# THE LYSIS, MATURATION AND CANALIZATION

# OF

# INTRAVASCULAR THROMBI.

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## SUMMARY.

I. The history of observations made on intravascular thrombi together with the progress made in effecting clot lysis in vivo are reviewed.

II. An account is given of:-

(a) the methods used for studying the effect on thrombi in rabbits of infusion of the lytic agent streptokinase;

(b) the methods used to create thrombi in canine arteries and veins for observation of the changes that occurred, with particular reference to organization and canalization.

III. The results of the investigations are presented as follows:-

(a) Thrombi in rabbits treated with streptokinase over four to ten hours showed a reduction in size but not complete lysis. Peripheral whole blood lysis was seen in the higher dose ranges and the fibrinogen level fell in all animals that were infused. Prothrombin time and clotting time showed no significant change.

(b) Thrombi in rabbits treated with streptokinase for a limited period of 4 hours and left in situ for three and six days showed no further significant change in size. 80% of control thrombi at six days were not evident macroscopically.

(c) Organization of canine thrombi created by different methods proceeds at a steady rate from fresh red cell clot to fibrous tissue. Canalization varies according to the nature of the circulation and to a lesser extent according to the injury inflicted on the vessel wall.

(d) Lack of organization and canalization of thrombi in occluded large and medium atherosclerotic vessels in man and its occurrence in venous thrombi is presented and discussed in relation to the above observations.

IV. The results of the investigations are discussed with regard to:-

(a) the use of streptokinase as a therapeutic agent;

(b) the factors concerned in organization of a thrombus;

(c) the relationship between organization and canalization;

(d) a comparison of artificial clot lysis and the natural process of canalization;

(e) the method of treatment of the thrombo-embolic disorders.

## STATEMENT.

This thesis is the report of work done by the author whilst holding the position of Research Fellow in Vascular Surgery at the Harvard Medical School, Boston, and Research Fellow of the National Heart Foundation of Australia in the Department of Surgery, University of Adelaide.

The results of this study have not been presented for the award of any other degree or diploma in any University and data published previously by other authors has been duly acknowledged in the text.

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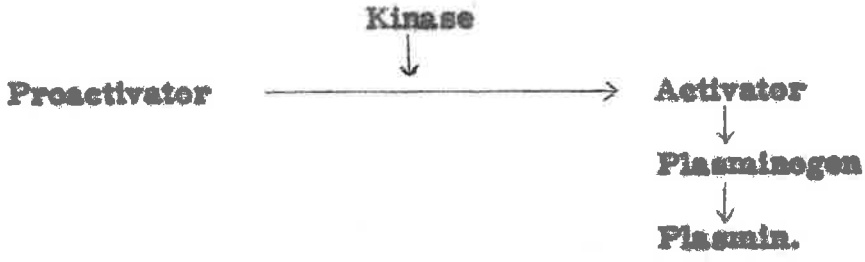
## INTRODUCTION.

There is in plasma a lytic system which can be expressed simply as follows:-




Plasmin is the active, circulating lytic compound.

The most convincing results of the ability of streptokinase to produce clot lysis in vivo have been produced by Johnson and McCarty (1959). However, no standard dosage was suggested, constant effectiveness recorded, or the results compared with the natural process of organization and canalization of thrombi.

Intermittent observations on the organization and canalization of intravascular thrombi have been made since the early eighteenth century. Although changes in a thrombus were described, there was no accurate knowledge regarding cell origin until the nineteenth century. At no time has there been any correlation of this work with the lack of organization and canalization in thrombi removed from medium and large arteriosclerotic vessels (Leriche 1947, Warren 1959), or with successful organization of venous thrombi with wide canalization and restoration of vessel blood flow.

It was the aim of this study to establish a rational therapeutic

approach to the use of streptokinase as a lytic agent, to compare with artificial lysis the natural process of canalization in restoring vessel patency after thrombosis, and to determine the relationship of canalization and organization in a thrombus.

# CHAPTER I.

## HISTORICAL SURVEY.

### A. THROMBOSIS:

### (1) Arterial.

The observations by Erasistratos of Chios (330 B.C.) that every organ was supplied by an artery, vein and nerve, was extended by Celsus who noted that haemorrhage ceased spontaneously or as a result of applied pressure, cautery and thread.

Not until Jean Louis Petit (1731) called attention to the 'couvercle' around the end of a divided vessel, and the 'bouchon' within the lumen did modern investigations on the healing of arteries begin. He gave the first account of clot formation in wounded arteries.

Hunter (1794) and Jones (1805) in England, Gendrin (1826) in France, and Stilling (1834) in Germany pursued the subject, with division of clot organisation into three stages by Stilling:-

(1) Soft and succulent becoming denser.

(2) Vascular spaces and sinuses developing.

(3) Fine connective tissue.

Weber (1864) elaborated further on this description by giving more cellular detail.

Virchow (1845) distinguished the 'sinus-like degeneration' of the thrombus from its vascularisation and thought the white cell might

be the originator of the connective tissue corpuscle in organization.

Vascular endothelium as the active cellular principle in organization was cited by Cohn (1860) and the idea was supported by Thiersch (1867). Warren (1886) observed both proliferation of the intima and invasion of the thrombus by cells from the media of canine arteries in which clots had been formed by ligation of the femoral or carotid artery. Welch (1900), as well, reported that the tissue forming cells were derived from the endothelium and other fixed mural cells.

Aschoff (1913, 1924) following on the work of Zahn in 1875 was more interested in the factors predisposing to thrombus formation and the gross structure of the thrombus. He investigated the formation of lines of Zahn in veins and the differences between white and red thrombi.

Winternitz (1933) during his investigations into arteriosclerosis cut serial sections of a thrombosed coronary artery. Vascularization of the cell mass was seen, and channels which traversed the outer vessel wall had free anastomosis with the vessels within the thrombus. In the same study, the anterior tibial artery from a gangrenous leg was injected and cleared. The lumen was found occluded in three places and connected with the general circulation through channels within the clot and its own side branches. There were no significant through channels connecting the lumen above and

below the thrombus to maintain adequate distal circulation. Vascularization of the thrombus, although noted by Virchow (1856), Winternitz (1938), has been investigated more recently by Akrawi and Wilson (1950) who have postulated that its blood supply is by capillaries connected with the vasa vasorum, and that these vessels play no part in a through circulation.

Duguid (1946) has seen wide channels with marginal organization in a coronary vessel following thrombosis, whilst Harrison (1948) has followed in rabbit pulmonary arteries the organization of fibrin emboli with contraction, fibrosis, and restoration of vessel patency.

In 1958 Dible stated that organization and canalization were distinct and independent processes, with the vascular endothelium able to enclose large masses of thrombus so that the lacunar channels would allow earlier through circulation than the ordinary process of organization.

Wessler and Freiman (1961) have observed pulmonary emboli of varying ages, and consider that spontaneous lysis as well as organization and canalization plays an important part in reducing the clot to small size, and restoring vessel patency.

(2) Venous.

With the progress of surgery in this century venous thrombosis and the subsequent risk of pulmonary embolus have gained much

attention.

