A QUANTITATIVE STUDY OF THE FATE OF OCCLUSIVE RED VENOUS THROMBI

G. B. D. SCOTT*

From the Department of Academic Pathology, Medical College of Virginia, Richmond, Virginia

Received for publication May 11, 1968

ALTHOUGH considerable attention has been devoted to the fate of experimental occlusive and mural thrombi in arteries (Williams, 1955; Still, Ghani and Dennison, 1967) and of arterial thromboemboli (Harrison, 1948; Heard, 1952; Barnard, 1953; Still, 1966; Casley-Smith, Ardlie and Shwartz, 1967), comparatively little has been paid to that of venous thrombi.

Robertson, Perrett, Colebeck and Moyes (1954) commented on the paucity of changes in the vessel wall in experimental portal vein thromboembolism in the dog while Filshie and Scott (1958) traced the organization of thromboemboli in the intrahepatic radicles of the portal vein of the rabbit. Wiener and Spiro (1962) described the organization of occlusive thrombi induced in the femoral veins of rats by sodium morrhuate and Stirling, Tsapogas and Girolami (1966) that of mural thrombi formed on gutters of nylon tubing inserted into the inferior vena cava of the rabbit.

Consequently, it was considered desirable to examine quantitatively the fate of occlusive venous thrombi, produced *in situ* without pre-existing phlebitis and with the minimum of trauma.

MATERIALS AND METHODS

New Zealand white rabbits of both sexes, weighing 1–2 kg., were fed on Purina chow with lettuce and unrestricted water. The skin over the subcutaneous vein in the lateral margin of the ear was shaved over a distance of approximately 3 cm. Care was taken to avoid obvious trauma and only those veins with small tributaries were selected.

Systemic injection of barium sulphate eluates of dog serum failed to induce intravascular coagulation in the ear vein in the presence of stasis, even in doses large enough to fill the whole of the portal vein with soft red clot under similar conditions (Scott, 1959). Thrombosis was induced therefore by " Topical Thrombin " (Parke-Davis).

The marginal vein was compressed by a light clamp applied to the ear near its base and approximately 0·1 ml. of thrombin (100 units per ml. of saline) injected into the vessel between 2·0–2·5 cm. distal to the clamp, a second clamp being applied just proximal to the site of injection. In several animals the mere insertion of a hypodermic needle into the vein replaced the injection of thrombin.

The formation of opaque i.v. clot could be observed by transilluminating the ear and, after 10 min., clotting had extended throughout the length of the vessel. A silk suture, passed through the whole thickness of the ear just distal to the proximal clamp but wide of the vein, was tied on the edge of the ear, thus compressing the vessel. The clamps were then removed and the flow of blood past the site of the distal one re-established by gently massaging the vein from the tip of the ear proximally.

The marginal veins of both ears were treated in this way and animals killed at intervals with Nembutal injected into a separate ear vein. A longitudinal strip of tissue, comprising

* Present address: Department of Morbid Anatomy, Royal Free Hospital, London, W.C.1.

the whole thickness of the ear and including ths suture and site of the distal clamp, was removed and fixed in glutaraldehyde to ensure that thrombi were not dislodged during subsequent manipulation. Two hr. later the strip was trimmed longitudinally to remove excess tissue. The proximal and distal 0·25 cm., representing the sites of the suture and distal clamp respectively, were cut off, thus discarding those parts of the vein most likely to have been traumatized. The remaining strip, now approximately 1·0 cm. long was cut transversely into blocks 1·0 mm. thick. These were embedded in a mixture of Araldite and Epon, the presence of the underlying ear cartilage facilitating their correct orientation. Sections 1 μ thick were stained with Richardson's methylene blue and examined by light microscopy. By examining all the blocks cut from the whole length of vein processed, it was possible to assess the fate of the whole thrombus, with particular reference to reduction of its total bulk, with some degree of accuracy. Where necessary ultrathin sections were examined by electron microscopy using an R.C.A. EMU3G.

RESULTS

Twenty-eight veins from 14 rabbits were examined at intervals of up to 14 days after the formation of thrombi, originally occupying the whole length of the vessel. The thrombi were of uniform composition throughout and initially occluded the lumen of the vein completely. They consisted of erythrocytes and showed a rim of fibrin in contact with the vein wall, thin tangential bands of fibrin sometimes intersecting the thrombus near its periphery, while small tufts lay between individual erythrocytes. Leucocytes were scattered evenly amongst the erythrocytes but sometimes clustered in and around the tangential bands and peripheral rim of fibrin. Sometimes small clumps of platelets adhered to intact endothelium, which was, however, more vesiculated than normal and contained occasional osmophilic inclusions (Fig. 1).

