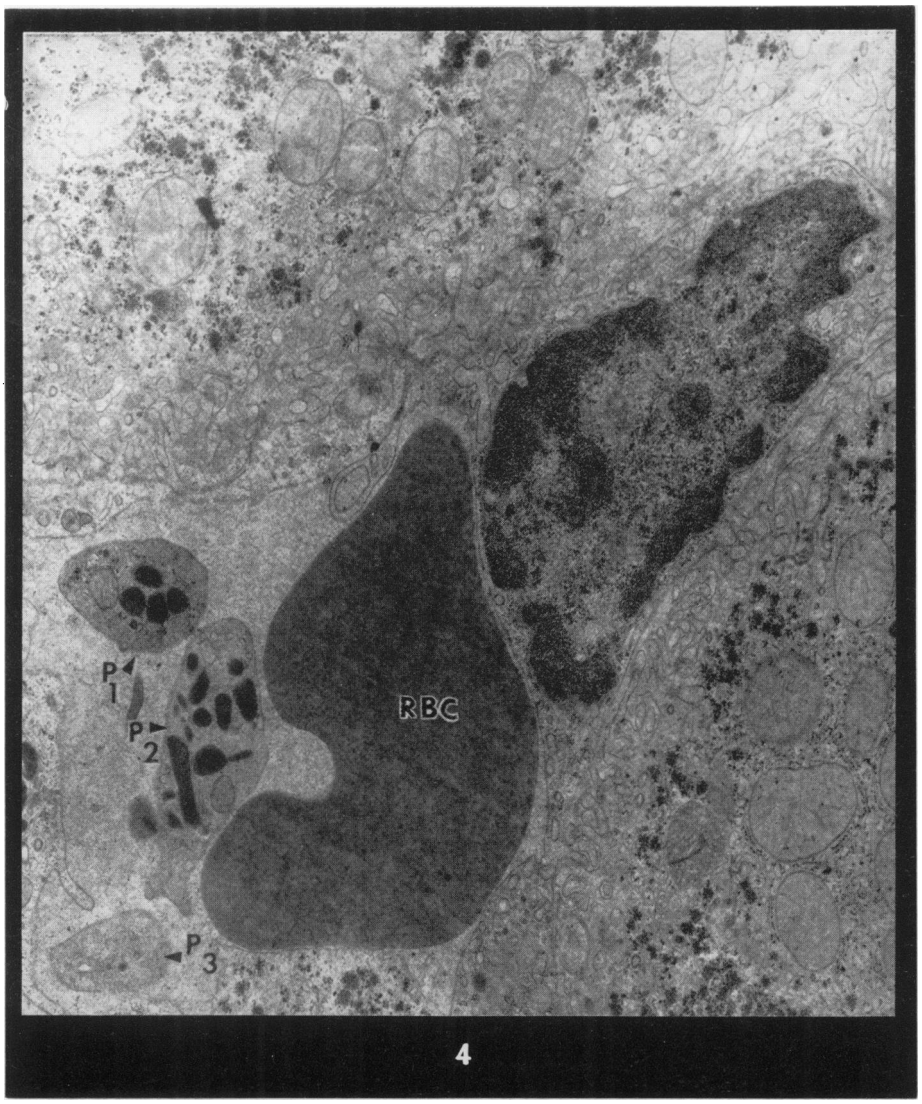

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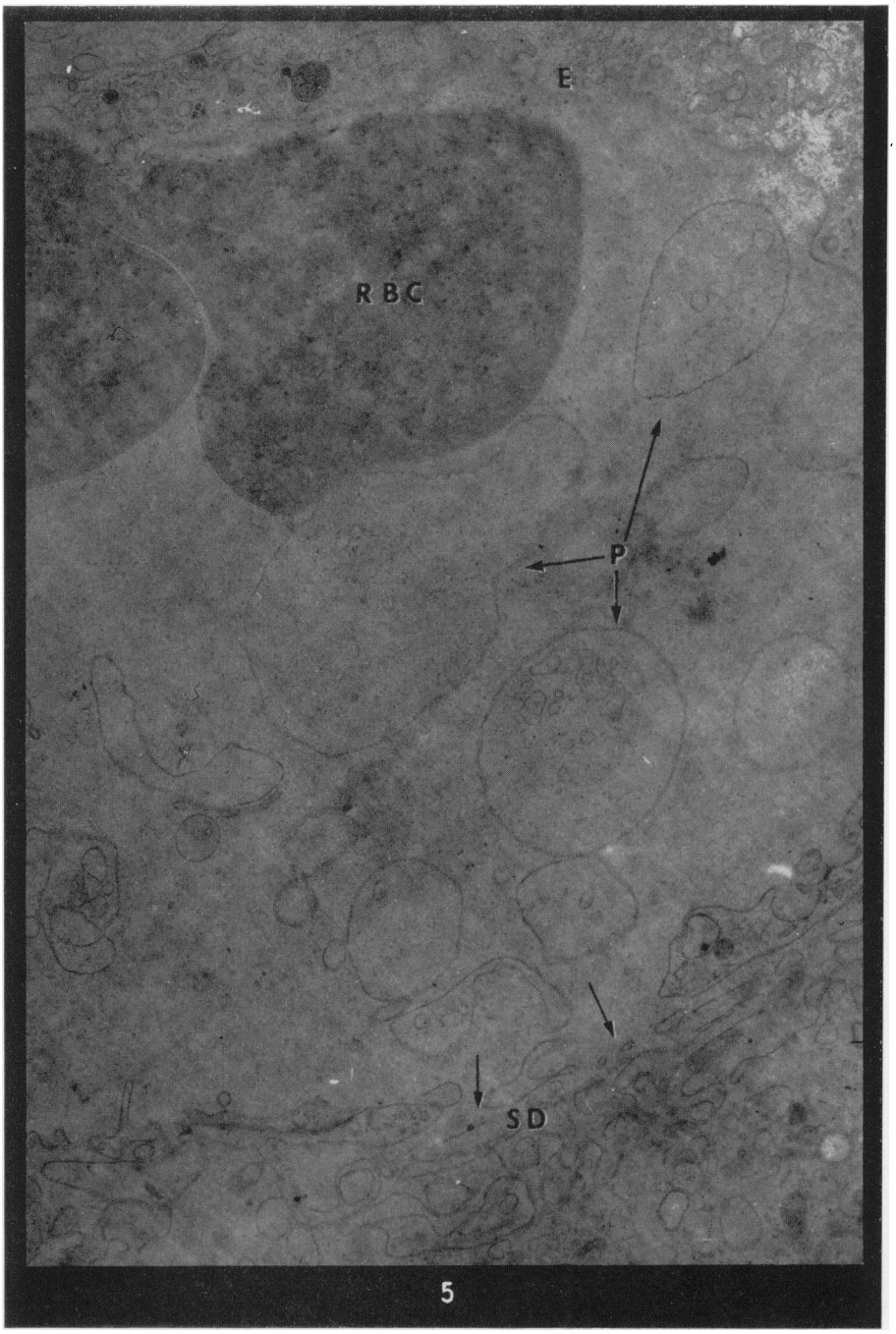

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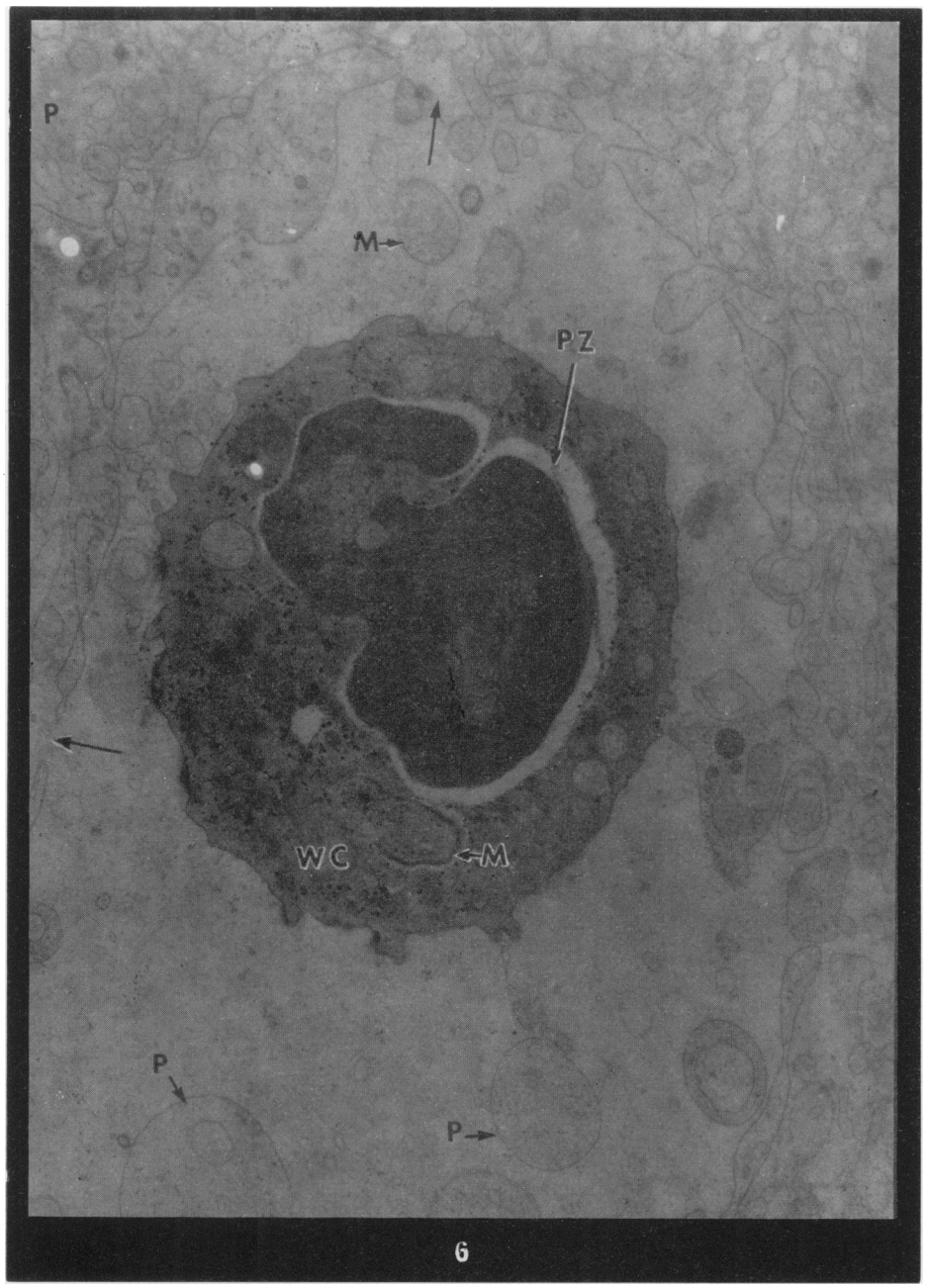