The gross structure of a thrombus and the changes in it had been investigated at the end of the nineteenth century by such men as Zahn (1875), Bizzozero (1882) Eberth and Schimmelbusch (1888). This enabled the later workers to concentrate on the clinical aspect of venous thrombosis and its cause.

Williams (1906) observed progressive venous thrombosis extending from the right median basilic and cephalic veins to the veins of the head and neck. In 1912 colonic obstruction due to venous thrombosis was reported by Carson and verified by Lockhart Mummery. The effect of damage to the vessel wall, infection and necrosis, slowing of the blood stream, and alteration in platelets have been investigated by Duckworth 1913, Barrett 1924, Dawbarn et al 1928, Wright 1942, 1951, Tullis 1953, Sharnoff et al 1960.

Edwards and Edwards (1937), Homans (1946), White and Warren (1949) reviewed separately the fibrosis and re-canalisation that occurred following thrombophlebitis. These changes were constant and always appeared after venous occlusion by a thrombus. Mehrotra (1953), Robertson (1954, 1956), Filshie and Scott (1958), Marin and Stefanini (1960) have all seen similar changes in thrombi produced in the experimental animal, whatever their site.

(3) Current Concept.

The earlier workers have noted the organization and

canalization of thrombi but have not explored the origin of the cell changes, the difference between arterial and venous canalization, and the lack of organization in human thrombi removed from medium and large atherosclerotic vessels by endarterectomy or in an amputation stump.

Dible has stated that organization and canalization are distinct independent processes but the factors concerned have not been elucidated.

It is the aim of this study to investigate these aspects of the changes in intravascular thrombi, and the factors concerned.

## B. THROMBOLYSIS - STREPTOKINASE.

### (1) Development of the agent.

The fluidity of blood following sudden death was noted by Morgagni (1761) and Hunter (1835). Its cause was recognised by Denys (1838) and Zimmerman who observed also that the fibrin of blood obtained from wet cupping redissolved in twelve to twenty-four hours. The activity was thought to be in the serum but its nature was not known (Denys 1889, Tagnon 1942), and to this phenomenon, Dastre (1893) applied the term fibrinolysis.

Tillett and Garner (1933) first observed that cultures of B. haemolytic streptococci, or cell free filtrates of these organisms, would lyse human plasma clots. The streptococcal extract was assumed to contain an enzyme specific for fibrinogen and fibrin and was termed fibrinolysin. Milstone (1941) found the extract had no fibrinolytic effect on pure fibrinogen, but that activity occurred if a small amount of human plasma globulin was added. Kaplan (1944) and Christensen (1945) showed that the streptococcal factor activated the enzyme precursor in the euglobulin fraction of human blood plasma or serum. To this streptococcal factor was given the name streptokinase. (Christensen and MacLeod 1945).

In 1945 Christensen established the streptokinase unit on a lytic basis. There have been variations in formation of the basic clot since then, but each manufacturer adheres to a standard lytic

procedure to determine the unitage of his product.

Clifton (1950) detected differing plasmin activity in the sera of several animal species using streptokinase, protamine, and spontaneous activation, and compared the results with the activation of human sera. He was unable to offer an explanation for this difference in activation pattern of the serum from various animal species.

It was Mullertz and Lassen (1953, 1955, 1956) who did much of the work to establish our present idea as to the precise action of streptokinase.

They activated human and bovine globulin by the addition of streptokinase, and found that the activity of the bovine globulin measured by casein digestion was less than that of human globulin. Activity to equal that of human globulin was produced by the addition of the activator found in spontaneously active human blood, and then testing the activity.

Further work established the likelihood that the initial action of streptokinase was with proactivator to form activator which then proceeded enzymatically as below:-

Astrup (1956) has suggested that plasmin, because of its lytic property, may be part of a homeostatic process maintaining the functional integrity of the vascular bed, and so directly involved in the aetiology of arteriosclerosis. The inactive naturally occurring lytic agent is plasminogen. This is present in Cohn's fraction III of plasma (1946), and has been produced in a form four hundred times more pure than it was originally by the acid extraction process of Kline (1953). The activation of plasminogen to plasmin by streptokinase may be in vitro or in vivo (Troll and Sherry 1955), and the ease with which activity is achieved depends on the proactivator content of plasmas.

Alkjaersig et al (1959) have suggested that the primary mechanism of thrombolysis involves the diffusion or adsorption of plasminogen activator to the thrombus, with activation of intrinsic clot plasminogen. The secondary mechanism involving digestion of the thrombus by extrinsic plasmin action is of little importance.

(2) Investigational Trials.

(a) Animal.

The first successful lysis of intravascular thrombi was reported by Johnson and Tillett (1952). They infused streptokinase in doses ranging from 15,000 units/kilo/hour to 40,000 units/kilo/hour into rabbits with preformed marginal ear vein thrombi.

The clots lysed in three to seven hours after commencement

of the infusion which had to be continued a further three to four hours to prevent reformation.

Sherry et al (1954) achieved complete lysis of thrombi in the femoral arteries of the dog in fifty per cent of cases, with streptokinase in doses of 20,000 units/kilo dissolved in thirty mls. of saline and injected intravenously over five minutes. Ambrus et al (1957) were less successful using varidase on $I^{131}$ labelled clots in dogs and rabbits even in the highest doses permitted by its solubility. The infusion resulted in neither fibrinolytic nor anticoagulation changes and there was no alteration in any blood coagulation factors. Streptokinase activated plasminogen on similar thrombi did result in clot lysis.

(b) Human.

Spontaneous lytic activity has been observed following heavy exercise (Cohen 1961), after the injection of epinephrine, or during anxiety and stress (Briggs 1947). Whether this is due to the tissue activator of Mullertz (1955, 1956), the labile fibrinolytic component of Fearnley (1955, 1958), or an undetermined blood component is unknown.

Tillet et al (1955) produced a lytic system in man both by continuous infusion of streptokinase, as varidase, and its intermittent administration over a dose range from 75,000 units to 150,000 units. Then Johnson (1957) followed this with further observations on the

effect purified streptokinase preparations produced on peripheral whole blood lysis with correlated temperature and cardiac changes. Doses of 10,000 units to 130,000 units were infused over a four hour period.

In a series of 61 patients who received one hundred and twenty eight streptokinase infusions in this cautious dose range, 15 patients showed peripheral whole blood lysis. This clot lysis occurred spontaneously from 45 - 240 minutes after blood withdrawal.

Temperature changes were in the range of $1^{\circ}$ - $3^{\circ}$F., and the electrocardiograms showed only minor and inconsistent changes.

The most successful report of systemic streptokinase in patients came from Johnson (1959). Continuous infusion was maintained for 28 - 72 hours producing complete lysis in fourteen out of twenty-five thrombi created in forearm veins. Pre-infusion occlusion was verified by inspection and venography and the patency confirmed similarly after infusion.

The streptokinase was infused at a dose range to give a circulating plasmin system, streptokinase system, or a streptokinase-plasmin system. It was the latter that resulted in complete lysis of eleven thrombi, with no reformation. The total dose range to maintain a streptokinase-plasmin system was from one million to two million units over an average of twenty-nine hours.

