# THE STRUCTURE OF HAEMOSTATIC PLUGS AND EXPERIMENTAL THROMBI IN SMALL ARTERIES

J. E. FRENCH, R. G. MACFARLANE AND A. G. SANDERS

*From the Sir William Dunn School of Pathology and the Medical Research Council Blood Coagulation Research Unit, Oxford*

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HAYEM in 1882 observed that during the flow of blood from a small incision in the wall of a vein in dogs, the mass which formed at the site of injury and eventually plugged the opening, consisted largely of aggregated platelets. He concluded that in flowing blood the platelets had the primary role in stopping haemorrhage, while the formation of visible fibrin was a secondary phenomenon. Lubnitzky (1885) examined histological sections of the haemostatic plug which formed in arterial wounds and illustrated areas which clearly consisted of platelets merging with areas of apparently structureless material.

Much experimental work on the physiology of haemostasis has followed, more recently by Roskam (1954, 1960), Zucker (1947, 1949), Hughes (1959*a*, *b*), Bounameaux (1959) and Jaques (1960). The findings have largely supported the importance of platelet aggregation, though varying emphasis has been placed on the supplementary role of vaso-constriction and fibrin formation. It appears to be agreed that within a few seconds platelets will adhere to the rim of a severed vessel and that, with continuing adherence of platelets, a loose plug or capsule is built up which eventually becomes consolidated by changes in the platelets themselves and by the formation of fibrin (Hughes, 1959 ; Lüscher, 1960).

The physiological role of the haemostatic plug is to arrest the bleeding from a disrupted vessel, but vascular injuries without actual disruption, and sometimes of minor extent, may lead to the formation of a similar mass or plug within the lumen of the vessel as a thrombus. Several observations on the development of thrombi in living vessels had indicated that the cells of the blood were concerned in this process (Wharton-Jones, 1851, 1852 ; Zahn, 1875) but it was Bizzozero (1882) who showed clearly that the platelets were the first of the formed elements to accumulate at the site of injury in small arteries or veins, and that they could form a mass which, if not swept away, progressively impeded the flow of blood. These observations were extended by Eberth and Schimmelbusch (1886) who emphasised that leucocytes appeared in the thrombus later than the platelets and that visible fibrin appeared only as the flow was greatly reduced or stopped by the platelet mass. Welch (1887) showed that when the experimental thrombi were examined at intervals after they had caused occlusion of the vessel, fibrin was found in increased amounts, as islands and bands between the masses of platelets. The important role of platelets in the early stages of experimental thrombus formation has been confirmed by subsequent observations, including those by Fulton, Akers and Lutz (1953) on vessels in the hamster cheek pouch, and by Honour and Ross Russell (1962) on vessels in the cerebral cortex and mesentery of rabbits.

The early work on experimental thrombi and haemostatic plugs was very

thorough so that other studies with the light microscope have added little that was new about the structural aspects of the problem. Questions about the attachment of platelets to damaged tissues and to each other, the occurrence of change in their morphology during this process, and the relationship of fibrin formation to platelet aggregation have remained unanswered for many years. The introduction of electron microscopy has provided a new approach to this problem and a number of workers have now published electron-micrographs of natural or experimental thrombi (Poole, French and Cliff, 1963). A detailed account of the fine structure of haemostatic plugs in vessels of the rabbit mesentery has been published by Kjaerheim and Hovig (1962) and of organising thrombi in the femoral vein of rats by Weiner and Spiro (1962). French and Poole (1963) have described the fine structure of artificial thrombi prepared from rat's blood by Chandler's method (Chandler, 1958). The results show morphological details of great interest, and suggest that a sequence of morphological changes in the platelets occurs during aggregation which might be integrated with other changes, such as fibrin formation.

In order to study these matters further and to compare the morphology of haemostatic plugs during their development with that of experimental thrombi, observations have been made with the electron microscope on injured vessels in the cheek pouch of the Syrian hamster. These vessels can be watched directly during the formation of the plug or thrombus and at the appropriate time can conveniently be fixed *in situ* for electron microscopy.

## MATERIALS AND METHODS

Golden hamsters (*Mesocricetus auratus*) of both sexes were used. Their weights ranged from 68–110 g. Anaesthesia was induced with ether and maintained with intraperitoneal pentobarbitone sodium (Abbot-Veterinary nembutal), 6·0 mg. per 100 g. body weight.

One cheek pouch was everted and pinned out over a transilluminated block on a microscope stage. Part of the upper surface of the pouch was removed by dissection with iridectomy forceps and scissors, and haemorrhage at this stage was prevented by using an electrocautery to seal vessels before they were cut. A suitable vessel on the lower side of the pouch was then selected and the loose areolar tissue and fat over it were removed. Only those preparations were used in which the circulation remained normal after this procedure.

The vessels to be studied were injured in various ways and their reactions observed with a binocular stereo-microscope at magnifications from × 5–× 75. Photomicrographs were taken at intervals with a Polaroid-Land camera before or after injury of the vessel, and also after fixation, so that the injured segment of vessel could be located for electron microscopy.

Arteries were transected as follows :

(a) One artery was transected with a knife made from a spicule of glass, after the tissue had been immobilised with a 2-pronged fork of fine stainless steel wire held in a micromanipulator (Fig. 1).

(b) Better results were obtained by vertical downward pressure with a very small steel blade, 0·2 mm. long, made from a razor blade stuck with araldite to a brass rod.

Injury of the vessel wall was induced by means of a micro-electrode as described by Fulton *et al.* (1953). A stainless steel electrode with a tip diameter of about 20 $\mu$, insulated as far as the tip with shellac, was used. The electrode was held in a micromanipulator and placed in contact with the wall of the vessel to be stimulated (Fig. 2 and 3). The electrode was connected to the −ve terminal of a square wave stimulator. The +ve terminal was connected to one foot or to one of the pins used for holding out the pouch. The frequency of the stimuli was usually 50/sec., the applied voltage 30 volts and the duration of the stimulus one second.

The tissue was fixed *in situ* with ice-cold 2 per cent osmium tetroxide buffered with veronal acetate-sucrose (Caulfield, 1957) and embedded in araldite after staining with phosphotungstic acid. The block was reorientated so that the vessel could be sectioned transversely

through the site of injury. Sections were examined and photographed with a Phillips 100B or E.M. 200 electron microscope.

## RESULTS

### *Normal structure of arteries in the pouch*

The arteries selected for observation were small vessels 30–50 $\mu$ in diameter. In transverse section these vessels were seen to be lined by a continuous layer of endothelial cells (Fig. 4). Most of the rest of the wall consisted of a single layer of smooth muscle cells, but between this and the endothelium there was a narrow zone containing fine fibrillary material and interrupted fibres of elastic tissue. External to the muscle layer there were a few collagen fibres, mostly cut transversely, and occasional elastic fibres (Fig. 5). In uninjured vessels platelets were seen within the lumen as discrete elliptical objects containing their characteristic osmiophilic granules.

### *Haemostatic plugs in transected arteries*

Bleeding usually began immediately from the proximal end of the cut vessel and was sometimes followed after an interval by retrograde bleeding from the distal end. The cut ends usually retracted slightly from the edges of the cut and there was a variable amount of vaso-constriction. Exuded blood was washed away by irrigation with McEwen's solution (McEwen, 1956), but further bleeding had usually stopped within $2\frac{1}{2}$–3 min. When fixation was not carried out immediately at this stage it could be seen that the column of blood in the artery, extending back to the first side branch, became stagnant when bleeding stopped. The flow proximal to the stagnant column was then diverted through the side branch (Fig. 1). At higher magnification, eddying movements of cells could be seen at the proximal end of the stagnant column. Some erythrocytes and presumably other cells left the main stream and were either returned to it by the eddy or were diverted into the stagnant column.