The subsequent changes in the thrombi and vein wall occurred at different rates in different vessels. After 2 hr., part of the circumference of a thrombus had retracted from the vein wall, the peripheral rim of fibrin adhering to the shrinking thrombus, while the endothelium remained in position. Fresh erythrocytes now lay in the space so formed and circulating leucocytes adhered to the now nonocclusive thrombus (Fig. 2). Although such separation was seen in only one of 4 thrombi aged 5 hr. or less, it was impossible to determine subsequently the exact proportion of originally occlusive thrombi which became nonocclusive or marginal in this way.

By 18 hr. erythrocytes and leucocytes in the depths of the thrombus had become swollen. The former were compressed into a regular mosaic pattern, and the latter had lost many of their features, those examples of both varieties lying near to or within fibrin bands appearing better preserved.

After 48 hr., thrombi were composed of amorphous material containing blurred erythrocytes and disintegrating leucocytes. The edges of some thrombi now had a loosely woven appearance suggesting lysis (Fig. 3). The occurrence of lysis was confirmed by finding either occlusive thrombus or small tangles of fibrin in less than half the blocks from vessels examined at this stage and the presence of occlusive thrombus and fibrin tangles in only 2 of 10 blocks from a vein a mere 18 hr. after thrombosis showed how fast thrombolysis could occur (Fig. 4).

Recognizable changes of organization appeared as early as 24 hr., part of the periphery of the thrombus, as well as the underlying vein wall, being invaded by mononuclear cells, polymorphonuclear leucocytes being absent. These mononuclear cells were scanty towards the centre of the thrombus whether the latter was still occlusive or had retracted from the vein wall (Fig. 5).

Unlike thrombolysis, organization proceeded slowly and after 5 days usually did not amount to more than a thin peripheral layer or crescent of mononuclear cells and fibroblasts which were seen often to be internal to an intact internal elastic lamina (Fig. 6), although the latter was always absent over some part of the area concerned. Many of the mononuclear cells invading the thrombus could be identified as macrophages by their large size, prominent mitochondria and osmophilic inclusions (Fig. 7). Numerous dead cells, with dark shrunken vesiculated cytoplasm, were present as well as occasional well-preserved erythrocytes. By 4 days many macrophages were becoming elongated (Fig. 8) and were beginning to resemble fibroblasts, the amount of cytoplasm increasing and their endoplasmic reticulum becoming more conspicuous. Initially such cells lay at right angles to the vein wall but ultimately lay parallel to it (Fig. 6). Similar cells were also present in the underlying media.

Total organization of occlusive thrombi was confined to only 2 of 14 veins examined after intervals of 4 days or more and was not seen before 7 days. In one of the two vessels total occlusion of the lumen was restricted to only one of several 1 μ sections from one of the 10 blocks examined, although in the other most of the length of the vessel was totally occluded. The fibrocellular tissue consisted of fibroblasts and several small spaces, lined by endothelium, were present. These contained occasional red cells and plasma and were all separated from the continuous internal elastic lamina by fibrocellular tissue (Fig. 9).

Shallow intimal accumulations of cells, covered by endothelium but devoid of overlying thrombus were seen as early as 4 days. They were usually localized

EXPLANATION OF PLATES

FIG. 1. Intact but abnormal endothelium covered by clump of platelets (top left). A muscle cell (bottom right) is separated from the endothelium by a thin, poorly defined internal elastic lamina. × 4450.

FIG. 2.—After 2 hr. a thrombus has retracted and become partially separated from the vein wall. Leucocytes adhere to its surface and fresh erythrocytes lie in the space between thrombus and vein wall. × 85.

FIG. 3.—After 48 hr. a thrombus, partially retracted from vein wall, shows loosely-woven appearance around its periphery, suggesting thrombolysis. × 85.

FIG. 4.—A vein, once filled with thrombus contains, after 18 hr., only two small masses of fibrin. A smaller mass of fibrin (arrow) adheres to the vessel wall. × 85.

FIG. 5.—A thrombus, 4 days old and containing scanty leucocytes, has retracted from the vein wall. Its base is undergoing organization and a layer of hypertrophied muscle is appearing (arrow). × 85.

FIG. 6.—The same thrombus showing a sharp line of demarcation between it and the layer of macrophages and fibroblasts, the later now lying parallel to the vein wall. × 200.

FIG. 7.—A group of macrophages, one of which contains osmophilic inclusions, invading a 5-day-old thrombus. × 4050.