Ambrus (1960) using streptokinase in ten cases of

thrombophlebitis, achieved complete clot solution in seven out of ten, partial in one, and no evident solution in the remaining two. Anlyan et al (1960) reported an improvement using Thrombolysin (Merck, Sharp and Dohme - streptokinase activated plasminogen) in eight of nine patients with clinical signs that altered on treatment - gangrenous finger tips, retinal artery thrombosis, absence of leg pulses. Fletcher and Alkjaersig consider that most of the activity of thrombolysin is due to its streptokinase content (personal communication 1961), and these same authors have reported in 1959 probable improvement in patients with myocardial infarction and other thromboembolic disorders on infusion with streptokinase.

(3) Current concept.

It seemed from these reports that streptokinase offered a ready means of activating the lytic system and producing clot lysis. Such solution would avoid the ischaemic complications of acute arterial occlusion, whether by thrombosis or embolism, secondary thrombosis after arteriotomy or venotomy, and the post-phlebitic syndrome that follows deep calf vein thrombosis. It was hoped to confirm this hypothesis.

## CHAPTER II.

## EXPERIMENTAL PLAN.

The question was whether intravascular thrombi treated with streptokinase could be successfully lysed to restore a normal through circulation, or whether the normal processes of organization and canalization might not be as efficient. The factors involved in these processes were to be explained, and the changes observed in human vessels correlated. The proactivator level in the rabbit is higher than in the dog. It was decided, therefore, to use the rabbit for the infusion experiments with streptokinase and the dog for observations on the organization and canalization of thrombi.

Phase I. Infusion experiments.

Series 1. Acute experiments in rabbits.

(a) Continuous streptokinase infusion with different dose ranges for periods from four to ten hours.

(b) Control infusion using normal saline over four hours.

The size of the thrombus was determined from its length and circumference both pre-infusion and post-infusion.

Series 2. Survival experiments in rabbits.

(1) Treated thrombi:-

An infusion of streptokinase in a massive dose range over four hours into two groups, each of six animals, with

(a) measurement of the length and circumference of

the thrombi pre-infusion and post-infusion.

(b) one group allowed to survive three days, and one group six days; the dimensions of the thrombi re-recorded, and the vessels with the thrombi removed for section.

(2) Untreated control thrombi:-

Two groups of six animals each, with thrombi formed in the right and left jugular veins, the size recorded, and the animals left to survive three and six days. At the end of this time the thrombi were recovered, their size recorded, and the vessel with its thrombus removed for section.

Series 3. Human Thrombi.

In four cases of superficial thrombophlebitis and two cases of deep vein thrombosis streptokinase has been infused continuously in varying doses over periods from eleven to sixty-two hours. Clot length in the superficial thromboses, peripheral whole blood lysis, fibrinogen levels, prothrombin and clotting time have all been recorded.

Phase II. Non-infusion experiments.

Series 4. Canine Thrombi.

The formation and examination of:-

(1) Reinjection venous and arterial thrombi

(2) Thrombi formed in intima-stripped veins and arteries.

(3) Reinjection thrombi in venous grafts incorporated in the arterial circulation.

Series 5. Human Thrombi.

(1) The arterial thrombi were obtained from:-

(a) atherosclerotic arteries, at operation, by thromboendarterectomy.

(b) amputated limbs removed because of vascular insufficiency.

(2) The venous specimens were obtained from late cases of thrombophlebitis. They had been the subject of a previous study which did not consider the organization and recanalization of thrombi.

# CHAPTER III.

## MATERIALS AND METHODS.

**Phase I. Infusion Experiments.**

Fifty-eight experiments were performed on forty-four rabbits (mean weight = 2.5 kg.) under nembutal anaesthesia.

Thrombi treated by systemic infusion of streptokinase or normal saline, and thrombi left in situ without infusion, were created by withdrawal and reinjection of blood.

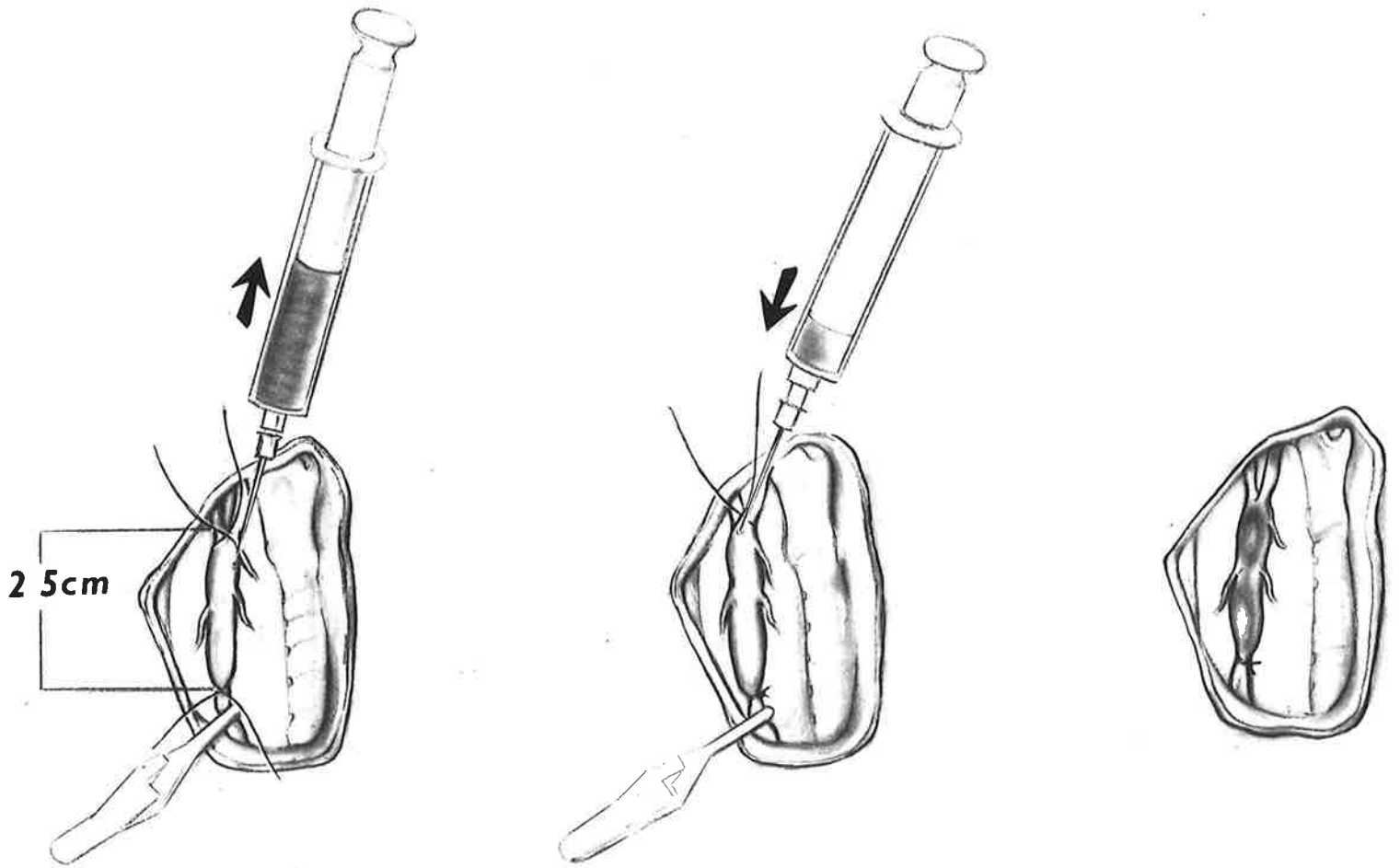
A. METHOD OF THROMBUS FORMATION. Fig. 1.