In serial sections, which approached the free end of the artery from the side of the cut, the haemostatic plug was seen first as a free mass, apparently projecting beyond the end of the artery. When fixation had been carried out immediately after bleeding had stopped, the plug consisted largely of platelets, in places tightly packed together and in others more loosely aggregated. The platelets had retained their identity at this stage, although a few at the edges of the plug appeared to have lost their granules. Leucocytes were scanty, while the grouping of erythrocytes within the centre of the plug suggested that up to the moment when bleeding stopped some flow had been continuing through the centre of the platelet mass (Figs. 6 and 7).

At the level where the plane of section passed through the cut end of the artery, the mass of platelets was seen partly within and partly outside the lumen (Fig. 8). At the edges of the cut, platelets were closely applied to the injured part of the wall and to collagen fibres in the surrounding tissue. Again the platelets were very closely packed together but had retained their form and outline. Fibrin could not be detected within the platelet mass, but a few wisps and strands were present at the edges (Fig. 9). The greater part of the lumen at this level, though very close to the cut edge, was not occluded when fixation was carried out immediately, and it appeared that, at the moment when bleeding stopped, the occluding plug had been formed by platelets which had aggregated in the tissues mainly outside the cut vessel.

In the specimens which were fixed at later intervals, up to 30 min. after bleeding had ceased, the plug beyond the cut end of the artery had a more compact structure. Platelets were the major component of the plug, but some were now distorted in shape and their boundary membranes less well defined than in the specimen fixed earlier (Fig. 10). At the edges of the mass, some of the platelets were without granules and were closely associated with collagen fibres (Fig. 11). Fibrin was now more prominent around the edges of the plug, but as in the other specimens it could not be seen among the closely aggregated platelets.

Sections through the part of the artery proximal to the cut, in which the column of blood had been stagnant since bleeding ceased, presented in one

## EXPLANATION OF PLATES

FIG. 1.—Drawing from a photograph taken after bleeding had ceased from an artery transected with a spicule of glass. The arrows indicate the final direction of blood flow. ×23 (approx.).

FIG. 2.—An artery with electrode in place before stimulation. The shadow in the upper part of the photograph is a wire loop held above the vessel. ×14 (approx.).

FIG. 3.—The same after stimulation and fixation. The wire loop was lowered immediately before fixation to prevent the thrombus (arrow) being dislodged. ×14 (approx.).

FIG. 4.—An uninjured artery, lined by a continuous layer of endothelial cells (E), has red cells (R) and platelets (P) in the lumen. ×3000.

FIG. 5.—Endothelium (E), elastic fibres (el), smooth muscle cells (sm) and collagen fibres (col) in the wall of an uninjured artery. ×18,000.

FIG. 6.—A haemostatic plug, situated distal to the cut end of an artery and fixed immediately bleeding ceased, shows aggregated platelets (P), a central group of red cells (R) and a leucocyte (L). ×3750.

FIG. 7.—Detail from haemostatic plug (above) shows platelets with granules (g) and intact boundary membranes. ×12,000.

FIG. 8.—A haemostatic plug at the level of transection. A mass of platelets (P) between the cut edges of the wall (W) is partly inside and partly outside the lumen (Lu) of the artery. ×3750.

FIG. 9.—Detail from haemostatic plug (above). The platelet mass (P) is closely associated with the damaged wall (W) and with collagen fibres (col). Fibrin strands (F) occur at the edge. ×10,000.

FIG. 10.—Edge of a haemostatic plug fixed 30 mins. after bleeding ceased. Non-granular bodies (ng) appear to be platelets which have lost their granules and are closely associated with collagen fibres (col). ×13,000.

FIG. 11.—Centre of haemostatic plug (above). Platelets are distorted in shape and many of the boundary membranes are indistinct. ×17,000.

FIG. 12.—Artery proximal to a haemostatic plug fixed 30 mins. after bleeding ceased. There is concentration of red cells (R) and platelets (P) in the lumen. ×3000.

FIG. 13.—Platelets (P) separated by amorphous material (am) in the lumen of the same artery (above) sectioned at a different level. ×10,000.

FIG. 14.—Fibrillary material (F), presumed to be fibrin, and part of a red cell (R) in the lumen of an artery proximal to a haemostatic plug, fixed 20 mins. after bleeding ceased. ×56,000.

FIG. 15.—An artery injured by electrical stimulus. A mass of platelets is attached to the wall at the point (arrows) where the endothelium (E) has been destroyed. ×3600.

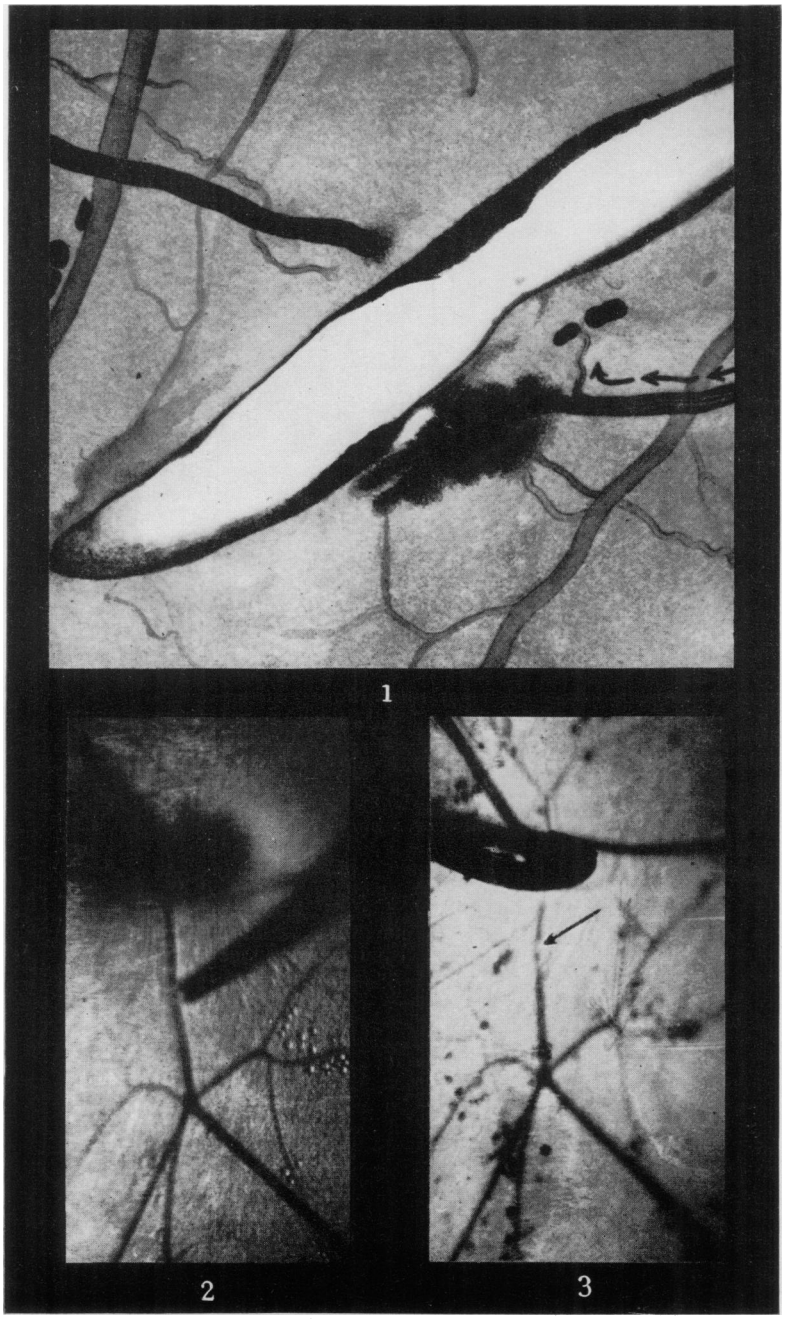
FIG. 16.—Part of an injured arterial wall showing an elastic lamina (el) and smooth muscle (sm). The endothelium has disappeared. A few of the platelets (P) in the lumen (Lu) are adherent. ×10,000.

FIG. 17.—A mass of platelets occupies the lumen of an artery injured at bottom right (arrows). A few red cells (R) and a leucocyte (L) are seen at the edges of the mass. Section at another level of same thrombus shown in Fig. 15. ×3200.