FIG. 8.—A macrophage, containing several small inclusions, invading a thrombus. The cell is becoming elongated. Intact erythrocytes are present as well as dark, shrunken, vesiculated cells and fibrin (bottom right). × 4050.

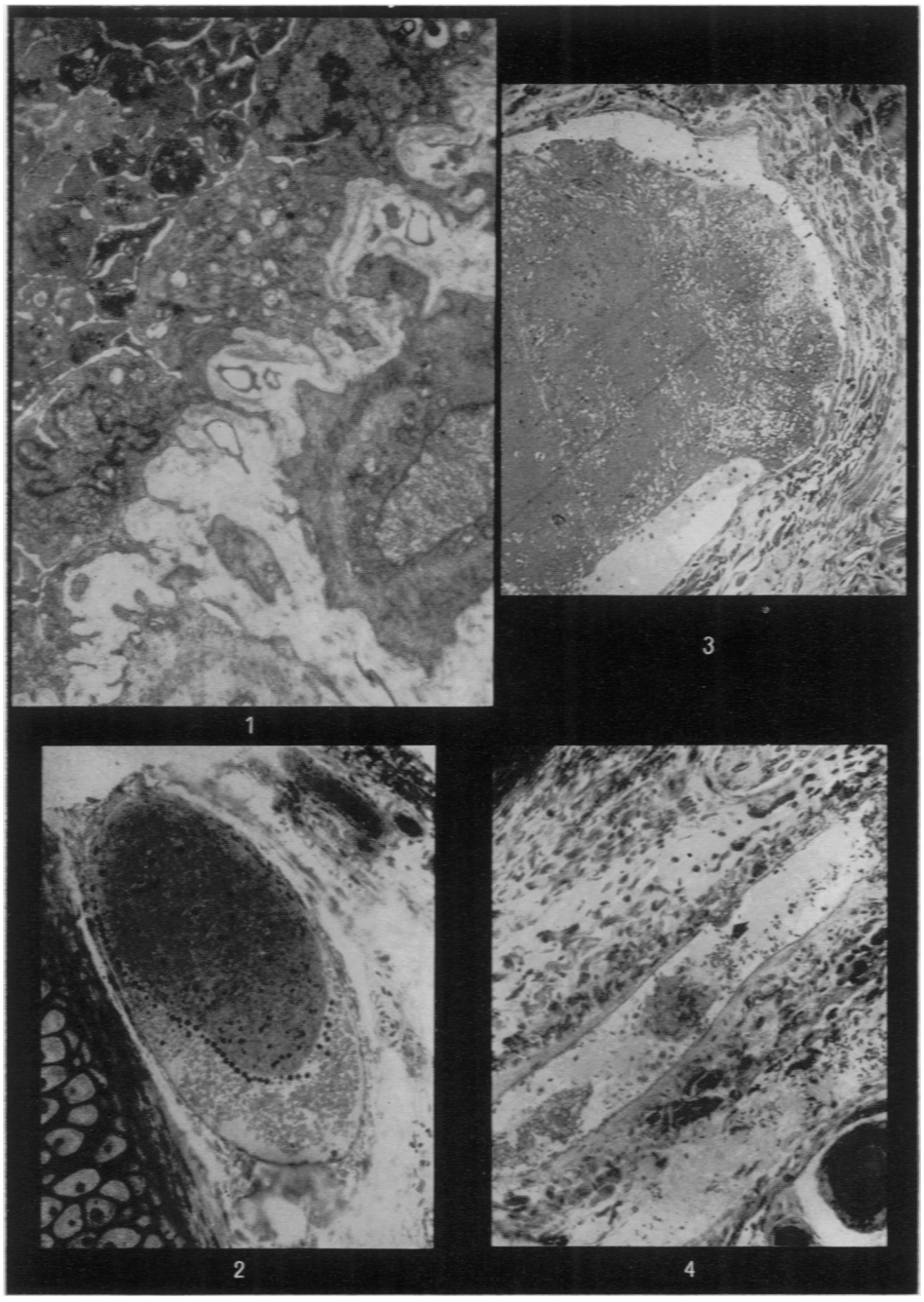
FIG. 9.—By 14 days this thrombus has become converted into fibrocellular tissue containing several small vessels. × 85.