The jugular vein on one side of the neck was exposed for 2.5 cms. taking care not to damage the tributaries. At the proximal end of the isolated segment a permanent partially constricting ligature was tied, and a bulldog clamp fastened. A temporary constricting ligature encircled the distal end. Two mls. of blood were aspirated from the isolated segment, the length allowed to refill, and then the temporary constricting ligature drawn tight. At the end of five minutes the aspirated and still fluid blood was reinjected into the blood filled segment. A soft and non-adherent clot usually formed within a few minutes. The procedure was repeated if a clot did not form. The temporary ligature and the bulldog clamp were removed when a suitable thrombus was present.

Figure 1.

Method of thrombus formation prior to systemic infusion of streptokinase.

Tributaries are left untied to allow free access of blood.




FORMATION OF REINJECTION THROMBUS (Streptokinase Infusion)

B. METHOD OF STREPTOKINASE INFUSION.

(a) Animal.

Streptokinase* was dissolved in normal saline or five per cent dextrose. The total volume of fluid administered continuously varied from forty mls. to one hundred mls. over four to ten hours. It was infused via a needle in a marginal ear vein, or by insertion of a fine polythene catheter into the non-thrombosed jugular vein. A steady infusion volume was maintained by the use of a bottle calibrated to the approximate volume per hour.

(b) Human.

Systemic infusion of streptokinase in normal saline or dextrose saline was via a convenient forearm vein.

Albumin was added to give a 0.5% to 1% concentration in the early cases to maintain the streptokinase activity. It was later discontinued as control solutions revealed no significant difference at specified intervals between the activity of streptokinase in solution with albumin, or without it. The volume of infusion was approximately 77 ccs/hour.

( * Streptokinase = Kinalysin, by kind generosity of Merck, Sharp & Dohme, Australia and New York).

C. STREPTOKINASE DOSAGE.

(a) Animal.

This was as follows:-

(1) A minimum dose range from 1250 u/kg. /hour to 2,000 u/kg. /hour over four hours. This level was determined by estimating the amount of streptokinase in 0.1 ml. solution which when mixed with 1 ml. of plasma, incubated for five minutes at 37.5°C. and then clotted with 0.1 ml. of thrombin (250 u/ml.) would give complete clot lysis in a water bath at 37.5°C. in ten minutes. This was considered a measure of the total streptokinase inhibitor and antibody in 1 ml. of plasma. The total neutralizing dose for plasma was then administered in the first twenty minutes and a fraction of the dose per hour given over the remaining period, to give an overall infusion dose as above.

(2) A moderate dose range from 3,500 u/kg. /hour to 25,000 u/kg. /hour for six to ten hours.

(3) Massive dose range from 35,000 u/kg. /hour to 80,000 u/kg. /hour for six hours (approximately).

To maintain its activity the solution of streptokinase was renewed in the early experiments four hourly, and later every two hours to maintain an even dose/volume infusion. Where it was possible a clotting time, prothrombin estimation, fibrinogen, and peripheral whole blood lysis were determined. Because of exsanguination and the

data from the experiments in groups 1 and 2 these investigations were not done in group 3.

Figs. 2 and 3 show the gradation in peripheral whole blood lysis as expressed by no lysis and three plus lysis. A polythene airway was inserted via the mouth into the trachea of those rabbits that were for survival.

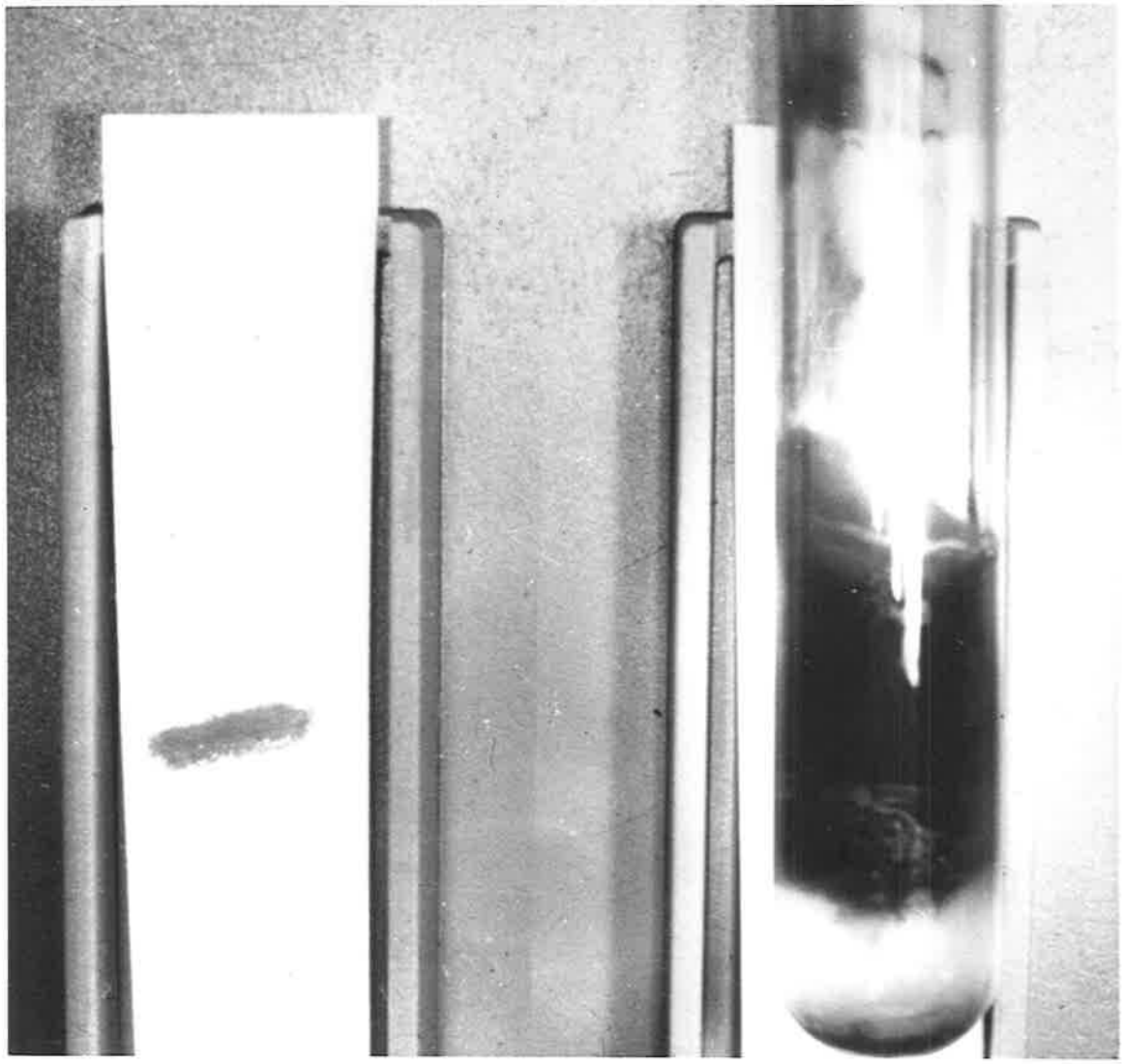
(b) Human.