FIG. 18.—Attached edge of thrombus illustrated in Fig. 15 and 17. The part of the wall shows injured smooth muscle cells (sm) and elastic fibres (el) of the original sub-endothelial zone. ×12,500.

FIG. 19.—Centre of thrombus (above). Platelets with intact boundary membranes (arrows) and containing granules (g) are closely packed together. ×26,000.

FIG. 20.—Part of the wall of a small vein near to an injured artery. A platelet (P) occupies a gap in the endothelium (E) at an intercellular junction. Other platelets in the lumen (Lu) are adherent to it. ×26,000.

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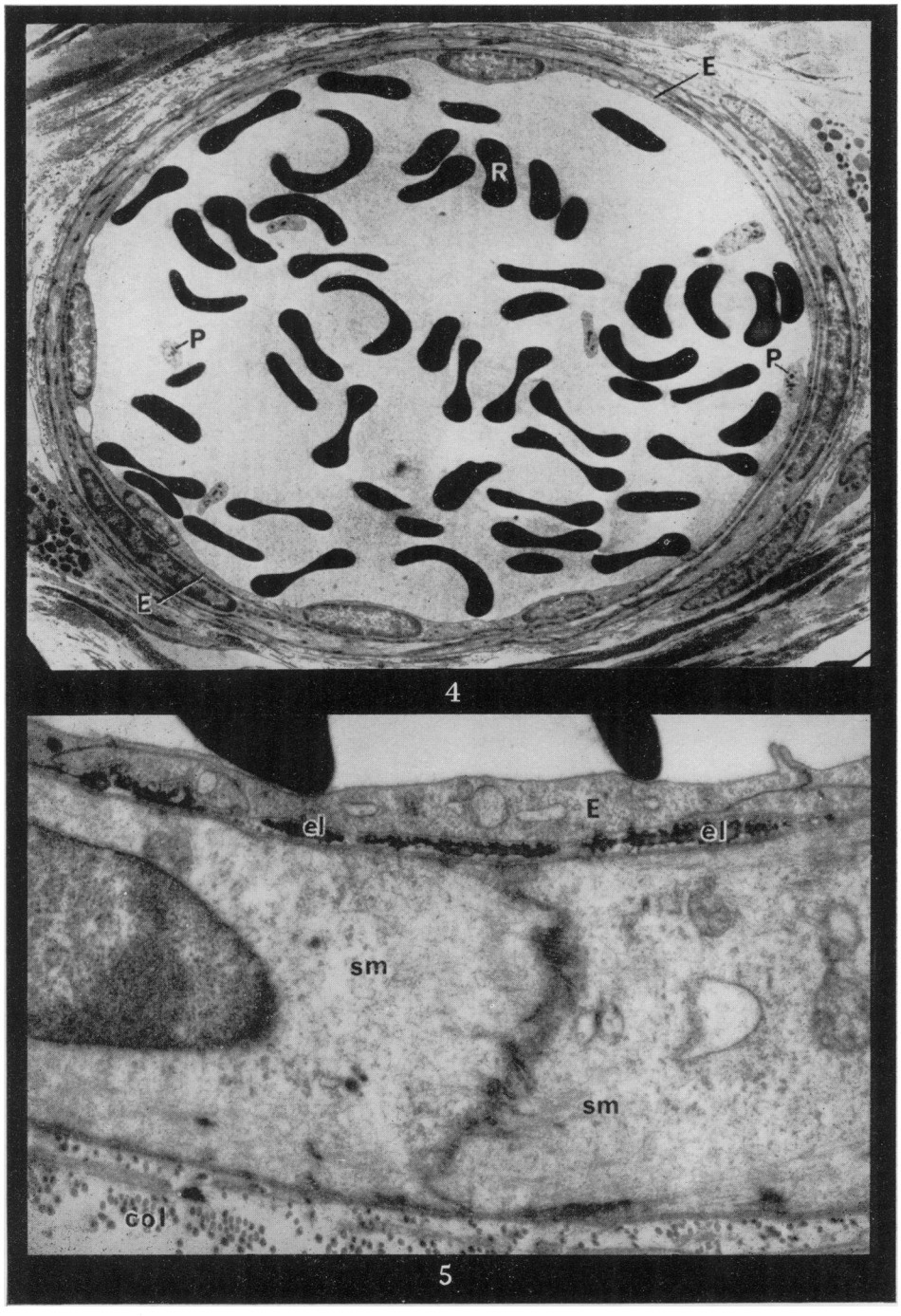


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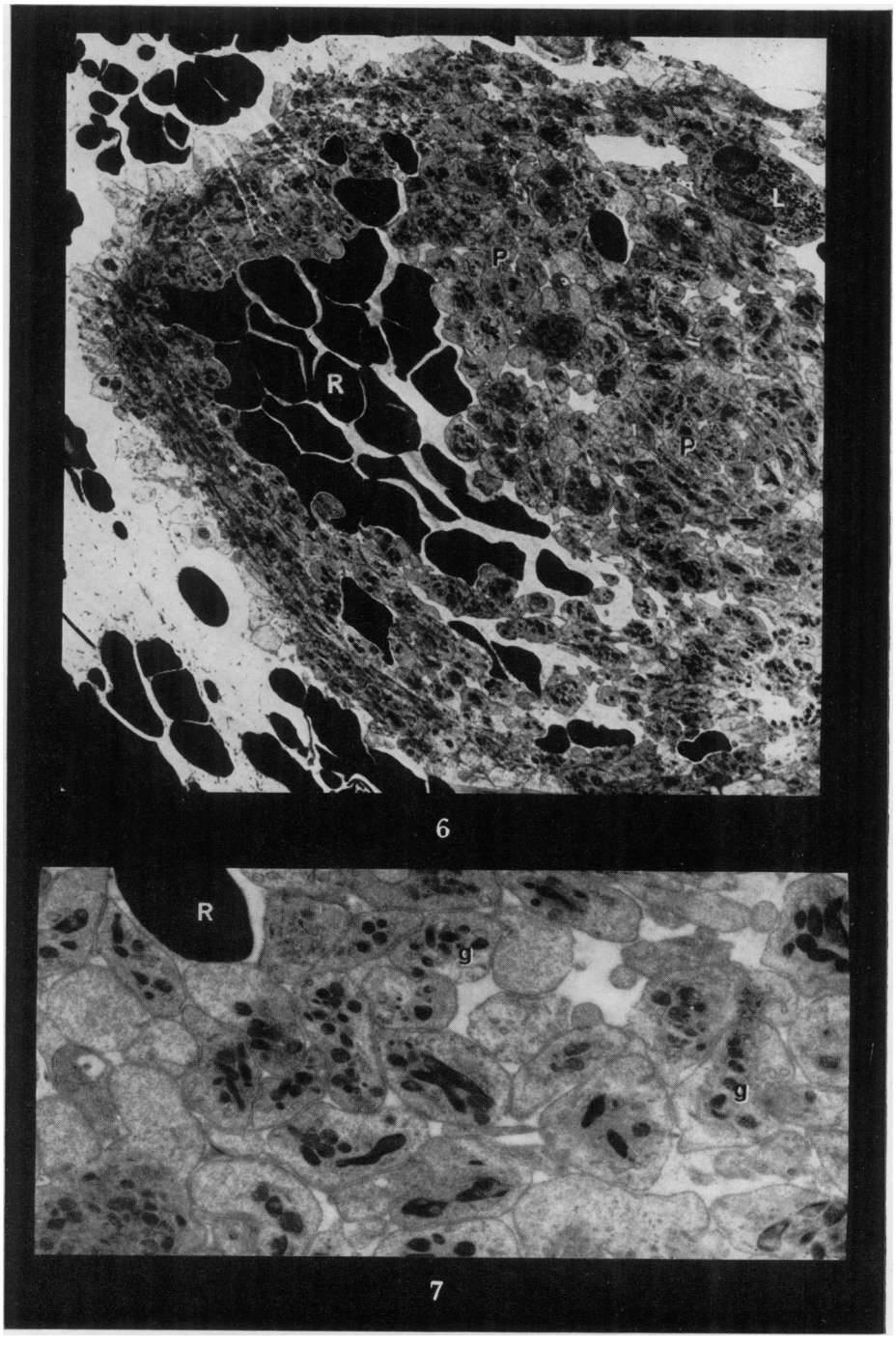