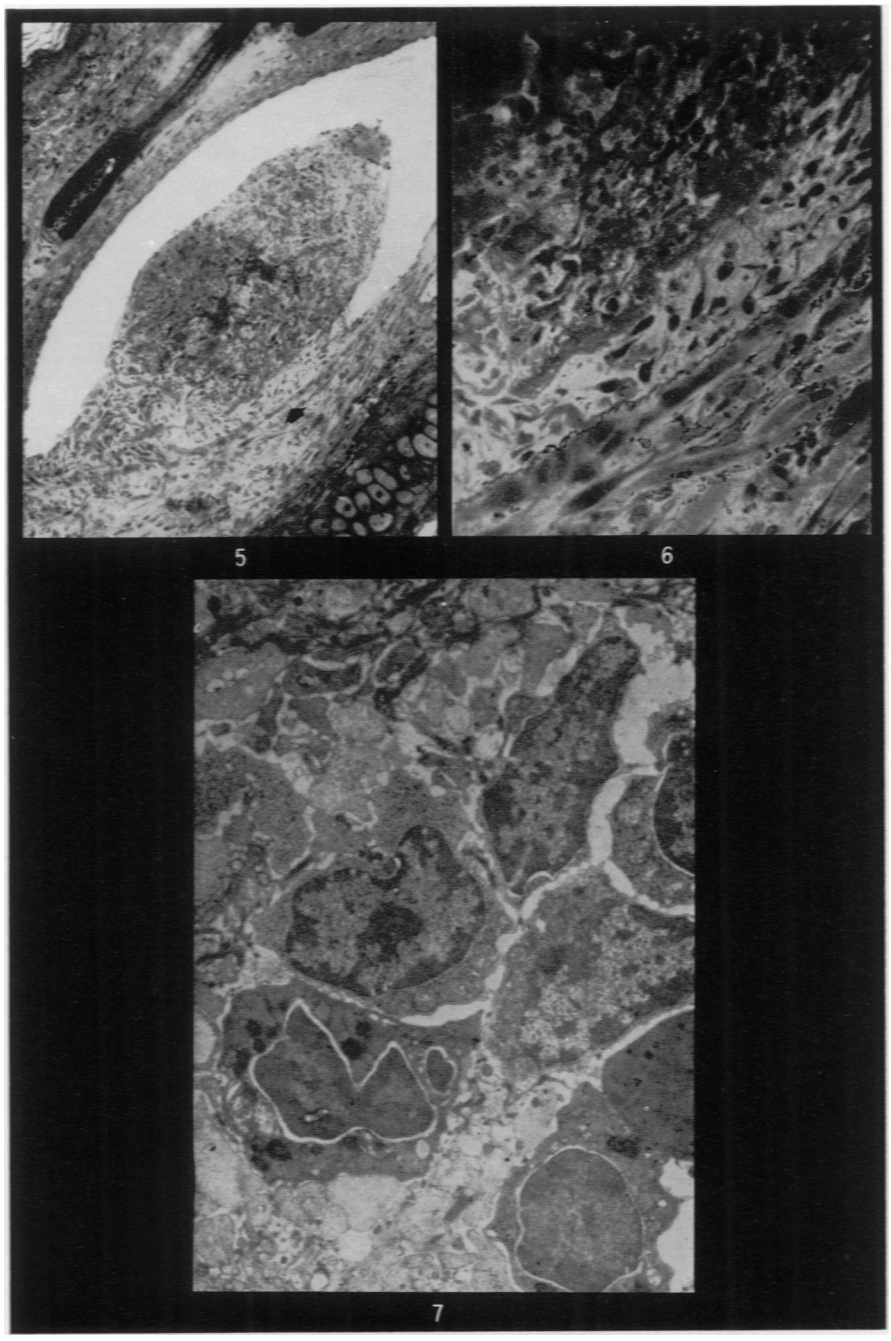
FIG. 10.—Seven days after thrombosis this vein contains a crescentic intimal accumulation of cells. The underlying muscle is hypertrophied (arrow). × 85.

FIG. 11.—A seven-day-old intimal thickening containing fibroblasts and well-formed collagen (C). × 4050.

FIG. 12.—Seen 4 days after thrombosis this intimal thickening contains a fibroblast (left); with prominent endoplasmic reticulum and two muscle cells (M) cut transversely. The internal elastic lamina is included (bottom). × 2850.

FIG. 13.—An intimal thickening, seen 4 days after thrombosis, is covered by a thrombus of more recent origin. × 200.

