SYMPOSIUM ON THROMBOSIS

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THE STRUCTURE OF NATURAL AND EXPERIMENTAL THROMBI

by

J. E. French, D.M., D.Phil.

Sir William Dunn School of Pathology, University of Oxford

WILLIAM WELCH (1899) in his classical article in Clifford Allbutt's *System of Medicine* defined a thrombus as " a solid mass or plug, formed in the living heart or vessels from constituents of the blood ". This definition emphasizes two points which must be taken into account when discussing thrombus structure. First, that we need to look no further than the constituents of the blood to find the elements which make up the structure of a thrombus; second, that the structure, at least in the beginning, is formed in flowing blood and not in a static system.

It has been known for a long time that two processes are concerned in the solidification of blood. Coagulation of the plasma, later defined in terms of the fibrinogen→fibrin reaction, is the most obvious; it was the first to be recognized and has been the most intensively investigated, since it lends itself to study *in vitro*. The other process is the aggregation of the formed blood elements, and, since a continuing supply of these elements is required for an appreciable mass to be built up, it is most readily observed in flowing blood. The process was recognized as early as the 1850s by Wharton Jones (1851, 1852) during direct observation on blood flow in vessels in the web of the frog's foot and in the bat's wing, but of course it was not until the blood platelets were identified later in the century that it could be appreciated that these particular formed elements had a predominant role in the process (Bizzozero, 1882).

It is perhaps unnecessarily obvious to point out that the significance of thrombosis is that it should occur in flowing blood and thereby impair or obstruct the circulation. In this sense the thrombus resembles the **haemostatic plug,** which forms at the cut end of a severed vessel and stops haemorrhage, and differs from the clot which forms when blood coagulates under static conditions. These differences are reflected in differences in the relative contribution of platelet aggregation and fibrin formation to the structure of the mass which is built up (Poole and French, 1961; Poole, 1964).

The clot which forms in a test-tube, or in a static column of blood in an already occluded vessel, has a homogeneous structure. It consists of a fibrin network in which the formed elements are distributed at random and few are aggregated. The thrombus, on the other hand, has, at least in parts, a more ordered structure in which pale and darker red zones alternate in a laminated pattern. Microscopically, the pale zones are seen in section as strands or islands of granular material which represents masses of aggregated platelets. The masses are fringed by leucocytes and fibrin and are separated from each other by a looser fibrin network in which red cells are entangled (Fig. 1).

Although the components are the same, there are differences in the gross appearances of thrombi as they occur in arteries and in veins. A typical thrombus in a leg vein consists of a pale head at the point of attachment

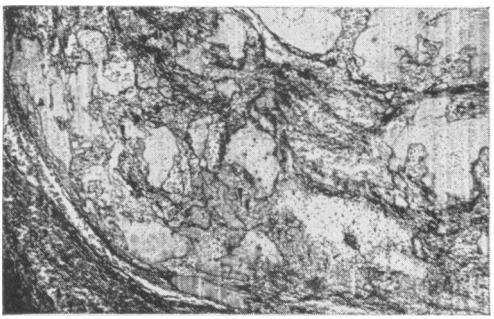

Fig. 1. Section through part of a human pulmonary embolus showing pale islands of granular material surrounded by a darkly stained rim of fibrin. Mallory trichrome. × 28.

to the vessel wall, a coarsely laminated neck and a dark red tail of varying length (Hadfield, 1950). Platelet aggregates form the major part of the pale head and extend as a coral-like structure into the neck. These are the parts of the thrombus which first obstruct blood flow, while the red tail, which is essentially a blood clot, forms later in the static column. Thrombi in arteries usually have a more compact structure; they are generally pale in colour and extend over a relatively short length of the vessel.

Examination of human material usually shows the fully developed thrombus; the stages in its development must therefore be deduced and, since it may have been *in situ* for days or weeks before being examined, its components have often undergone degenerative changes which make them difficult to identify. It is very largely as the result of animal experiments, in which the thrombus formation can be observed directly in living vessels, or in which tissues can be fixed at known stages, that it is possible to describe the sequence of events in thrombus formation and to state that the granular material which is seen in thrombi is of platelet origin.