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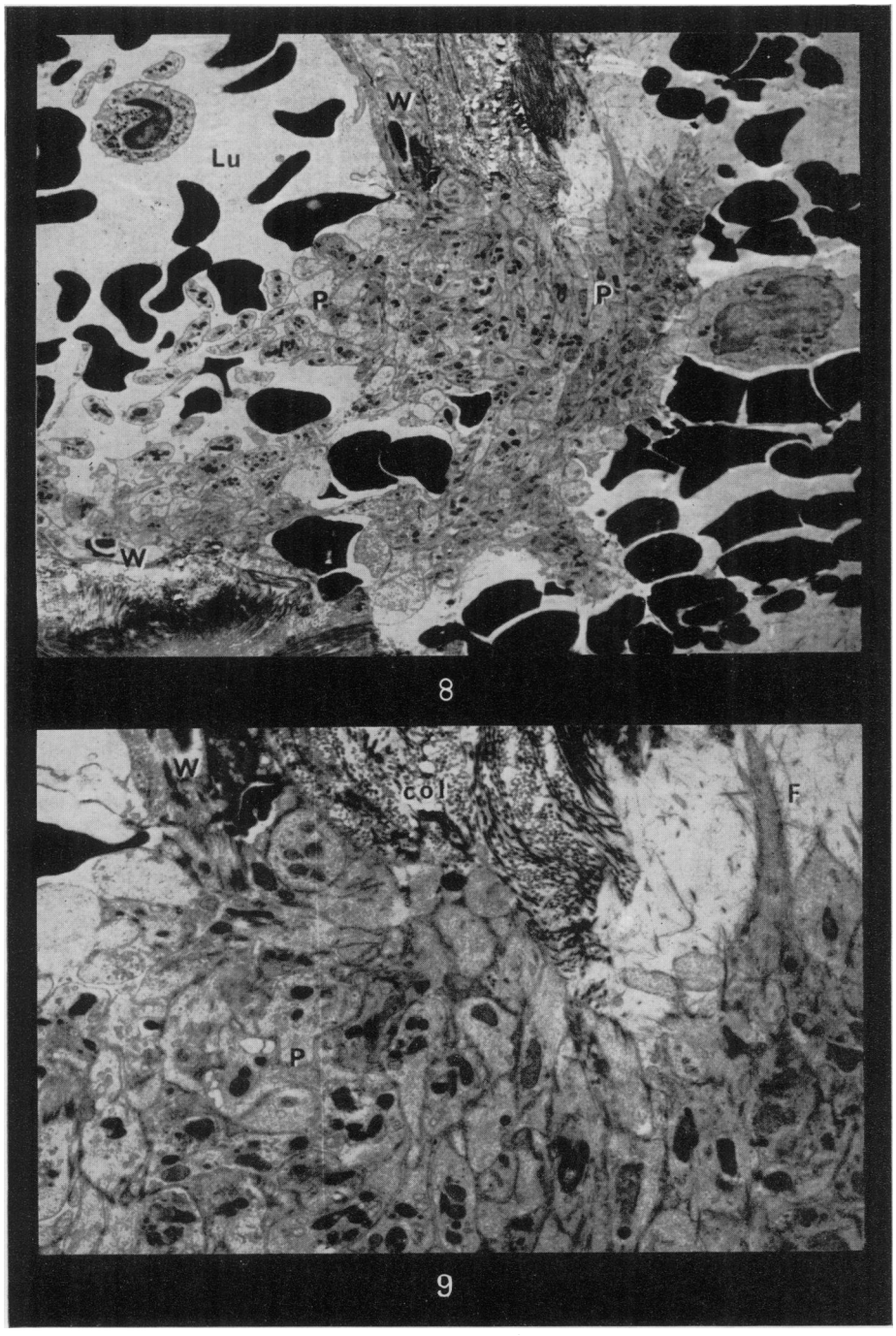


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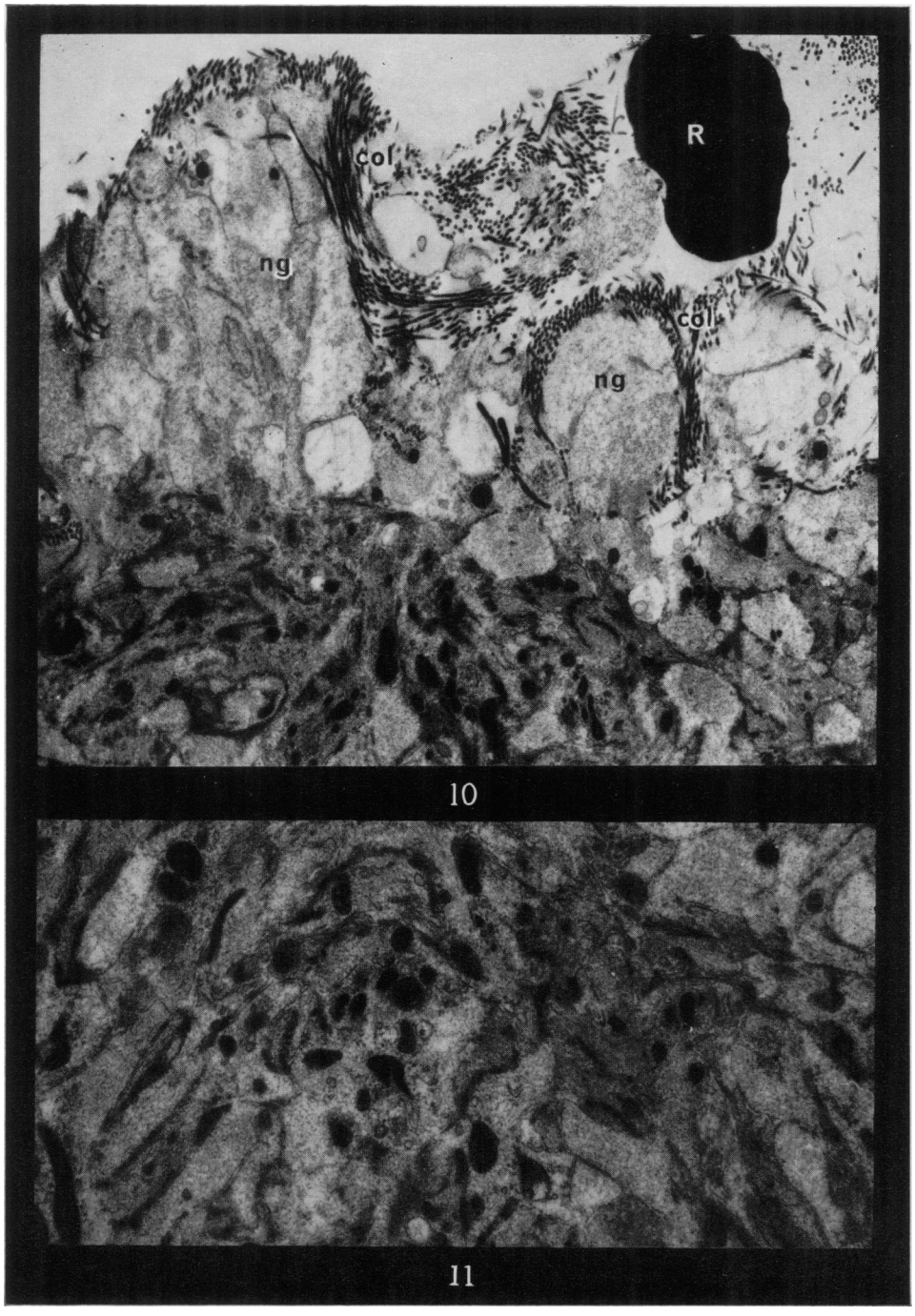


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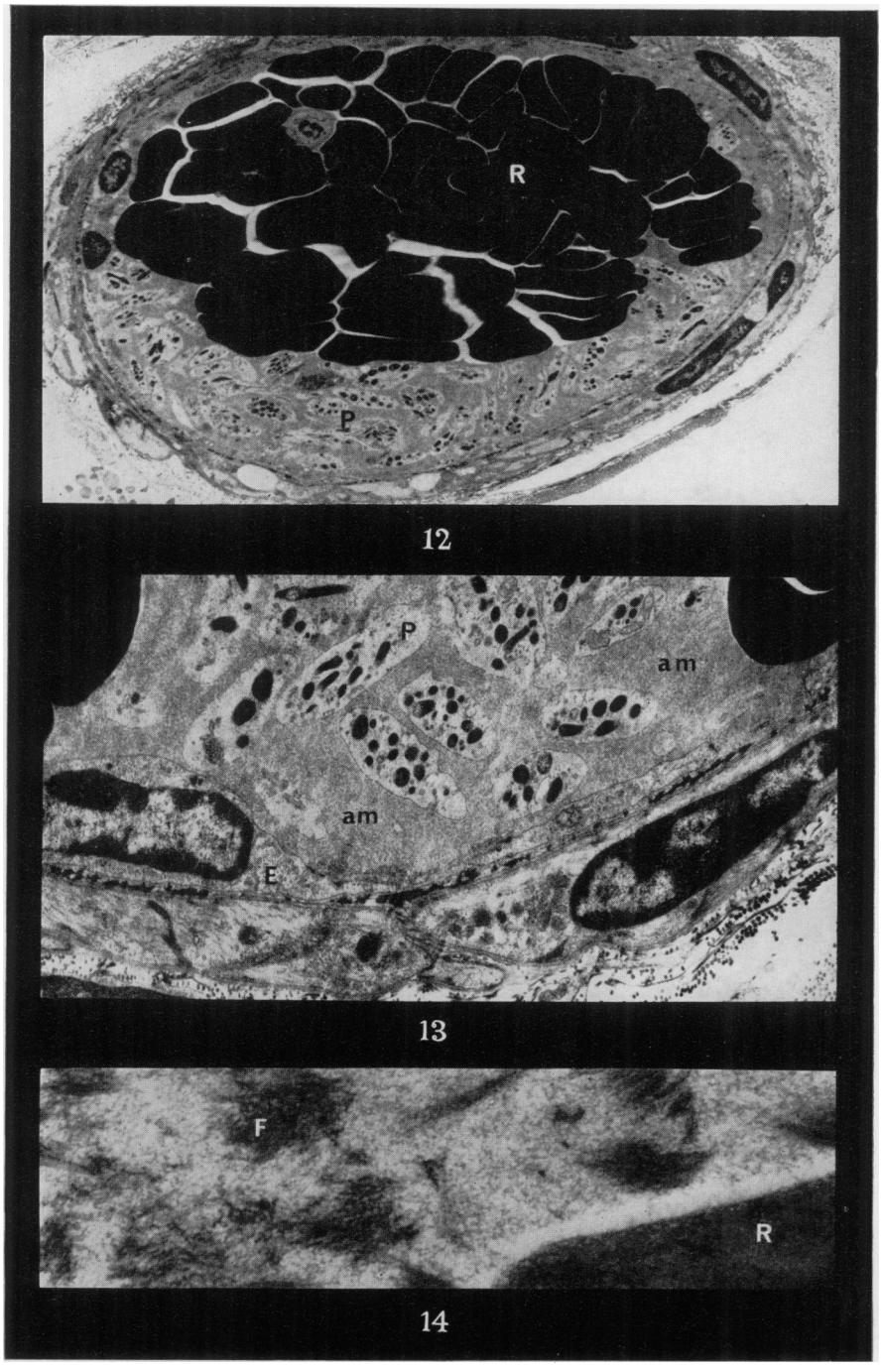


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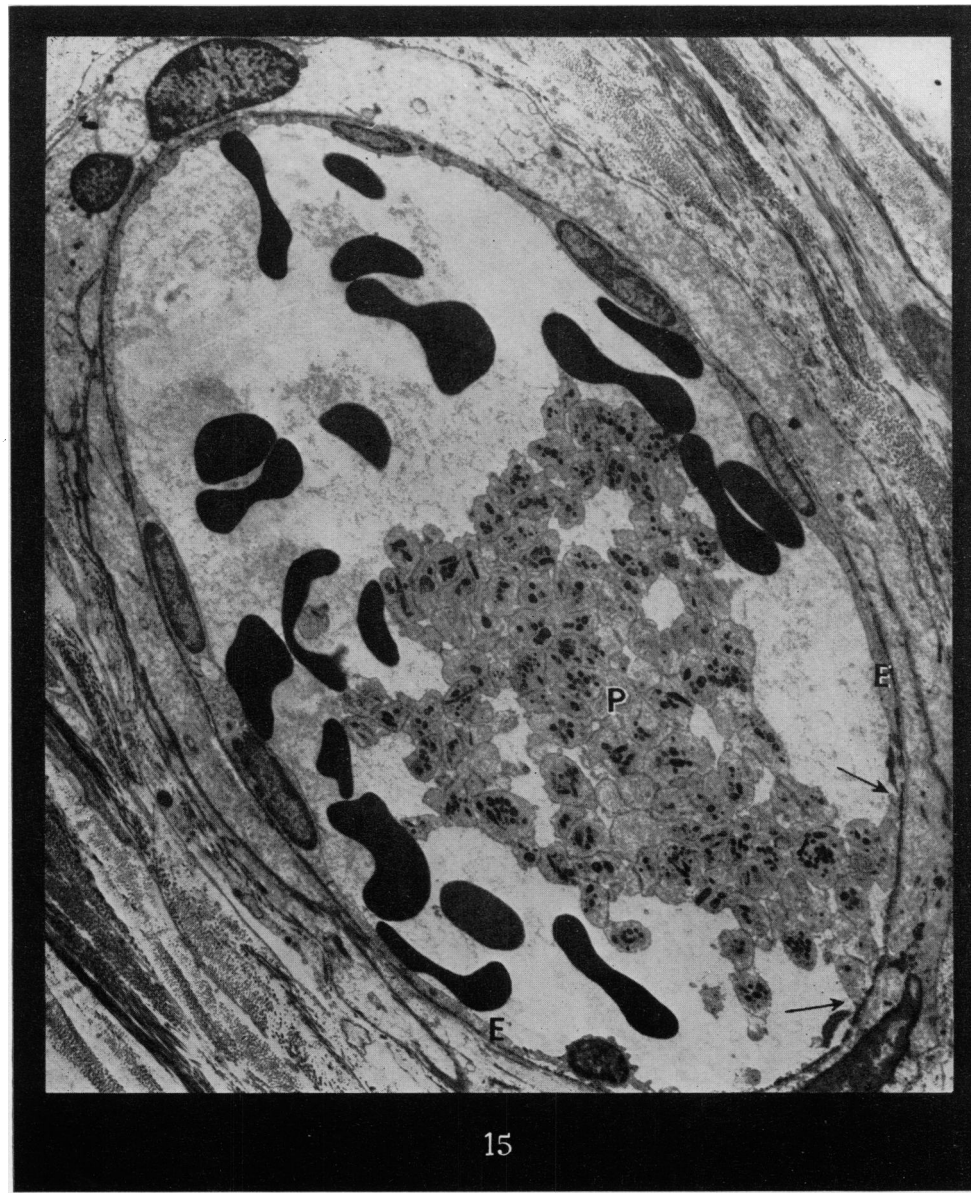


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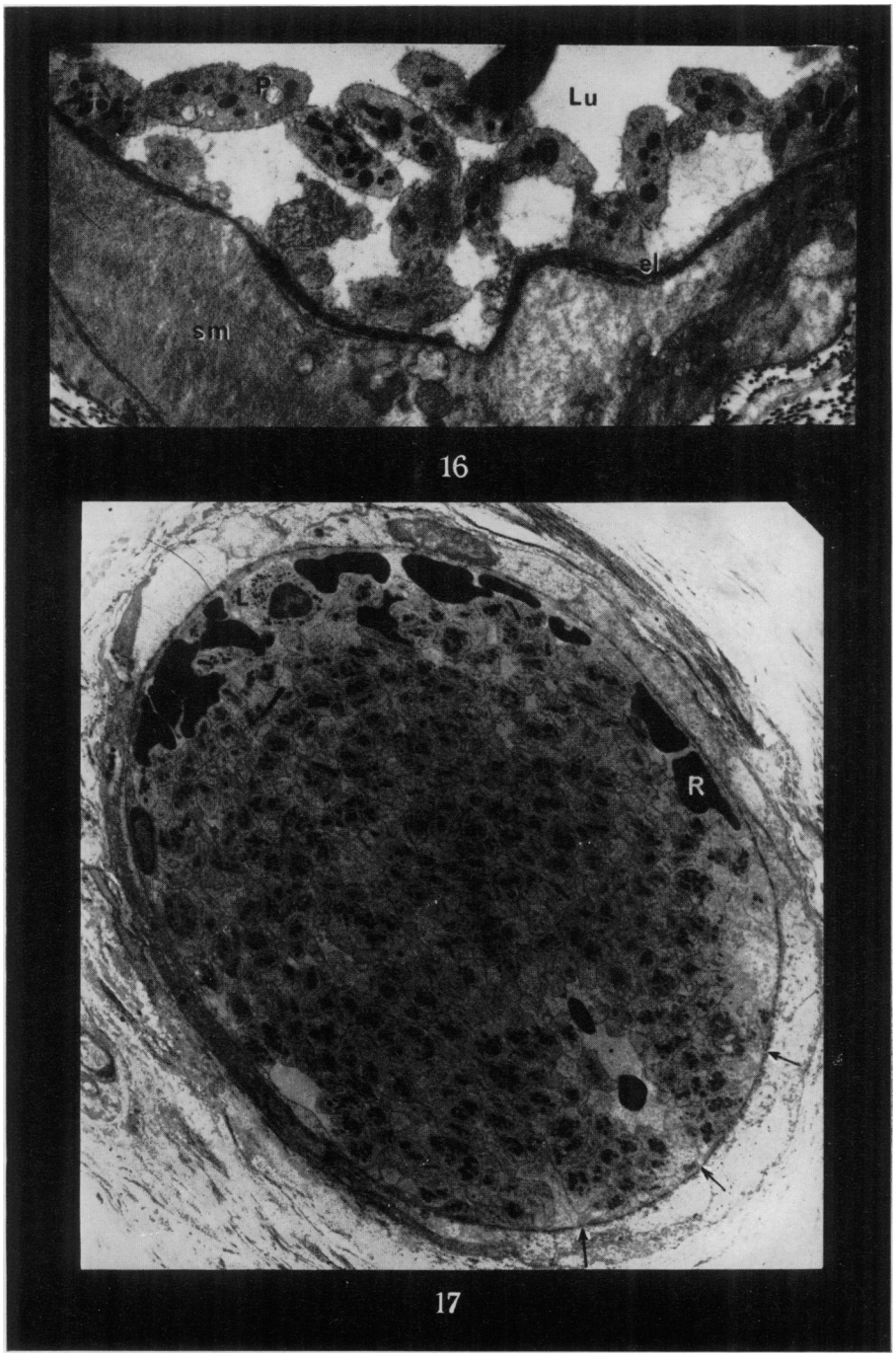


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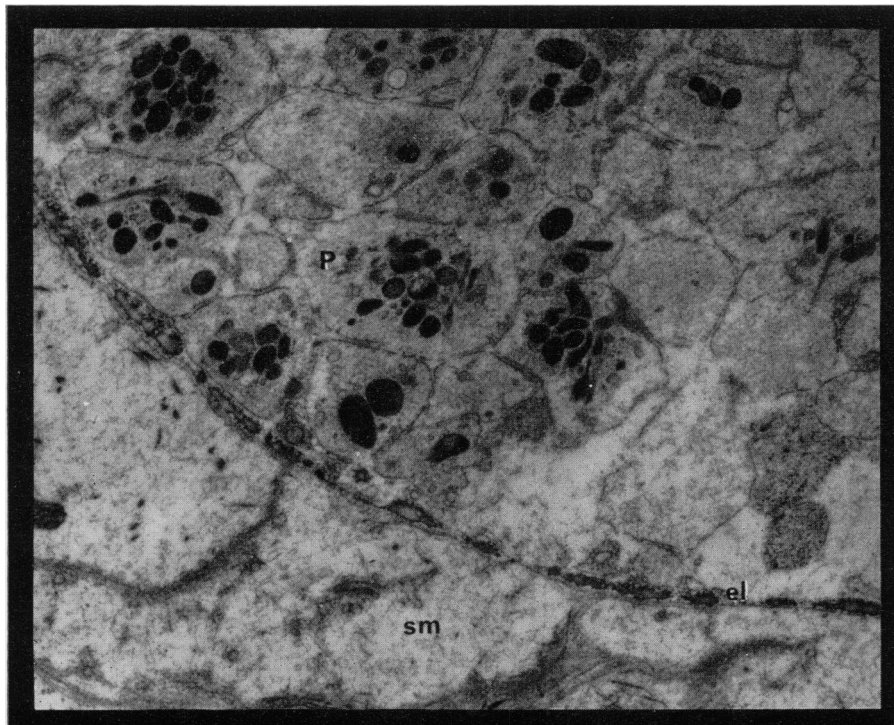


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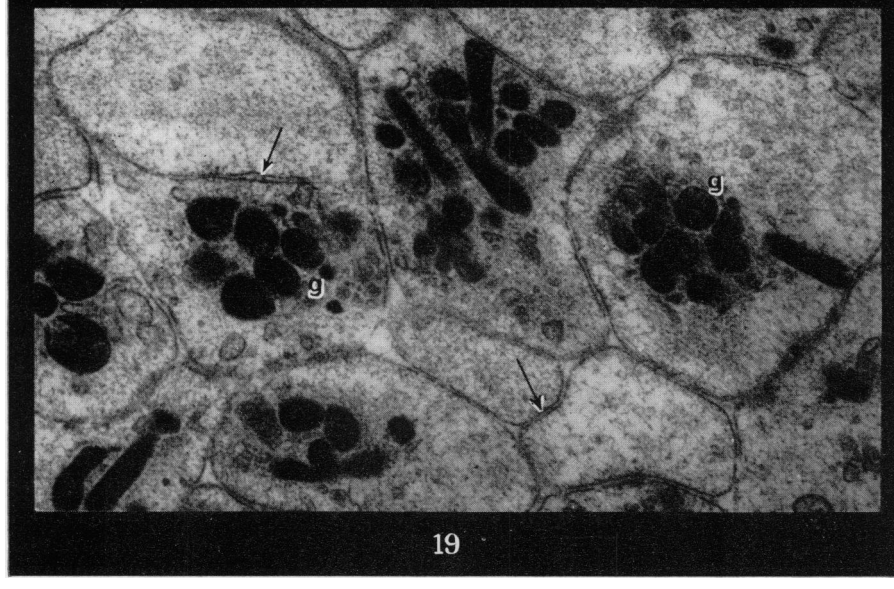


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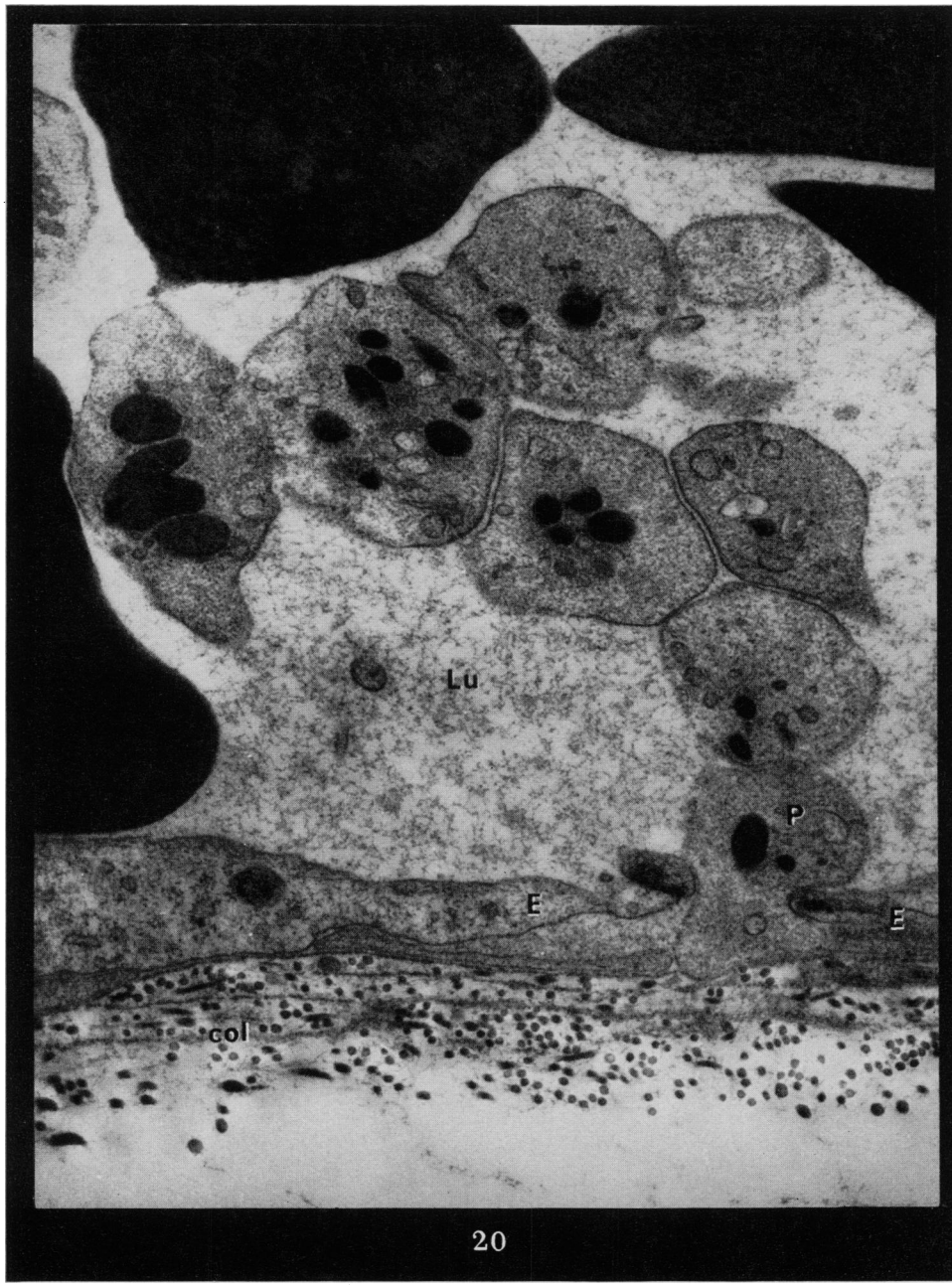


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French, Macfarlane and Sanders.

specimen a remarkable appearance at this later stage. There was a concentration of erythrocytes and platelets in the lumen of the vessel (Fig. 12), but the platelets, instead of being compactly arranged, appeared here to be embedded in a matrix of amorphous material (Fig. 13). In another specimen, small islands of fibrillary material, which was presumably fibrin, were seen scattered in similar amorphous material in the lumen of the artery proximal to the cut (Fig. 14).

*Thrombi in arteries injured by electrical stimulus*

Constriction occurred at the site of stimulation. This appeared within a few seconds of the cessation of the stimulus and persisted for several minutes. In some cases, but not all, mural thrombi appeared at the site of injury as " white bodies ". The usual sequence of events was for these to increase in size until they were nearly blocking the lumen, when they were swept away by the blood stream and another body would then begin to form at the same site. Since the aim was to fix the white body *in situ* rather than to observe the formation of emboli, the vessel was occluded distally by pressure with a wire loop immediately a white body of suitable size had developed, to prevent it being dislodged, and the fixative was then applied (Fig. 3).

In transverse sections of the stimulated artery, the endothelium on the side of the vessel closest to the electrode had disappeared or was represented only by tattered fragments (Fig. 15). Muscle cells on the side of the injury were swollen and their cytoplasm either vacuolated or less dense than normal. Platelets were attached on the luminal surface where the wall had been denuded of endothelium. However, the bare area was not always completely covered by platelets and the number attached varied from a few scattered loosely over the surface (Fig. 16) to large numbers which occupied most of the lumen of the vessel (Fig. 17). The mass, seen as a " white body " by direct vision with the dissecting microscope, consisted almost entirely of closely packed platelets. Only one leucocyte was seen in serial sections taken through the mass illustrated in Figs. 15 and 17, and there was no recognisable fibrin. A few erythrocytes were present at the edges of the mass, on the opposite side to its site of attachment, and there were occasional erythrocytes in spaces within the mass. This distribution of erythrocytes was consistent with the observation that, at the time the fixative was applied, blood flow had not been stopped completely by the white body.

At the points of attachment, platelets appeared to be closely apposed to elastic tissue and sub-endothelial fine fibres (Fig. 18). Within the mass the platelets had retained their identity and were each bounded by an intact membrane. They were very closely packed together so that only a narrow gap, 200–300 Å across, separated adjacent membranes along most of their length. The granules were not distributed uniformly throughout the cytoplasm of the platelets and this appeared to explain why some profiles showed only scattered vesicles in a cytoplasm which was otherwise free of organelles (Fig. 19). Conclusive evidence of complete loss of granules from any of the platelets in these early thrombi was not obtained.

*Changes in adjacent veins*

Thrombi were sometimes seen in veins which had not been injured deliberately but were near to the injured artery. In sections, occasional small veins were

seen to be partly or completely occluded by a compact mass of platelets, at the edges of which a few leucocytes were adherent. In some there was no clear evidence of injury to the endothelial lining of the vein in the plane of section, but in others slight mechanical damage to the wall was indicated by adherence of leucocytes or diapedesis of leucocytes or red cells. In one vein clearly showing these features of injury a platelet which was attached to the wall was seen to be inserted in a gap between two endothelial cells and to have a further group of platelets attached to its free surface (Fig. 20).

## DISCUSSION

These experiments support the view that the early stages of the formation of a haemostatic plug and an experimental thrombus in small vessels are closely similar processes (Poole and French, 1961). In each case the mass which first impedes blood flow consists of an aggregate of closely packed platelets. These aggregates, as previously shown by Kjaerheim and Hovig (1962) for haemostatic plugs and by French and Poole (1963) for artificial thrombi, are characterised by very close packing of the platelets and the absence of any fibrillary fibrin within the aggregates. In the experimental thrombi, which were fixed at the stage when blood flow had not been arrested by the platelet mass, no fibrin was visible at all, but in the haemostatic plugs, fixed immediately haemorrhage ceased, traces of fibrin were seen at the edges of the aggregates and fibrin had increased in amount at the later stages.

At the centre of the aggregates in both situations the adjacent surface membranes of the platelets were so close together that the gap between them would not be resolvable with the light microscope. In the early stages, the boundary membranes appeared to be intact and there was no evidence that individual platelets had immediately fused together in the sense that had been proposed from studies with the light microscope. With the passage of time it is probable that breakdown of membranes and disorganisation of cytoplasmic structure does ocur. The appearance of the haemostatic plugs fixed 30 min. after their formation, and of artificial thrombi also fixed after 30 min. (French and Poole, 1963) suggests the beginning of this process. Weiner and Spiro (1962) have described disintegration of platelets at 48 hr., as well as of erythrocytes and leucocytes, in thrombi which had been induced in rats by injection of sodium morrhuate into a femoral vein.

In artificial thrombi produced by Chandler's method, the marginal zone of the platelet aggregates shows a fringe of platelets which have apparently lost their granules (French and Poole, 1963). Loss of granules also occurs in the platelet aggregates produced by thrombin *in vitro* (Parmeggiani, 1961); it has been described by Kjaerheim and Hovig (1962) in haemostatic plugs in rabbits, and by Florey, Greer, Kiser, Poole, Telander and Werthessen (1962) in mural thrombi on the surface of fabric grafts in the aorta of baboons. Loss of platelet granules could be seen at the edges of the haemostatic plugs in the present experiments but was a less striking feature of the aggregates than it is in artificial thrombi prepared from rat's blood (French and Poole, 1963) or from hamster's blood (French and Poole, unpublished). No clear evidence of loss of platelet granules was seen in the experimental thrombi, though at the time of fixation the thrombi had been present *in situ* for at least as long as the

haemostatic plugs fixed immediately bleeding ceased. The findings indicate that degranulation occurs later than platelet aggregation and that the delay may be increased under conditions *in vivo* when blood continues to flow past the platelet mass.

At present it is not clear to what extent morphological changes in platelets themselves or the formation of fibrin around the aggregates are associated with increased cohesion of the mass, but in the early experimental thrombi, in which neither of these changes was seen, direct observation showed that the platelet mass could readily be disrupted by the blood stream.

In addition to the attachment of the platelets to each other, the platelets also attach to tissues at the site of injury. In the haemostatic plugs, platelets were seen in close association with the injured arterial wall and with collagen fibres in the perivascular tissue. In the experimental thrombi, platelets were closely applied to sub-endothelial tissue, which included elastin and fine fibrillary elements, but few or no typical collagen fibres. The muscle in the wall was damaged by the electrical stimulus in these experiments, so that platelets were close to these damaged cells though not in actual contact with them. The possibility that substances released from injured cells may increase the adhesiveness of platelets has recently been investigated by Honour and Mitchell (1964). There is also evidence that aggregation of platelets can be induced by saline extracts of collagenous tissue (Hovig, 1963) and that platelets will adhere to sub-endothelial arterial fibres and to collagen fibres *in vitro* (Bounameaux, 1959 ; Zucker and Borrelli, 1962).

In contrast to the situation in the arteries, it is well recognised that in veins thrombi may occur when there is little evidence of damage to the wall as shown by conventional histological methods. However, when the endothelium of veins which have been subjected to the mild injury is viewed *en face*, it has been claimed that platelets are first seen to be adherent in the region of the cellular borders, though the cells themselves remain intact (Samuels and Webster, 1952). The demonstration with the electron microscope that platelets may be seen inserted in small gaps between cells at the endothelial junctions in small veins suggests one probable explanation of the alleged greater adhesiveness of the intercellular region following mild injury.

In the experiments on haemostasis, platelets and erythrocytes were concentrated in the segment of artery in which blood flow had ceased. Copley and Lalich (1941) and Macfarlane and Sanders (unpublished) have noticed that exudation of clear fluid follows the cessation of bleeding from the cut end of the tail of a mouse. This suggests that the haemostatic plug is permeable to the plasma for a short time after it has stopped the passage of red cells and that this filtration process could lead to the concentration of red cells and platelets which was observed proximal to the plug.

## SUMMARY

The structure of haemostatic plugs and experimental thrombi has been studied by electron microscopy in injured small arteries in the cheek pouch of the Syrian hamster.

The haemostatic plug which formed when an artery was transected was mostly outside the lumen of the vessel. When fixed immediately after bleeding had ceased it consisted of a mass of closely packed platelets which had retained

their structure and had intact boundary membranes. Fibrillary fibrin was seen only at the edges of the platelet mass. When fixed 30 min. after bleeding had ceased, some platelets were distorted in shape, their boundary membranes less distinct, and some at the margins of the mass had lost their granules. Platelets at the margins of the mass were closely associated with collagen fibres and with damaged parts of the vessel wall.

The thrombi which formed in arteries damaged by an electrical stimulus consisted almost entirely of a mass of closely packed platelets. The mass was attached to the damaged part of the wall where it had been denuded of endothelium. In adjacent small veins occasional groups of platelets were adherent to the wall at sites of minimal injury.

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## REFERENCES

BIZZOZERO, J.—(1882) *Virchows Arch.*, **90**, 261.
BOUNAMEAUX, Y.—(1959) *C.R. Soc. Biol. (Paris)*, **153**, 865.
CAULFIELD, J. B.—(1957) *J. biophys. biochem. Cytol.*, **3**, 827.
CHANDLER, A. B.—(1958) *Lab. Invest.*, **7**, 110.
COPLEY, A. L. AND LALICH, J. J.—(1941) *Amer. J. Physiol.*, **135**, 547.
EBERTH, J. C. AND SCHIMMELBUSCH, C.—(1886) *Virchows Arch.*, **103**, 39 ; **105**, 331, 456.
FLOREY, H. W., GREER, S. J., KISER, J., POOLE, J. C. F., TELANDER, R. AND WERTHESSEN, N. T.—(1962) *Brit. J. exp. Path.*, **43**, 655.
FRENCH, J. E. AND POOLE, J. C. F.—(1963) *Proc. roy. Soc. B*, **157**, 170.
FULTON, G. P., AKERS, R. P. AND LUTZ, B. R.—(1953) *Blood*, **8**, 140.
HAYEM, G.—(1882) *C.R. Acad. Sci. (Paris)*, **29**, 1907.
HONOUR, A. J. AND ROSS RUSSELL, R. W.—(1962) *Brit. J. exp. Path.*, **43**, 350.
*Idem* AND MITCHELL, J. R. A.—(1964) *Ibid.* **45**, 75.
HOVIG, T.—(1963) *Thromb. Diath. Haem.*, **9**, 248.
HUGHES, J.—(1959*a*) *Thromb. Diath. Haem.*, **3**, 34.—(1959*b*) *Ibid.*, **3**, 177.
JAQUES, L. B.—(1960) *Internat. Rec. Med.*, **173**, 481.
KJAERHEIM, Å. AND HOVIG, T.—(1962) *Thromb. Diath. Haem.*, **7**, 1.
LUBNITZKY, S.—(1885) *Arch. exp. Path. Pharm.*, **19**, 185.
LÜSCHER, E. F.—(1960) *Vox Sang. (Basel)*, **5**, 259.
MCEWEN, L. M.—(1956) *J. Physiol.*, **131**, 678.
PARMEGGIANI, A.—(1961) *Thromb. Diath. Haem.*, **6**, 517.
POOLE, J. C. F. AND FRENCH, J. E.—(1961) *J. Atheroscler. Res.*, **1**, 251.
*Iidem.* AND CLIFF, W. J.—(1963) *J. clin. Path.*, **16**, 523.
ROSKAM, J.—(1954) ' Arrest of Bleeding.' Springfield, Illinois (Charles C. Thomas). —(1960) *Schweiz. med. Wschr.*, **90**, 947.
SAMUELS, P. B. AND WEBSTER, D. R.—(1952) *Ann. Surg.*, **136**, 422.
WEINER, J. AND SPIRO, D.—(1962) *Exp. mol. Path.*, **1**, 554.
WELCH, W. H.—(1887) Reprinted in : ' William Henry Welch, Papers and Addresses ', (John Hopkins Press), Baltimore, 1920. Vol. 1, p. 47.
WHARTON-JONES, T.—(1851) Guy's Hosp. Rep., 2nd Series., **7**, 1.—(1852) *Phil. Trans.*, **142**, 131.
ZAHN, F. W.—(1875) *Virchows. Arch.*, **62**, 81.
ZUCKER, M. B.—(1947) *Amer. J. Physiol.*, **148**, 275.
*Idem* AND BORRELLI, J.—(1962) *Proc. Soc. exp. Biol. N.Y.*, **109**, 779.
ZUCKER, H. D.—(1949) *Blood*, **4**, 631.