For initial cases, the loading dose of streptokinase was determined by the same method as that used in the animals and then a proportion of this administered per hour for the determined period. This dose was later increased empirically to a maximum of 177,000 u/hour and the time of infusion extended from eleven and one half hours to as long as sixty-two hours. The solution of streptokinase was renewed four hourly to maintain its activity. A record was kept of the prothrombin level, fibrinogen, peripheral whole blood lysis and clotting time.

Figure 2.

A negative result for peripheral whole blood lysis.

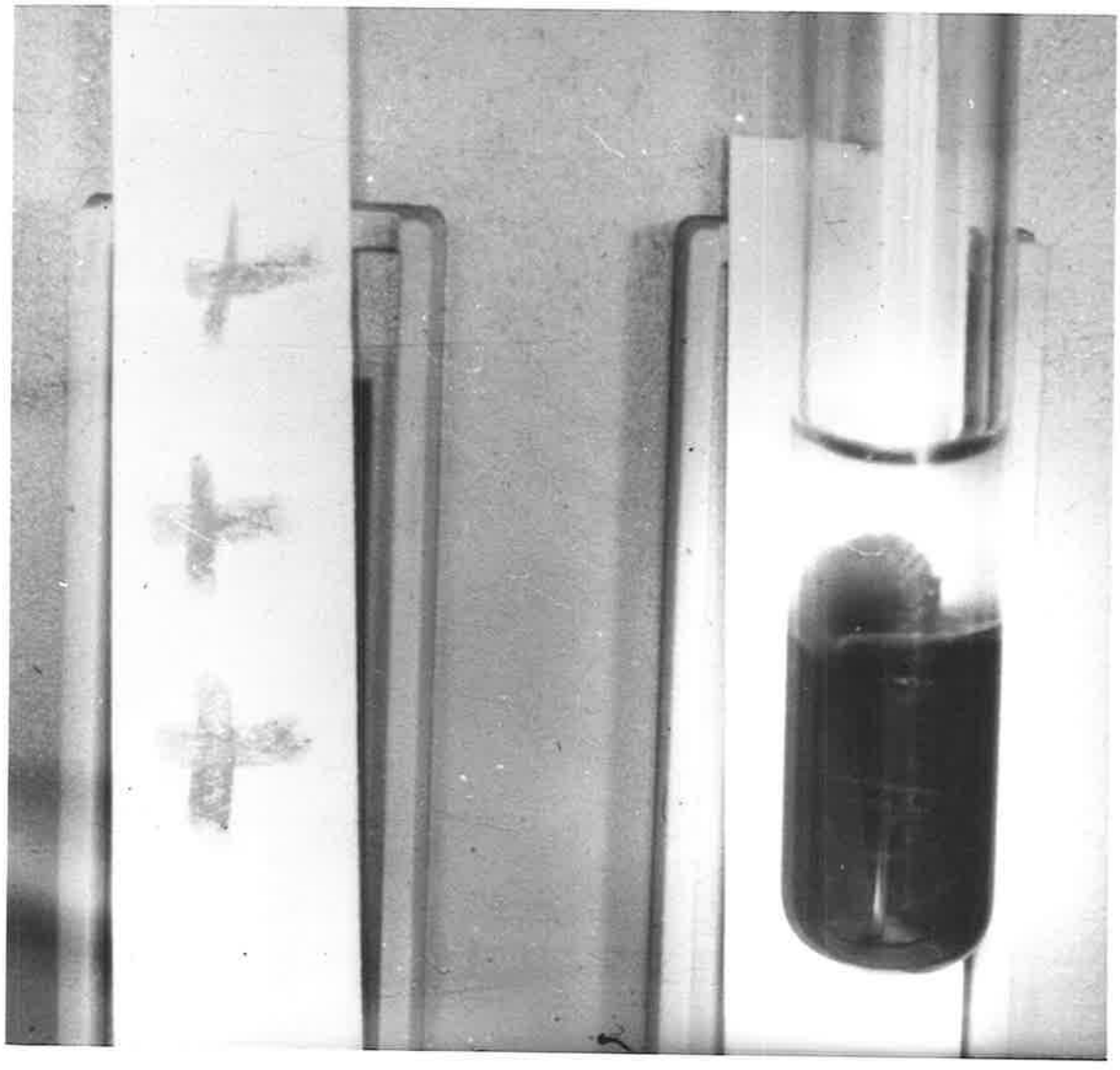
After twenty-four hours in a water bath at 37.5°C., this clot shows only retraction with no evidence of lysis. Serum is present in the bottom of the test tube.



## Figure 3.

A positive result for peripheral whole blood lysis.

This specimen of peripheral whole blood shows three plus lysis after allowing to clot and standing in a water bath at 37.5° C. for twenty-four hours.
Some residual clot can be seen on the upper red cell margin with serum above.
Four plus lysis indicates complete clot lysis with no residuum at all.



D. EXAMINATION OF THROMBI: In situ.

(a) Animal.

After the thrombus had been made in the vessel its length and circumference were measured. At the end of the infusion these measurements were repeated. In the animals allowed to survive, whether post-infusion or controls, the same measurements were recorded, and again at three and six days. From these could be determined the clot volume according to the formula, $V = R^2 X L$. Blood flow through the vessel was decided by naked eye inspection.

(b) Human.

Six cases of venous thrombosis were treated with the length of the vein clot recorded in four, and the surrounding area of inflammation marked. These were noted before the infusion commenced, after each twelve hours of continuous infusion, and at the end.

(2) Microscopic Examination.

(a) Animal Thrombi.

Six thrombi treated by systemic streptokinase in massive doses over four hours, and twelve untreated control thrombi were left in situ for three days, removed, fixed in formalin solution, stained with Haematoxylin and Eosin, and examined histologically. An equal number treated similarly were left for six days and examined in the same way.

(b) Human Thrombi.

No human thrombi were available for microscopy after infusion.

Phase II. Non-infusion experiments.

A. CANINE THROMBI.

On forty-five dogs (mean weight = 12 kg.), one hundred and thirty-nine experiments were performed under nembutal anaesthesia, with a clear airway maintained by an endotracheal tube of medium size.

(1) METHOD OF FORMATION OF THROMBI.

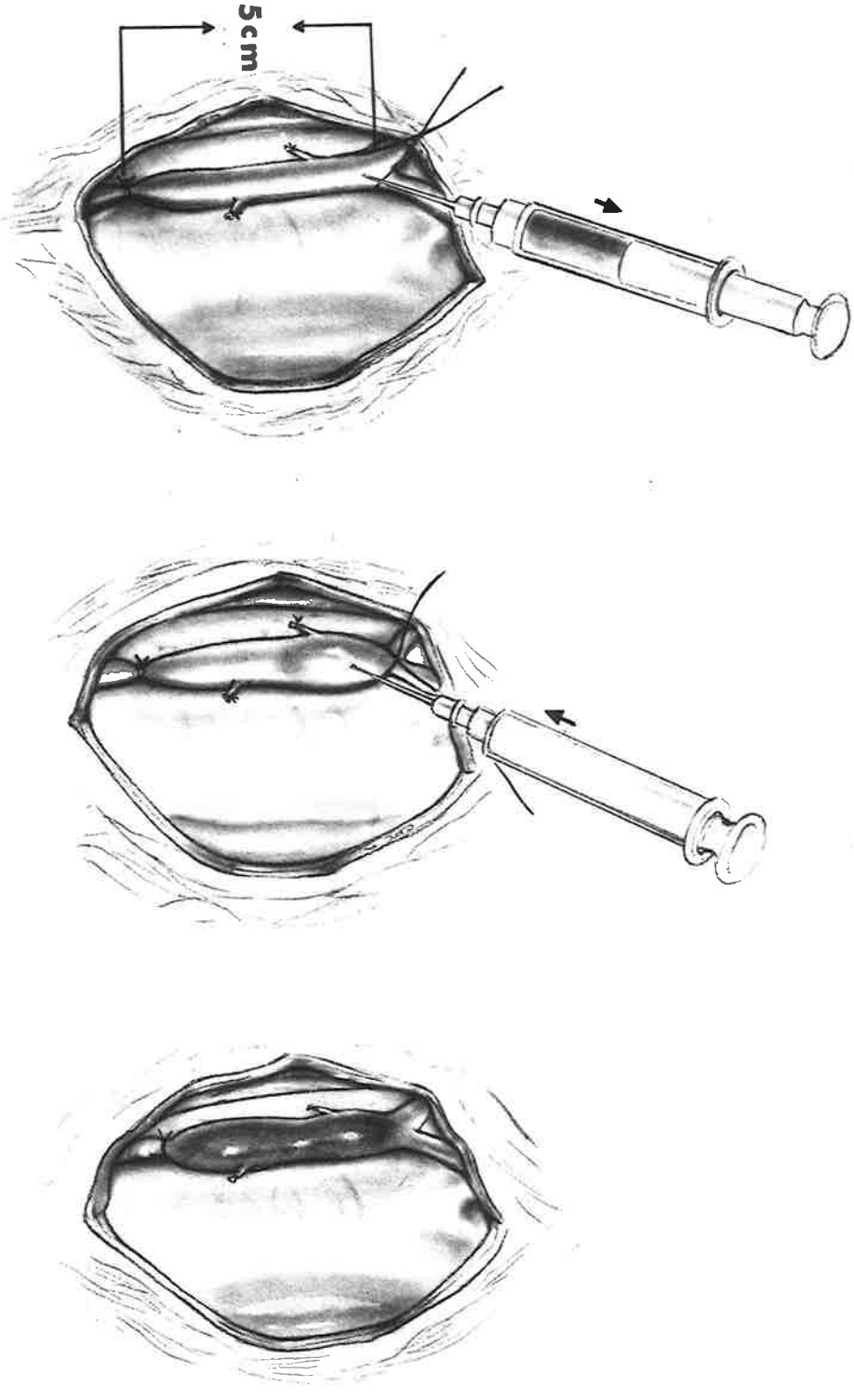
Thrombi were created in the jugular vein, carotid artery, the femoral artery and femoral vein using the following methods:

(a) Reinjection Thrombi (54 experiments) Fig. 4.

A five centimetre length of the vessel was cleared of all tributaries by their ligation and division. A permanent occluding ligature was tied at the proximal end of the isolated vein, 2 mls. of blood were aspirated distal to the obstruction, the vein allowed to refill, and the five centimetre length isolated by a second temporary and more distal ligature. At the end of five minutes the aspirated and still fluid blood was reinjected and mixed with the blood contained in the isolated segment. A soft non-adherent clot usually formed within a few minutes. If this procedure failed, blood was reaspirated and reinjected. When aclot had formed the temporary ligature was removed. A similar thrombus was formed in an arterial segment

Figure 4.

Canine reinjection thrombi, not submitted to streptokinase infusion, were created in vein segments, with the tributaries ligated and divided.




FORMATION OF REINJECTION THROMBUS

except that as the blood flow was in the opposite direction, the permanent tie was placed distally, so forming both the venous and arterial thrombi in the direction of blood flow.

(b) Thrombi in intima-stripped vessels (43 experiments) Fig. 5.

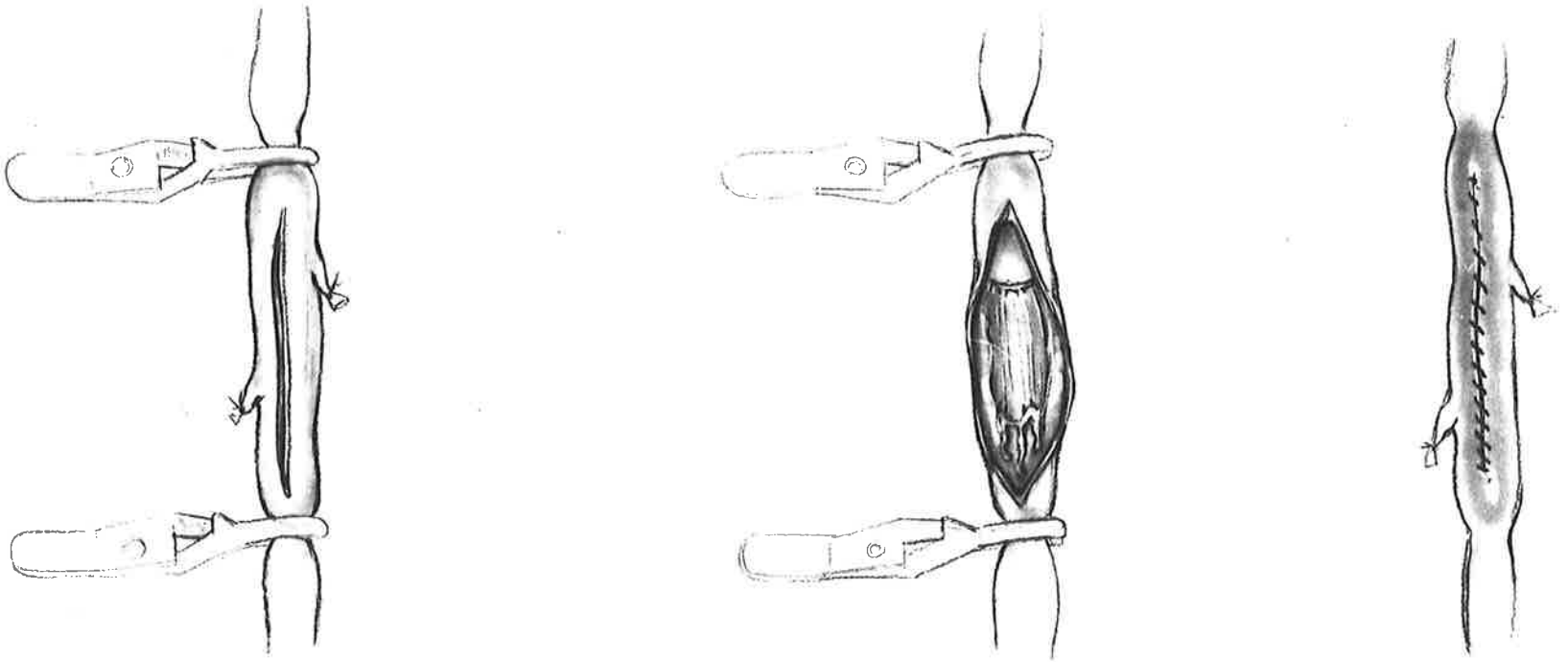
A five centimetre length of vessel was cleared of its tributaries by ligation and division. A bull dog clamp was applied at each end and the vessel then opened along its length for three to four centimetres. At the upper end of this incision using a very fine, sharp point, a shallow incision was made across the full width of the vessel. With a fine tipped, angled probe the intima was stripped off the vessel wall for the full distance of the arteriotomy or venotomy opening. Small strands were removed and the vessel wall closed using 50 black silk. The distal clamp on the vein and the proximal clamp on the artery was then removed to allow inflow of blood. After two minutes (approximately) the second clamp was removed and by five minutes a firm, adherent, occluding thrombus formed in the vessel over the length of the intima-stripped wall.