The basic experiments establishing these points were carried out in the latter part of the last century. Bizzozero (1882) described the changes observed after a crush injury to small arteries or veins in the mesentery of a guinea pig as follows:

" After a few moments the formation of a thrombus begins. The platelets carried in the blood stream are stopped as soon as they have arrived at the injured area of the vessel wall; at first a few but soon increasing to hundreds. Many leucocytes are also stopped and the mass soon fills the lumen of the vessel and progressively impedes the flow of blood. If the flow is strong it breaks down the obstacle carrying the whole or part of the thrombus with it. But platelets again stop and accumulate so that within a quarter of an hour the process may be repeated three to four times."

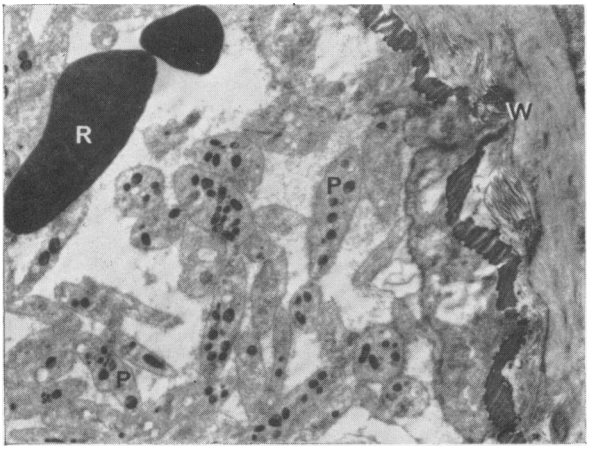

Fig. 2. A group of platelets (P) and a red cell (R) at the site of a crush injury to the wall (W) of a mesenteric artery in a rat. Electron micrograph. × 5,600.

These observations were extended by Eberth and Schimmelbusch (1886) and by Welch (1887), who emphasized that leucocytes appeared in the experimental thrombi later than the platelets and that visible fibrin did not appear until the blood flow was greatly reduced or stopped.

At the same time similar observations were made which demonstrated the important contribution of blood platelets to the structure of haemostatic plugs. Hayem (1882) noted that during the flow of blood from a small incision in the wall of a vein in a dog, the mass which formed at the site of injury and eventually plugged the opening consisted largely of aggregated platelets. Lubnitzky (1885) examined histological sections of haemostatic plugs in arterial wounds and illustrated areas which clearly consisted of aggregated platelets merging with areas of apparently structureless or granular material.

In a paper delivered before the Pathological Society of Philadelphia in 1887 Welch took up the then very controversial question of whether these observations in small vessels in experimental animals were relevant to the

situation in man, where, as has already been pointed out, fibrin and leucocytes are often very conspicuous components of thrombus structure and the granular component is by no means always clearly of platelet origin. He was able to show, first, that when experimental thrombi were examined at later intervals the number of leucocytes and the amount of fibrin increased, the latter as islands and bands between the masses of platelets, and that with time the experimental thrombi acquired all the characteristics of human thrombi. Secondly, he was able to satisfy himself that, in human thrombi which had recently formed, the granular component was composed of more or less altered platelets.

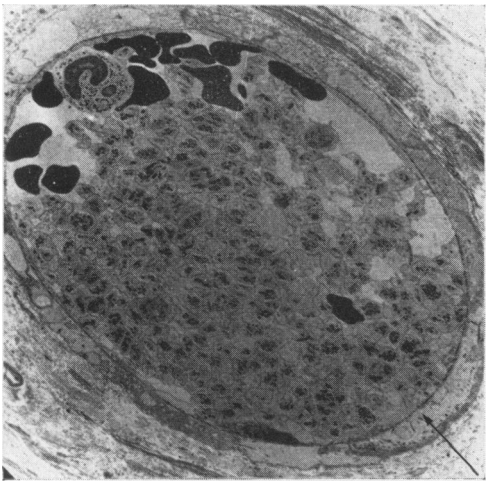

Fig. 3. A mass consisting of tightly packed platelets in the lumen of a small artery in a hamster cheek pouch. The arrows indicate the site of an electrical injury. Electron micrograph. × 1,900.

Subsequent studies on the structure of experimental thrombi and haemostatic plugs have confirmed this earlier work and there is no reason for not accepting it as established in pathology. The electron microscope has been used to fill in some points of detail beyond the resolution of the light microscope and to illustrate the platelets more clearly and hence more convincingly. It has not so far led to any major reappraisal of the work based on light microscopy, but has served to re-emphasize observations which until recently have not always been given the prominence they deserve. The platelets as seen by electron microscopy in sections of normal vessels are elliptical objects bounded by a well-defined membrane. They have no nucleus but contain cytoplasmic organelles of various kinds. The most prominent are the dense osmiophilic granules, but in addition there are a few mitochondria of relatively simple structure, vesicles of various sizes, and sometimes other granules or inclusions. The

appearance of platelets at the site of a crush injury in a mesenteric artery of a rat (essentially a repetition of Bizzozero's experiment) is shown in Figure 2.

Several investigations of the fine structure of experimental thrombi and haemostatic plugs have been carried out; these include descriptions by Kjaerheim and Hovig (1962) of haemostatic plugs in vessels of the rat mesentery, by Weiner and Spiro (1962) of organizing thrombi in the femoral veins of rats, and by French and Poole (1963) of artificial thrombi prepared from rats' blood by Chandler's method (Chandler, 1958). Recently we have made observations on the fine structure of thrombi and haemostatic plugs in the cheek pouch of the Syrian hamster (French *et al.*, 1964). This is a preparation which has previously been used by Fulton

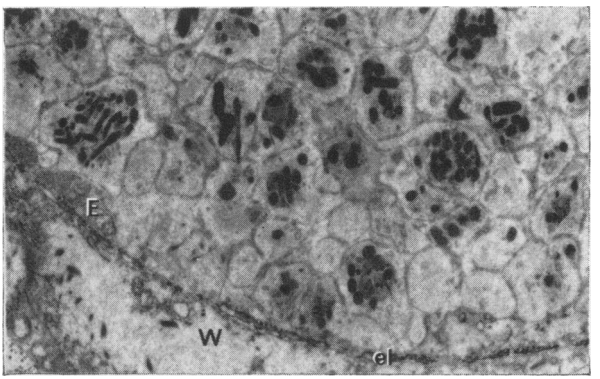

Fig. 4. A platelet mass attached to an injured artery wall (W). Much of the endothelium (E) has been destroyed and elastic fibres (el) are exposed to the lumen. Electron micrograph. × 5,600.

et al. (1953) for direct observations on thrombus formation and haemostasis in living vessels, and provides a convenient tissue for electron microscopy.

When a small artery in the pouch is injured by an electrical stimulus, a mural thrombus consisting entirely of platelets begins to form at the site of injury (Fig. 3). The mass increases in size until it nearly occludes the lumen, but is then usually swept away and does not produce a permanent stoppage of blood flow. It is notable that when examined at this stage no fibrin is seen within the platelet mass or at its edges, and only an occasional leucocyte is adherent at the edge. The platelet mass is adherent to exposed sub-endothelial fibres at its point of attachment to the damaged wall, and, throughout the mass, the platelets are very tightly packed together; indeed the gap between adjacent boundary membranes is so narrow that it would not be resolved by the light microscope (Fig. 4).

It is understandable that observations by light microscopy have suggested that platelets rapidly fuse together during the formation of a thrombus, but the electron microscope shows that at this early stage this is not so.

It is appropriate to mention a few other points about the behaviour of platelets in injured arteries. If the flow is brisk a mass of the size shown does not always form even when the endothelial lining is com-