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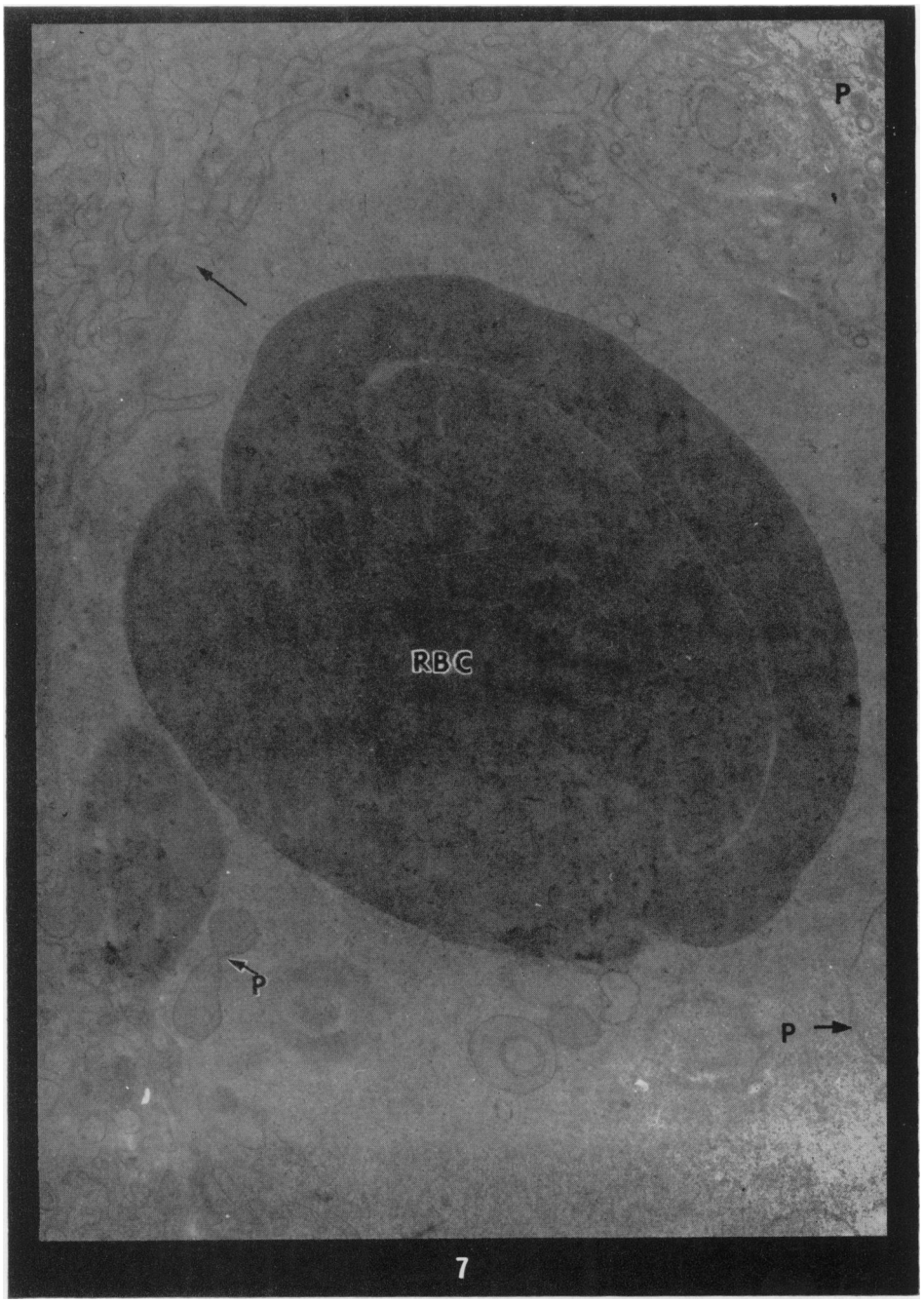

Stewart.

JENNINGS, M. A., MARCHESI, V. T. AND FLOREY, H.—(1962) *Proc. R. Soc. B.*, **156**, 14.
MAJNO, G. AND PALADE, G. E.—(1961) *J. Biophys. Biochem. Cytol.*, **11**, 571.
MASUCCI, F. D. AND BERMAN, H. J. (1965) *American Zoologist*, **5**, 730.
MCKAY, D. G., MARGARETTEN, W. AND CSAVOSSY, I.—(1966) *Lab. Invest.*, **15**, 1815.—(1967) *Surg. Gynec. Obstet.*, **125**, 825.
MOVAT, R. Z., URIUHARA, R. AND MACMORINE, D. L.—(1964) *Life Sci.*, **3**, 1025.
MUSTARD, J. F. AND PACKHAM, M. A.—(1968) *Haematologica*, **1**, 2, 168.
THOMAS, L.—(1965) *in* ' The Inflammatory Process '. New York (Academic Press).