(c) Reinjection Thrombi in Venous Grafts (42 experiments) Fig. 6.

A five centimetre length of vein was grafted by end to end suture into an artery of approximately equal diameter. Thrombi were produced in the vein segment by the reinjection technique and the

Figure 5.

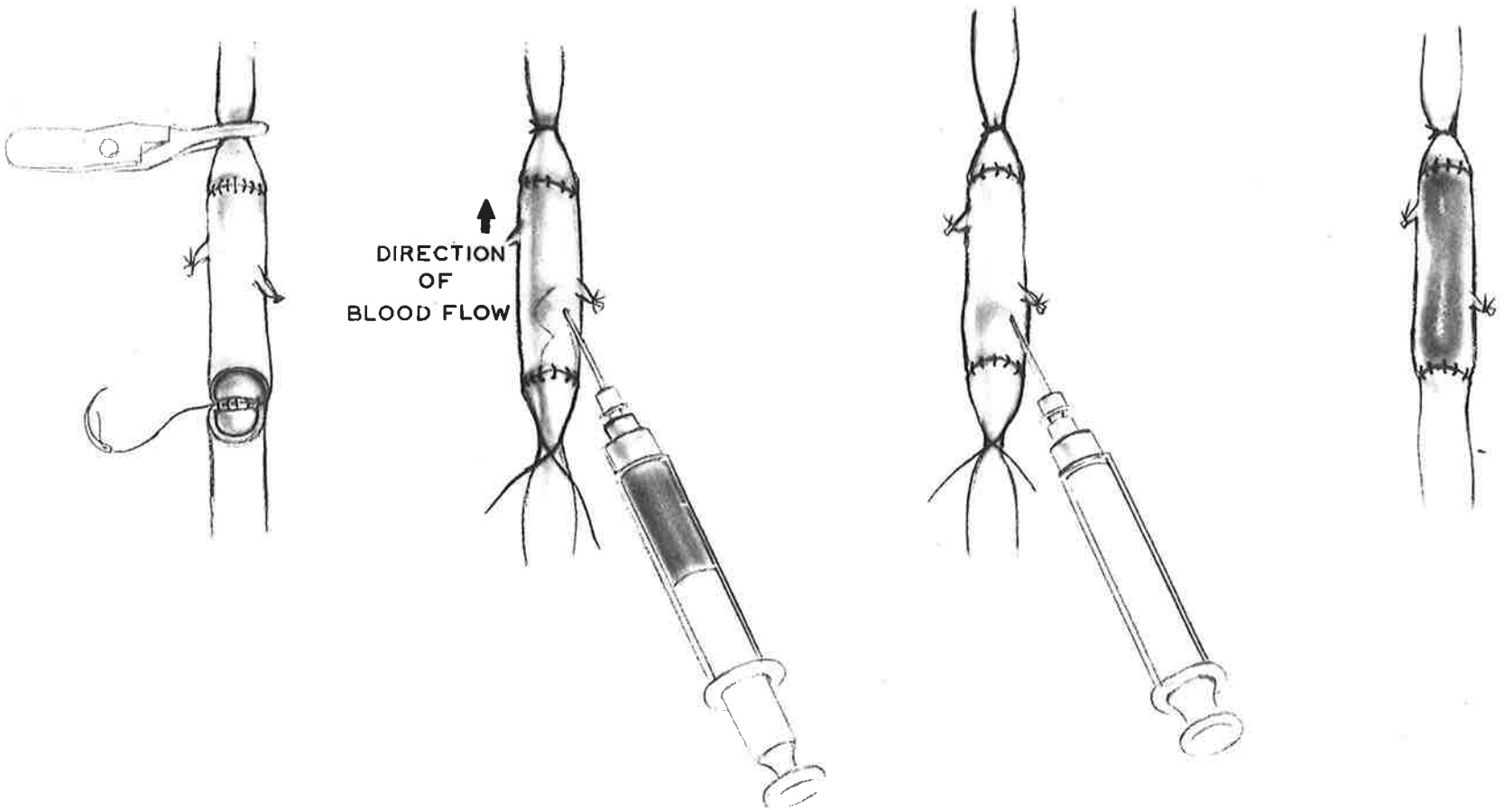
The intima was stripped with a fine pointed probe, aiming to remove just down to the internal elastic lamina of both arteries and veins.
In some specimens part of the inner media was removed as well.



FORMATION OF A THROMBUS IN AN INTIMA STRIPPED VESSEL

**Figure 6.**

A reinjection thrombus in a vein graft was formed by the same method as in Figure 4.




FORMATION OF A REINJECTION THROMBUS IN A VEIN GRAFT

permanent ligature was left on the artery distal to the vein segment.

(2) METHOD OF EXAMINATION OF THROMBI. (Fig. 7).

(a) India ink in a 1% agar solution was injected into vessels containing thrombi of twenty-one days and over, in situ and before death, to determine the degree of recanalisation.

Just beyond the thrombus in the direction of blood flow the vessel was opened. Two polyethylene tubes, 0.034 inches diameter, were inserted and tied in position close to but not encroaching on the thrombus. One was led to a graduated mercury gauge, the other to the solution.

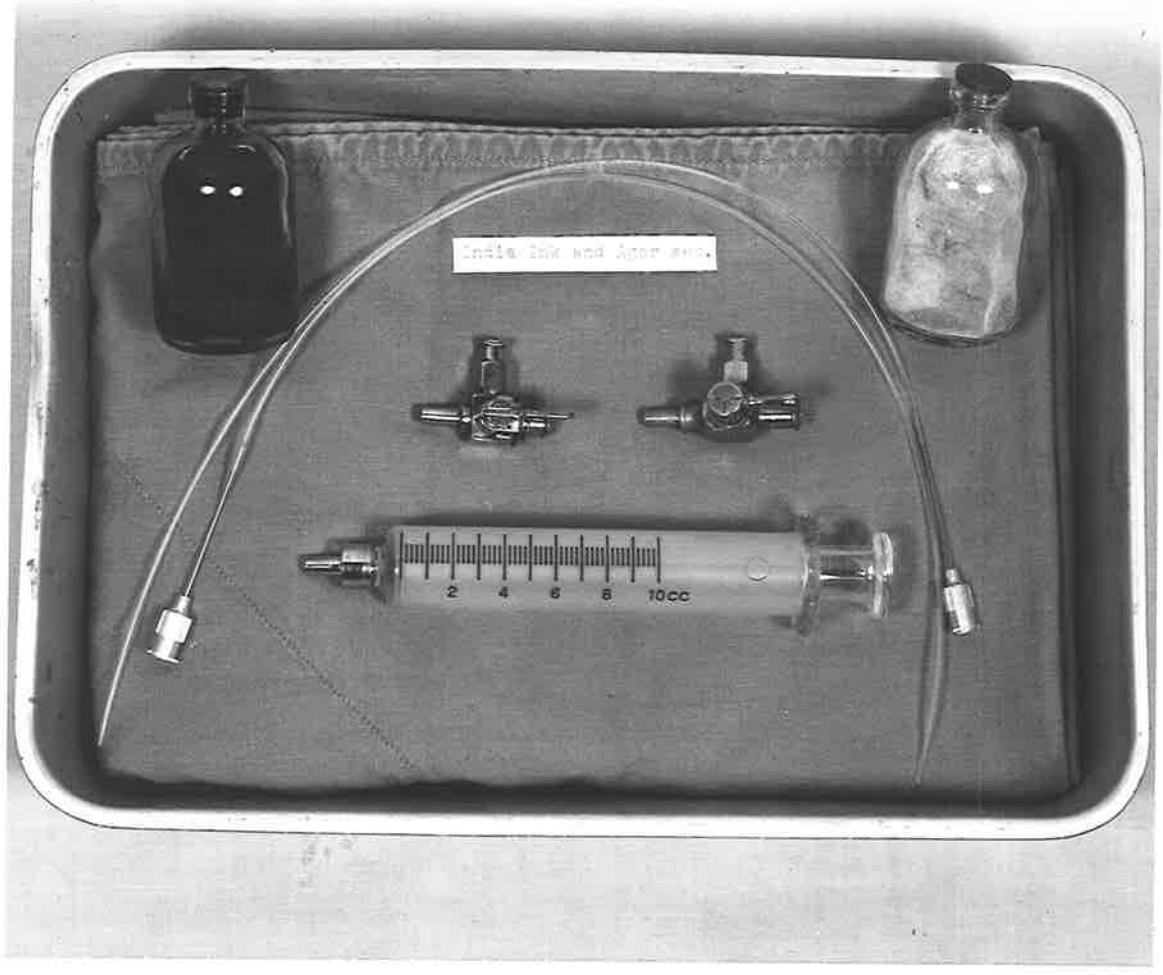
The pressure for arterial introduction was 140 mms. of Hg; for the veins 10 mms. of Hg. The pressure was maintained in each for two minutes, released, and the specimens removed for fixation and staining.

(b) Thrombi that varied in age from fresh to older than forty days were removed at an elected time, examined by naked eye, fixed in a solution of 15% formalin, 1% acetic acid, 2.5% $ZnCl_2$ and stained with Haematoxylin and Eosin for routine histologic survey. Periodic acid Schiff to delineate vascular reticulin, and elastic tissue (orcein) Masson as a differential stain for elastic tissue, muscle and collagen were also used.

Figure 7.

Apparatus to inject India ink in 1% agar solution.

One polyethylene tube was the route for instillation. The second tube was connected to a graduated mercury manometer which recorded the pressure levels produced.


India Ink and Agar mix.
2
4
6
8
10cc

Specimens were removed at the following times:-

1 day

3 days

5 days

14 days

21 days

28 days

40 days

greater than 40 days.

B. HUMAN THROMBI.

Ten arterial thrombi were examined for organization and canalization. These specimens were removed at operation by thromboendarterectomy, and from the vessels of limbs amputated because of vascular insufficiency. Nine venous thrombi for examination were obtained from long established cases of femoral phlebo-thrombosis.

The specimens were examined macroscopically, and microscopically after staining with Haematoxylin and Eosin. The origins of the thrombi can be seen in Table I.

## TABLE I.

## HUMAN THROMBI.

(1) Arterial thrombi analysed

(a) Specimens removed by thromboendarterectomy

| | |
|---|---|
| Femoral and popliteal | 5 |
| Anterior descending coronary | 1 |
| Common and internal carotid | 2 |

(b) Thrombosed atherosclerotic arteries removed from amputation specimens

| | |
|---|---|
| Below knee | 1 |
| Above knee | 1 |

All the occlusive human arterial specimens examined were present in atherosclerotic vessels. These specimens had a minimum and maximum age.

(2) Venous thrombi.

| | |
|---|---|
| Superficial femoral vein | 7 |
| Saphenous vein | 1 |
| Deep femoral vein | 2 |

This table shows not only the origin, but also the most common sites for peripheral venous thrombosis.

# CHAPTER IV.

## EXPERIMENTAL OBSERVATIONS.

Phase I. Infusion experiments (Table II).

Series 1. Acute experiments in rabbits.

These animals will be considered according to the dose of streptokinase/kg./hour that was administered.

(a) Minimum dose range (Fig. 8).

The thrombi in these animals showed a reduction in clot size between twenty-five per cent and fifty per cent but complete clot lysis did not occur in any specimen. The prothrombin time altered insignificantly, the fibrinogen level showed a slightly greater fall than the controls, and the peripheral whole blood lysis was incomplete in twenty-four hours, in all samples.

(b) Moderate dose range (Fig. 9).

Three out of six thrombi treated showed a fifty per cent to seventy-five per cent reduction in their original size. The remaining three were reduced by twenty-five per cent. Four plus whole blood lysis was achieved in four of the six rabbits during the time of infusion and occurred in the first ten hours after the specimen was taken. The prothrombin and clotting times varied little from normal, and there was a thirty per cent to fifty per cent drop in the fibrinogen levels in four of the six specimens. No samples were measured in the

## EXPERIMENTAL OBSERVATIONS.

### TABLE II.

**Phase I. Infusion experiments.**

**Analysis of 58 experimental thrombi in 46 rabbits.**

**(1) 22 acute experiments.**

| Dose range of S.K. Infusion | Minimal | Moderate | Massive | Total No. |
|---|---|---|---|---|
| Number | 4 | 6 | 8 | 18 |
| