CONCERNING THE ORGANIZATION OF THROMBI

Erasmus Wilson Demonstration on 4th June 1970

by

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THE FATE OF thrombi is a subject that has interested me for a number of years, both through the examination of human material and by animal experimentation. The subject is not only of considerable clinical importance in view of the frequency of thrombotic disease but raises a number of fundamental pathological questions as well.

The fate of mural thrombi

Many thrombi begin life by being mural and become replaced progressively by fibrocellular tissue, and the origin of the cells concerned in this process will be referred to later. There can now surely be no doubt that many of the localized venous intimal thickenings, which are such a common microscopical finding in leg veins, result from the organization, total or partial, of mural thrombi[1]. On the other hand, thrombi may start off by being occlusive and then become converted into the mural variety through their retraction from the vessel wall, the lumen being re-established as shown by the presence of fresh red cells in the gap between the thrombus and vessel wall, while the surface of the thrombus exposed by retraction becomes covered by leucocytes. Like their mural counterparts, these retracted occlusive thrombi become replaced by fibrocellular tissue to a greater or lesser extent so that in both instances the end result is a localized intimal thickening, sometimes containing fat-laden phagocytes, the fat resulting from the breakdown of red cells and platelets in the thrombus. Many of these venous intimal thickenings have a laminated appearance when stained for elastic tissue, pointing to their being formed as a result of several episodes of thrombosis.

Experimental 'thrombin' thrombi

It is appropriate to say a word here about the thrombotic masses produced experimentally in vessels following the injection of thrombin. These masses consist predominantly of red cells but have a peripheral rim of fibrin, containing platelets and leucocytes, while tangential bands of fibrin transect the periphery of the central red cell mass. Microscopically these experimental thrombi are basically similar to the red propagated thrombus forming the bulk of the thrombotic mass in cases of phlebothrombosis, proving that they are indeed a relevant experimental tool[2, 3].

Thrombotic disintegration

There is massive evidence that many thrombi and thrombo-emboli disintegrate although the exact mechanism for this is not clearly under-

stood. A semi-quantitative study of the fate of 'thrombin' thrombi formed in the marginal ear vein of the rabbit revealed that over 80% of their total mass disintegrated rapidly, while the extent to which they were replaced by fibrocellular tissue was minimal[3]. This massive disintegration is not confined to the macroscopical thrombi forming in veins but also applies to the microthrombi, composed of fibrin and platelets, produced in the generalized Schwartzmann reaction by the intravenous injection of two suitably spaced doses of lipopolysaccharide. Shortly after the administration of the second dose it is possible to identify large numbers of such microthrombi in the liver, lungs, spleen and kidneys. Three days later, however, it is only with the greatest difficulty that any evidence of them can be found, the inference being that the vast majority disintegrate[4]. Finally, pulmonary angiograms have shown that in some episodes of massive non-fatal pulmonary embolization the whole or virtually the whole of the thrombotic mass in the pulmonary artery undergoes progressive disintegration ultimately[5].

What is the mechanism for this massive disintegration of thrombotic material? Plasminogen tissue activator is produced by vascular, particularly venous, endothelium. Plasminogen is a normal constituent of plasma, and when fibrin is formed becomes attached to it. On being activated to plasmin it brings about fibrinolysis, at least under experimental conditions[6]. It has been suggested by many that the production of plasminogen tissue activator by endothelium may be responsible for thrombotic disintegration and this question will be discussed later.

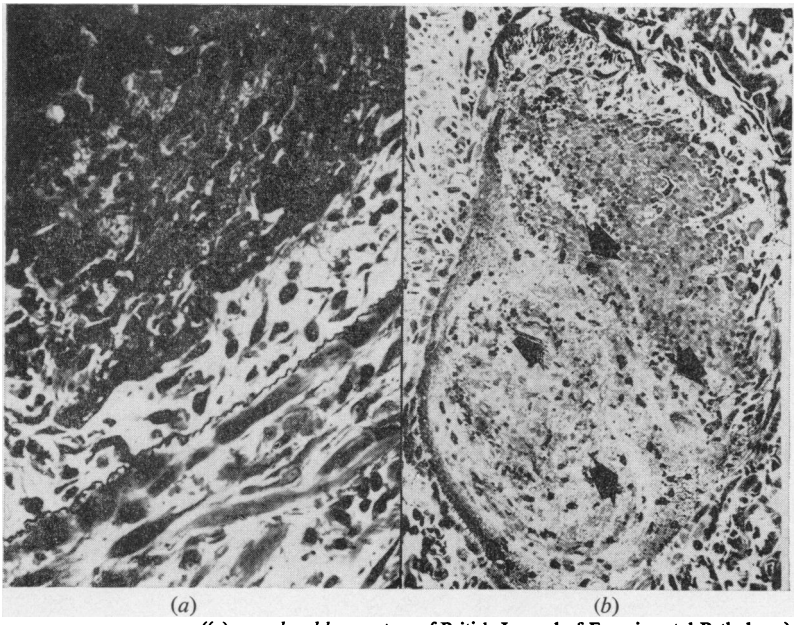
If indeed endothelium be responsible for the disintegration of thrombi in this way, this process must surely depend on the ability of vascular endothelium to remain viable when covered by thrombus and the longer the endothelium remains thus covered the less likely will it be able to bring about the disintegration of the thrombus.

The fate of permanently occlusive thrombi

The kernel of this lecture, the question of occlusive thrombi which neither retract from the vessel wall to any significant extent nor undergo early and massive disintegration, will now be considered. In a search for evidence pointing to the factors determining the extent to which such thrombi undergo organization and vascularization, a large number of naturally occurring and experimentally induced thrombi and thromboemboli were examined.

In some it was obvious that the cells concerned in the organization, whatever their origin, tended to be more numerous at the periphery of the thrombotic mass than in its centre and sometimes these cells remained confined to this peripheral zone (Fig. 1*a*)[3]. They appeared either unable to penetrate into the thrombotic mass or, if they could do so, were unable to survive and differentiate therein.

Frequently, this peripheral cellular zone became demarcated sharply from the central mass, being divided from it by a layer of flattened cells resembling endothelium. Although nucleated cells were still present at the edge of the residual thrombotic mass they appeared unable to participate in the organization, and ultimately the central mass disintegrated[7]. A similar sequence of events took place in 'thrombin' thrombi induced in the femoral artery and vein respectively of the dog, the edge of the thrombus becoming replaced by a rim of cells, and the



(*a*) (*b*)

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Fig. 1. (*a*) Four-day-old experimental venous thrombus showing peripheral zone of cells. Section 1μ thick from plastic embedded material (Methylene blue × 260). (*b*) Five-day-old experimental venous thrombus divided into two red cell masses by fibrin band containing nucleated cells (arrows). Marginal organization is occurring at one edge. Montage prepared from three photomicrographs of 1μ thick section from plastic embedded material (Methylene blue × 100) (*Arch. Path.* 1969, **87**, 643).

centre of the thrombus disintegrating[8]. This similarity between the events in an artery and a vein raised the question of the possible rôle, if any, of the particular vessel in determining the extent of organization.

The other extreme was observed when the cells concerned were scattered throughout the whole thickness of the thrombus and in places were forming small vascular lacunae[9]. In such cases the progressive proliferation of such cells led to the whole thickness of the thrombus being replaced by fibrocellular tissue containing a variable number of vessels of differing sizes, usually small. In between these two extremes instances were

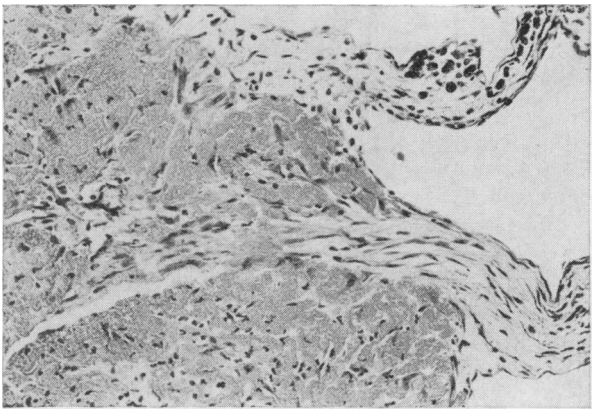

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Fig. 2. Band of fibrous tissue growing across an organizing red cell thrombus. Large vascular spaces have been formed through disintegration of part of thrombus (Haematoxylin and Eosin × 125) (*Arch. Path.* 1969, **87**, 643).

encountered where the cells concerned were able either to penetrate into or exist within the thrombus to a differing extent in different areas. In Figure 1 (*b*) two red cell masses are divided by a zone of what proved, on electron-microscopy, to be fibrin containing elongated cells. A further stage in this process is shown in Figure 2 where, in an organizing venous thrombus, a band of fibrocellular tissue is traversing a red cell mass, massive disintegration of thrombus having taken place leading to the formation of large cavernous vascular spaces. The end-point of this process is seen in Figure 3, where the vascular lumen has become transsected by a fibrocellular band and disintegration of the residual thrombus has occurred.

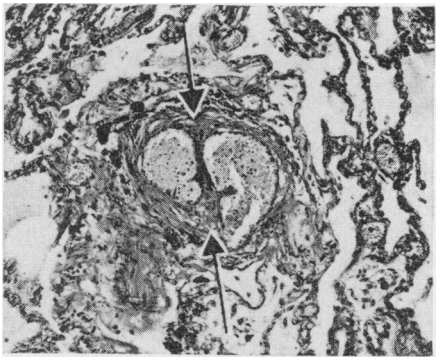

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Fig. 3. A small pulmonary artery whose lumen has become transected by a band of fibrous tissue (arrows) (Haemotoxylin and Eosin × 80) (*Arch. Path.* 1969, **87**, 643).

Classification of organized occlusive thrombi

The variety of appearances presented by occlusive thrombi in all stages of organization and revascularization suggested that the final results could be grouped broadly into three main types as shown in Figure 4 [8]. In marginal organization the vessel wall is lined by a thin layer of fibrocellular tissue, which is either intermittent or continuous in which event the terms partial or complete marginal organization are used respectively.

At the other extreme there is occlusive organization where the whole of the lumen of the vessel is blocked by fibrocellular tissue containing small vascular lacunae of varying size[9]. In between there is septate organization where the lumen is transected by one or more, sometimes

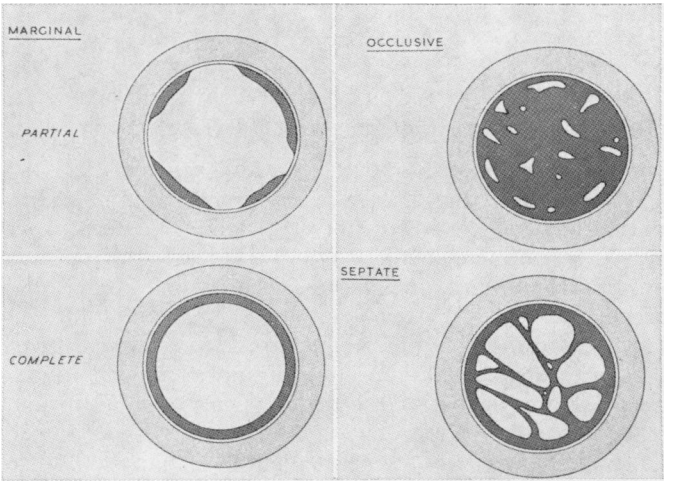

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Fig. 4. Chart showing marginal, occlusive and septate organization (*Arch. Path.* 1969, **87**, 643).

branching, fibrous septa, thus producing large cavernous vascular spaces. Transitions occur between these varieties, for it is obvious that examples of occlusive organization can become converted into the septate variety through the dilatation and coalescence of the vascular spaces therein. Conversely it is possible that septate organization may veer towards the occlusive type through secondary thrombosis of the large vascular spaces, such secondary thrombi themselves undergoing organization. Finally, elements of two or more of these types can sometimes be seen in the same thrombus.

Nevertheless these three varieties undoubtedly exist in their extreme and classical forms and the very existence of such forms throws light on one of the main, if not the main, factors determining the extent to which

individual thrombi are replaced by fibrocellular tissue. The extent of such replacement depends on the initial location of the leucocytes within the thrombus and their ability to survive and proliferate within the thrombus. Marginal organization seems to occur in those thrombi in which the leucocytes are concentrated in the periphery of the thrombus, occlusive organization results when the cells are scattered throughout the thrombus, while septate organization seems to arise when bands of fibrin containing leucocytes transect the thrombotic mass.

Thus the extent to which thrombi organize depends very largely on their structure[8]. The close relationship between the initial distribution of the leucocytes within the thrombus and the ultimate distribution of the fibro-cellular tissue is surely further evidence that the cells concerned are derived predominantly from the leucocytes, particularly the monocytes. However, in marginal organization in veins it is sometimes impossible to escape the likelihood that cells from the intima may play some part (Fig. 1*b*).

Modes of vascularization of organizing occlusive thrombi

These studies have also shown that new vascular spaces can be formed, not only by the proliferation of vaso-formative cells leading to the formation of vascular lacunae, but also through failure of areas of thrombus to be replaced by fibro-cellular tissue, and this raises the question of the mechanism of thrombotic disintegration. The possible rôle of fibrinolysis in this process has already been mentioned. In support of this is the fact that leucocytes alighting on artificial graft material and forming new endothelium are capable of elaborating fibrinolytic agents[10] and thus it may be that polymorphs within the thrombus bring about disintegration of the latter through the production of proteolytic enzymes. While this is an attractive idea it must be pointed out that, in the studies described here, those thrombi or parts of thrombi undergoing disintegration seemed to be those either totally or relatively bereft of leucocytes.

The rôle of the vessel in the organization of occlusive thrombi

The question of the possible rôle of the type of vessel, and thus differences in intraluminal pressure in determining the type of organization, was investigated by occluding the femoral arteries and veins of dogs by means of ligatures and inducing ' thrombin ' thrombi upstream and downstream from them[2]. The animals were killed at intervals of up to four weeks, and after fixation the vascular bundles were cut horizontally into blocks 2–3 cm. thick and a section examined from the same aspect of each block, thus providing a picture of the state of affairs from one end of the specimen to the other through the various vascular segments. Thrombi were found in all cases upstream from the venous ligatures, although seldom downstream, and were basically similar to the ' thrombin '

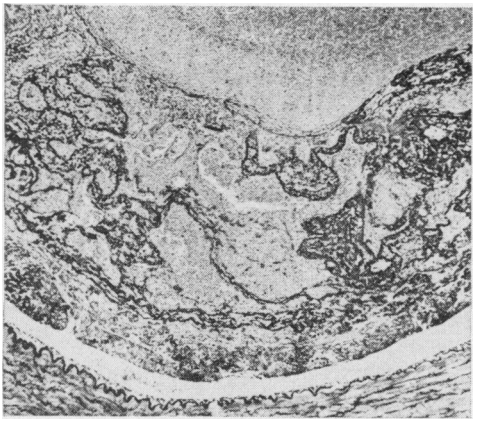

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Fig. 5. Experimental arterial thrombus showing compact rim composed of of layers of fibrin, platelets and leucocytes demarcated sharply from central red cell mass (Verhoff and Van Giesen × 40).

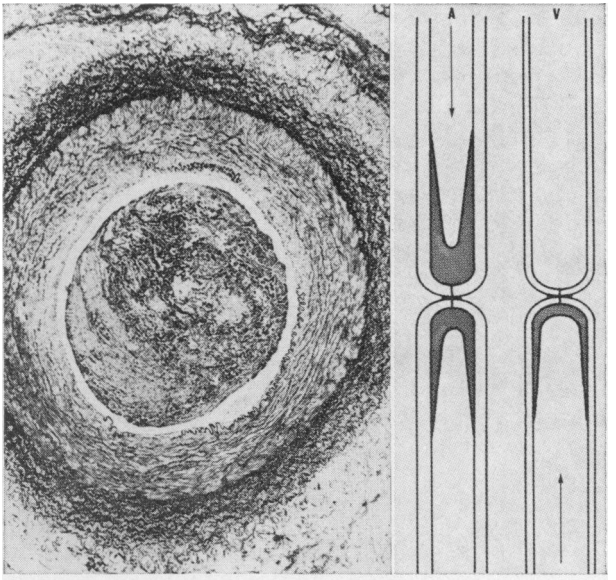

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Fig. 6. (*a*) Experimental arterial thrombus close to occlusion. The whole thickness of thrombus is composed predominantly of layers of fibrin, platelets and leucocytes (Verhoff and Van Giesen × 20). (*b*) Diagram of occluded femoral artery and vein showing relative thickness of fibrin and cellular zone (dark grey areas). (Not drawn to scale.)

thrombi already described[2, 3]. They possessed a thin peripheral layer of fibrin containing platelets and leucocytes, and tangential bands of similar composition tended to be concentrated near the periphery. Close to the occlusion these bands extended through practically the whole thickness of the thrombus. With the passage of time, organization of venous thrombi was only marginal and even then mainly partial; although close to the occlusion, where the fibrin stretched across the whole thrombus, it was sometimes occlusive. Although basically the same situation occurred in arteries, there were certain important differences. Considerably upstream from the point of occlusion the vessel was lined by a thin dense rim of similar composition to that seen in veins, sharply demarcated from a central mass of red cells devoid of leucocytes. The closer to the point of occlusion the thicker this rim became (Fig. 5), until finally the whole lumen was filled with a dense mass of fibrin, platelets and leucocytes with small islands of red cells therein (Fig. 6*a*). In sharp contrast to the findings in veins, the extent to which these thrombi underwent organization was much greater. The resultant rim of marginal organization was always much thicker and increased in thickness as the occlusion was approached until finally the whole lumen was filled with fibrocellular tissue. Occasionally a band of fibroblasts containing one or two small vessels transected a thrombus but such bands did not apparently survive the disintegration of unorganized thrombus, as at no time was septate organization the final result. The results of this experiment are summed up in Figure 6 (*b*), the thickness of the initial layers of fibrin, platelets and leucocytes always being thicker in arteries, both upstream and downstream from the occlusions, than in veins, while the resultant organization closely paralleled this pattern. It would appear that the more vigorous pulse pressure in the arterial as opposed to venous segments—and this would be enhanced by the greater recoil of the vessel wall—results in fibrin, platelets and leucocytes becoming packed into a thicker and denser layer leading to greater replacement of the thrombus concerned by fibrocellular tissue.

Thus, to sum up, it would appear that the extent of the organization of occlusive thrombi depends on their structure, which in its turn depends on the pulse pressure in the vessel concerned.

ACKNOWLEDGEMENTS

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PROCEEDINGS OF THE COUNCIL IN NOVEMBER

At a meeting of the Council on 12th November 1970, with Sir Thomas Holmes Sellors, President, in the Chair, the Mitchiner Medal for 1971 was awarded to Colonel John H. H. Oliver, T.D., Q.H.S., M.R.C.S., L.C.R.P., R.A.M.C. (V.), of Tenbury Wells, Worcestershire.

Professor J. G. Robson was appointed Joseph Clover Lecturer for 1972.

The appointment of Dr. Christopher Pedler of the Institute of Ophthalmology as the Edridge-Green Lecturer for 1970 was reported.

The Begley Prize was awarded to Dr. R. N. Clayton, M.R.C.S., of the London Hospital Medical School.

Diplomas of Membership were granted to 182 candidates.

Licences in Dental Surgery were granted to 68 candidates.

Diplomas in Orthondontics were granted to 26 candidates.

The following Diplomas were granted, jointly with the Royal College of Physicians:

Child Health (119), *Industrial Health* (21), *Medical Radio-diagnosis* (55), *Tropical Medicine and Hygiene* (31), *Ophthalmology* (32), *Laryngology and Otology* (1), *Psychological Medicine* (1).

Hospitals were recognized under paragraph 23 of the Fellowship Regulations and details are available on application to the Examinations Secretary.

After the meeting the Victor Bonney Lecture was delivered by Professor J. A. Stallworthy on ' The surgery of endometrial cancer—in the Bonney tradition'.