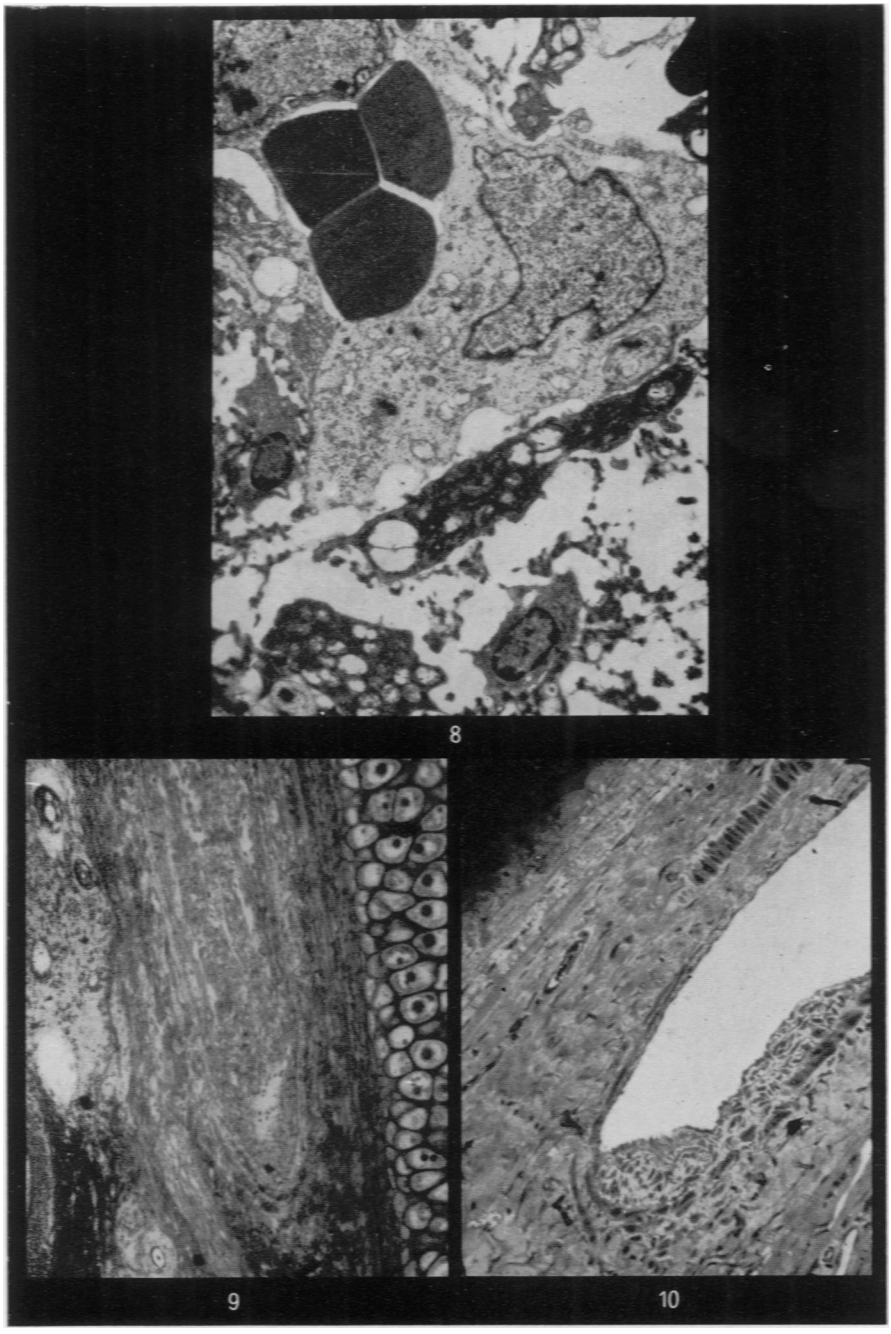

1
2
3
4



5

6

7

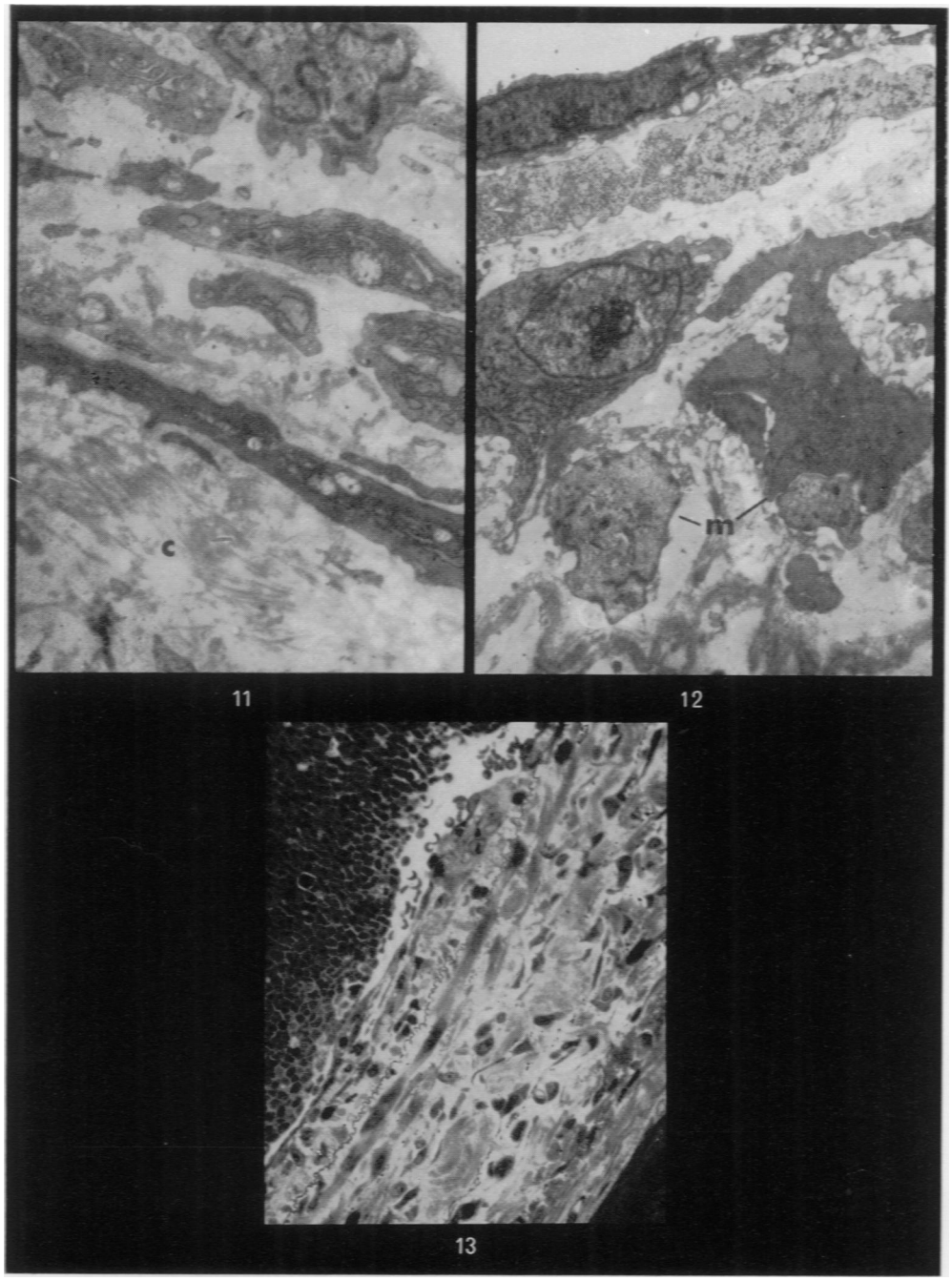


8

9

10

Scott.


c
m
11
12
13

(Fig. 10) but on occasions lined the whole circumference of the vein. They consisted of macrophages and elongated cells now recognizable as fibroblasts through their prominent endoplasmic reticulum (Fig. 11). Scanty round or irregularly shaped plain muscle cells, not seen in the intima of normal veins, were also present. When compared with many of those lying in the media, their appearance suggested that they had been cut transversely and thus lay in the long axis of the vessel (Fig. 12). Initially the various cells were separated by amorphous featureless material, but with time well-formed collagen fibres were much in evidence. The overlying endothelium contained occasional osmophilic inclusions.

On occasions a rim of hypertrophied muscle fibres lay beneath the base of the lesion (Fig. 10). The cellularity of these thickenings decreased and their collagen content increased with age. Only one protuberant lesion was encountered and with one exception the underlying internal elastic lamina was always intact although serial sections were not cut.

In one instance, 4 days after the initial thrombosis, a flat endothelialized intimal thickening was covered by an occlusive thrombus whose appearance was more in keeping with one of 24 hr. duration (Fig. 13).

After 4 days either residual thrombus or intimal thickenings or both were never encountered in more than 2 of the 10 or 12 blocks comprising the total length of vein examined, the 2 vessels showing total organization of their thrombi to a greater or lesser extent being the only exceptions.

Thrombi and intimal thickenings were not seen in vessels subjected to dummy experiments and the endothelial changes described by T'sao and Spaet (1967) following partial venous constriction were absent in those controls and in those parts of the test vessels devoid of thrombi or intimal thickenings.

DISCUSSION

In this study the method of producing thrombosis was designed to minimize the trauma to the vein wall since Wright, Kubik and Hayden (1953), showed by venography that phlebitis delayed the speed of recanalization of thrombosed veins.

Although complete compression of the vein proximal to the thrombus was continued during the whole period of the experiment, the presence of small side branches or tributaries distal to this point made it possible for blood to flow through the length of the vessel after retraction of the thrombus. Visualization of the thrombi by transillumination, the reason for choosing the ear vein, ensured that only those vessels apparently thrombosed along their whole length were included.

The changes observed, while occurring at different rates in different vessels, revealed that massive destruction and removal of thrombus was the rule, that its replacement by fibrocellular tissue was minimal (Figs. 5 and 6) and that both processes invariably proceeded simultaneously in the same vessel.

The loose woven appearance at the edge of some thrombi after 48 hr. (Fig. 3) suggested thrombolysis and the rapid reduction of some thrombi to between 50–20 per cent of their original volume in between 18–48 hr. confirmed this. Indeed, Holemans and Tysinger (1965) found increased fibrinolytic activity in venous blood 5–10 min. after the onset of occlusion while Kwaan and Astrup (1965), using the method developed by Todd (1959) showed that plasminogen

tissue activator (PTA) was produced by venous endothelium, lysis of occlusive thrombi beginning immediately after their formation. They also showed that sclerosing agents halted the production of PTA and delayed thrombolysis, which would explain why veins thrombosed by sodium morrhuate take longer to recanalize when treated with anticoagulants than those thrombosed by thrombin alone (Wright, *et al*, 1953). Thus, one of the factors promoting recanalization in phlebothrombosis as opposed to thrombophlebitis must be the ability of the endothelium to produce PTA.

The massive thrombolysis seen in the present study indicates the survival of the endothelium (Fig. 1) and its ability to continue producing PTA after being covered by thrombus for several days. The localized areas of organization must therefore represent those segments of the venous intima that have become irrevocably damaged by overlying thrombus. Thrombolysis is by no means confined to veins since Dalal, Shah, Steth and Deshpaude (1965) produced angiographic proof of recanalization in 9 cases of cerebral artery occlusion and Wessler, Freiman, Ballon, Katz, Wolff and Wolff (1961) demonstrated massive lysis of experimental thromboemboli in the canine lung.

In the present study the flat fibrocellular intimal thickenings (Figs. 10 and 13), involving part or whole of the circumference of the lumen of the veins, are similar to the thin zones of granulation tissue seen at the periphery or base of organizing thrombi (Figs. 5 and 6). The former are obviously derived from the latter through dissolution of the overlying thrombus and are identical with may of the lesions seen in leg veins (Scott, 1956), supporting the thesis that many such lesions are of thrombotic origin. The situation illustrated in Fig. 13 shows that further episodes of thrombosis can occur on the surface of such intimal thickenings.

The finding after 4 days or longer, of either residual organizing thrombus or intimal thickenings or both in no more than 2 of 10 or 12 blocks comprising the whole length of 12 out of 14 veins indicates that the volume of original thrombus had been reduced by at least 80 or over 90 per cent, no account being taken of thrombus in the discarded distal and proximal portions of the vessel.

The minimal organization of the thrombi in this study is in sharp contrast to that seen in the central artery of the ear of the rabbit by Williams (1955) although this may be due to the difference in vessels and to the trauma apparently found necessary to induce thrombosis in this particular artery.

A comparison of this minimal and marginal organization with the massive angiomatous structures seen in the leg veins in phlegmasia cerulea dolens (Moore and Scott, 1955) and following portal vein thrombosis in children (Gibson and Richards, 1955; and Parker and Seal, 1955) suggests an element of phlebitis in these conditions, and such differences raise the whole question of the factors determining the extent to which thrombi will organize.

While attention was not paid specifically to the exact origin of the cells responsible for the organization of thrombi, appearances suggested one from the vessel wall and it is hard to imagine that the scanty and moribund leucocytes lying in the centre of the thrombi (Fig. 5) could produce the lacunae demonstrated by Dible (1966) in organizing arterial thrombi and indeed no such structures were ever seen.

In recent years there has been growing evidence of the frequency of phlebothromboembolism. Sevitt and Gallacher (1961) demonstrated macroscopical thrombi in the veins of the lower extremities of 65 per cent of subjects dying as the

results of trauma but without pulmonary emboli, while Scott (1956) found numerous microscopical thrombi in varying stages of organization in the calf veins of subjects dying from conditions other than thromboembolic disease or trauma.

Freiman (1965) saw evidence of recent or old organized thromboemboli of various sizes in 64 per cent of unselected autopsies and the study of Morrell, Truelove and Barr (1963) and the more than threefold increase in the number of fatal cases of pulmonary thromboembolic disease reported annually to the United Kingdom Registrar General over the last 20 yr. suggests that phlebothromboembolism is actually increasing.

Since the method if inducing thrombus in the present experiments resulted in thrombi which initially occluded the whole length of the vein, it can be deduced that a considerable proportion of venous intimal thickenings and organizing mural thrombi (Scott, 1956) are formed from thrombi, which were originally totally occlusive and occupied a much larger segment of the vessel than their ultimate dimensions would indicate. These conclusions and the observation of the massive lysis of experimental phlebothromboemboli (Wessler, *et al.*, 1961) points to phlebothromboembolism being an even commoner and more massive occurrence than the current literature suggests.

SUMMARY

A quantitative study of the fate of occlusive red cell venous thrombi produced by thrombin showed that thrombolysis was massive, organization of the thrombi minimal, and that both processes often proceeded simultaneously in the same vessel. The thrombotic origin of many venous intimal thickenings was confirmed and the results of the study pointed to phlebothrombosis being a more massive and commoner occurrence than is suggested in the present evidence.

My thanks are due to Miss Mary Lynne Altemus for technical assistance.

REFERENCES

BARNARD, P. J.—(1953) *J. Path. Bact.*, **65**, 129.
CASLEY-SMITH, J. R., ARDLIE, N. G. AND SHWARTZ, G. J.—(1967) *Br. J. exp. Path.*, **48**, 501
DALAL, P. H., SHAH, P. M., STETH, S. C., DESHPAUDE, C. K.—(1965) *Lancet*, i, 61.
DIBLE, J. H.—(1966) ' Pathology of Limb Ischaemia'. Pathological Monographs No. 3, Ch. 3, Edinborough (Oliver and Boyd).
FILSHIE, I. AND SCOTT, G. B. D.—(1958) *J. Path. Bact.*, **76**, 71.
FREIMAN, D.—(1965) ' Pulmonary Embolic Disease', p. 81, Eds. Sasahara, A. A. and Stein, M., New York (Grune and Stratton).
GIBSON, J. B. AND RICHARDS, R. C.—(1955) *J. Path. Bact.*, **70**, 81.
HARRISON, C. V.—(1948) *J. Path. Bact.*, **60**, 289.
HEARD, B.—(1952) *J. Path. Bact.*, **64**, 13.
HOLEMANS, R. AND TYSINGER, M. F.—(1965) *Proc. Soc. exp. Biol. Med.*, **118**, 488.
KWAAN, H. C. AND ASTRUP, T.—(1965) *Circulation Res.*, **17**, 477.
MOORE, R. F. AND SCOTT, G. B. D.—(1955) *Br. J. Surg.*, **43**, 591.
MORRELL, M. T., TRUELOVE, S. C. AND BARR, A.—(1963) *Br. Med. J.*, ii, 830.
PARKER, R. A. AND SEAL, R. M. E.—(1955) *J. Path. Bact.*, **70**, 97.
ROBERTSON, H. R., PERRELT, T. S., COLEBECK, J. C. AND MOYES, P. D.—(1954) *Surg., Gynec. Obstet.*, **98**, 705.

SCOTT, G. B. D.—(1956) *J. Path. Bact.*, **72**, 543—(1959) *Br. J. exp. Path.*, **40**, 567.
SEVITT, S. AND GALLACHER, N. G.—(1961) *Br. J. Surg.*, **48**, 475.
STILL, W. J. S.—(1966) *Lab. Invest.*, **15**, 1492.
STILL, W. J. S., GHANI, A. R. AND DENNISON, S. M.—(1967) *Am. J. Path.*, **61**, 1013.
STIRLING, G. A. TSAPOGAS, M. J. AND GIROLAMI, P. L.—(1966) *Br. J. Surg.*, **53**, 232.
TODD, A. S.—(1959) *J. Path. Bact.*, **78**, 281.
T'SAO, C. AND SPAET, T.—(1967) *Am. J. Path.*, **51**, 789.
WESSLER, S., FREIMAN, D., BALLON, J. D., KATZ, J. H., WOLFF, R. AND WOLFF, E.—(1961) *Am. J. Path.*, **38**, 89.
WIENER, J. AND SPIRO, D.—(1962) *Exp. mol. Path.*, **1**, 554.
WILLIAMS, G.—(1955) *J. Path. Bact.*, **69**, 199.
WRIGHT, H. P., KUBIK, M. M. AND HAYDEN, M.—(1953) *Br. med. J.* i, 1021.