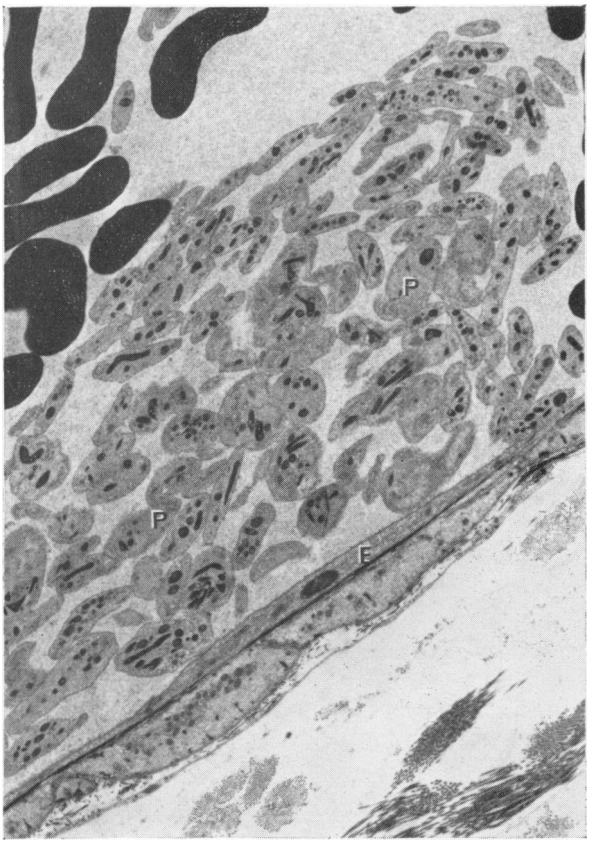

Fig. 5. Loosely aggregated platelets (P) in a side branch of an occluded artery. The endothelium (E) is intact. Electron micrograph. × 4,200.

pletely destroyed. Sometimes only a few platelets adhere to the damaged surface. On the other hand, when there is disturbance of flow, platelets may congregate in regions where there is no obvious injury to the wall. This is seen for example in the eddy currents at the mouths of side branches when flow is diverted from the main vessel by an occlusion of its lumen (Fig. 5).

In veins, it is well recognized that thrombi may occur at sites where there is little evidence of damage to the wall as shown by conventional histological methods. However, Samuels and Webster (1952), by studying the endothelial surface of veins subjected to mild injury, concluded that platelets could adhere in the region of the endothelial cell borders though

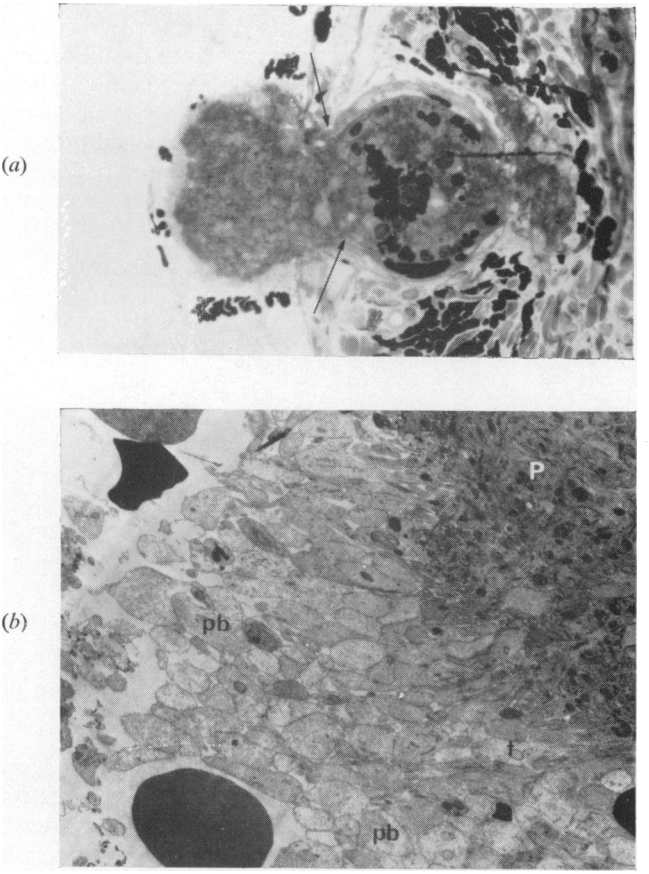

Fig. 6. (*a*) A haemostatic plug as seen by light microscopy. The plug is partly inside and partly outside the lumen. The arrows indicate the position of the cut. × 440. (*b*) The edge of the plug by electron microscopy shows a fringe of pale bodies (pb) and tightly packed platelets (P). × 4,800.

the cells themselves remained intact. In the hamster cheek pouch, small veins, near to the injured artery but not deliberately injured themselves, showed small groups of platelets in the lumen and, in some cases, these were attached to the wall by insertion into small gaps at the junctions between endothelial cells. This raises the possibility that in venous

thrombi a platelet mass may have quite small sub-endothelial roots of attachment which cannot readily be seen with the light microscope.

In order to follow Welch's arguments further and to make the comparison with natural thrombi, it is necessary to observe the structure of the experimental thrombus at later stages. This requires that the experimental thrombus can be retained *in situ* and located again at later intervals. So far we have not succeeded in doing this satisfactorily in the hamster cheek pouch, and it will probably be necessary to use a different experimental model. However, the haemostatic plug at the cut end of arteries in the pouch is an analogous structure to an occluding thrombus; it is not subjected to continuing blood flow and, since it is attached firmly to perivascular tissue, it remains in position and can be identified again later. We have used these plugs to observe the changes which occur in platelet

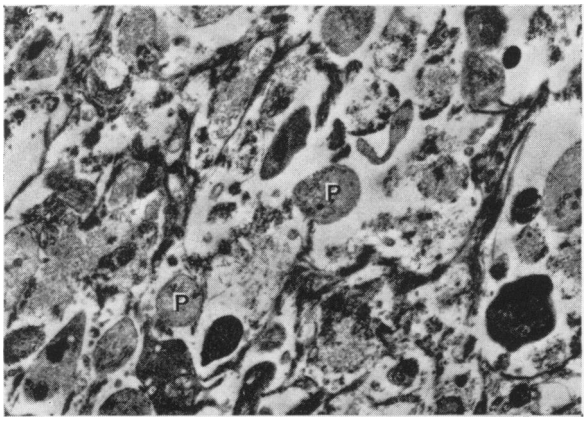

Fig. 7. Part of a haemostatic plug at 24 hours, showing altered platelets (P) and strands of fibrin (F). Electron micrograph. × 10,000.

masses with time. When fixed immediately bleeding has ceased, the plug, like the early thrombus, consists of a mass of closely packed platelets which have retained their structure and have intact boundary membranes. Traces of fibrillary fibrin can be seen at the edges of the mass but not within it. Fixed at 30 minutes, the platelets are more tightly packed and some are distorted in shape. At the edges of the mass there is now a fringe of paler bodies which appear to be either platelets which have lost their granules or pseudopod-like projections from the platelets deeper in the mass (Fig. 6). Fibrillary fibrin is more prominent at the edges and, particularly near to the margin of the cut arterial wall, appears to extend into the interstices in the fringe of pale bodies. At 24 hours, platelets can still be identified as circular or elliptical profiles in the plug. They appear to contain fewer granules, they are more loosely arranged and there is fibrillary fibrin extending through the spaces between them (Fig. 7).

It should be emphasized that the thrombi or plugs examined so far in these experiments have been very small objects. In larger thrombi there may well be differences in the relative proportions of the components and in the rate at which changes occur with time. With human material obtained at a post-mortem or surgical operation it is usually not possible to know precisely the age of a thrombus and, although surgical specimens can be fixed rapidly, it is probable that with post-mortem material further degenerative changes in the platelets or additional fibrin formation occur after death. These factors limit the applications of electron microscopy in the study of natural thrombi and so far little work on human material has been reported. Levene and Levene (1957) illustrated an area of a human pulmonary embolus in which the granular component was shown

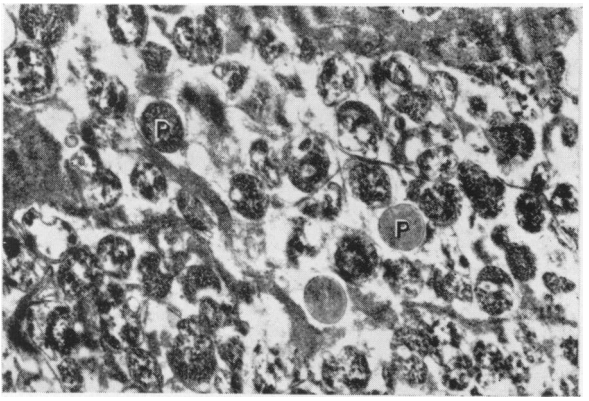

Fig. 8. Part of a human thrombus obtained at post-mortem from the surface of a prosthetic mitral valve. The round bodies appear to be altered platelets (P) separated by strands of fibrin (F). Electron micrograph. × 10,000. (*I am indebted to Professor P. R. Allison and Dr. A. H. T. Robb-Smith for permission to use the material illustrated in Figure* 8.)

to consist of platelets. The appearance of thrombus material, which was obtained at post-mortem from the surface of a prosthetic mitral valve, is shown in Figure 8. The platelets are not well preserved, but the general pattern of arrangement of altered platelets and fibrin is similar to that seen in the experimental haemostatic plugs which had remained *in situ* for 24 hours.

In conclusion, recent work has substantiated the view that the first event in thrombosis is the attachment of blood platelets to the vessel wall at a damaged site. If the local conditions of blood flow are favourable more platelets then adhere, at first loosely but later becoming closely packed together and forming a mass which restricts blood flow. At this stage the individual platelets are still discrete and have retained their normal form. Changes are then observed at the edges of the platelet aggregates which

include loss of platelet granules, sticking of leucocytes and fibrin formation. Fibrin formation probably reinforces the platelet mass and, when blood flow is stopped, its formation in the resulting static column may add very considerably to the total size of the thrombus. At still later stages, degenerative changes occur in the platelets and fibrin formation becomes still more extensive throughout the mass. Natural human thrombi are likely to show marked degenerative changes since they are usually examined at a late stage in their development.

REFERENCES

BIZZOZERO, J. (1882) *Virchows Arch.* **90,** 261.
CHANDLER, A. B. (1958) *Lab. Invest.* **7,** 110.
EBERTH, J. C., and SCHIMMELBUSCH, C. (1886) *Virchows Arch.* **103,** 39; **105,** 331, 456.
FRENCH, J. E., and POOLE, J. C. F. (1963) *Proc. Roy. Soc. B.*, **157,** 170.
———— MACFARLANE, R. G., and SANDERS, A. G. (1964) *Brit. J. exp. Path.*, **45,** 467.
FULTON, G. P., AKERS, R. P., and LUTZ, B. R. (1953) *Blood*, **8,** 140.
HADFIELD, G. (1950) *Ann. Roy. Coll. Surg. Engl.* **6,** 219.
HAYEM, G. (1882) *C.R. Acad. Sci. (Paris)*, **29,** 1907.
KJAERHEIM, Å., and HOVIG, T. (1962) *Thrombos. Diathes. haemorrh. (Stuttg.)* **7,** 1.
LEVENE, M., and LEVENE, C. I. (1957) *J. clin. Path.* **10,** 200.
LUBNITZKY, S. (1885) *Arch. exp. Path. Pharm.* **19,** 185.
POOLE, J. C. F. (1964) *Scientific Basis of Medicine Annual Reviews*, p. 55.
———— and FRENCH, J. E. (1961) *J. Atheroscler. Res.* **1,** 251.
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*, **1,** 47, Baltimore: Johns Hopkins Press.
———— (1899) in *A System of Medicine*, **6,** 155, edited by C. Allbutt, London: Macmillan.
WHARTON JONES, T. (1851) *Guy's Hosp. Rep.* 2nd ser. **7,** 1.
———— (1852) *Phil. Trans.* **142,** 131.

PLATELETS IN THROMBOGENESIS: MECHANISM AND INHIBITION OF PLATELET AGGREGATION

by

G. V. R. Born, M.A., M.B., Ch.B., D.Phil.

Vandervell Professor of Pharmacology, Royal College of Surgeons of England and University of London

IT IS GENERALLY believed that thrombosis has two causes: damage of the vessel wall by disease or by injury and alteration in the blood which leads to the adhesion to the site of damage of circulating platelets and their rapid aggregation into clumps or " white bodies ". These bodies slow or stop the flow of blood which thereupon clots. The classical thrombus consists, therefore, of a white " head " consisting of aggregated platelets and a red " tail " consisting of clot. This paper is concerned only with the second cause.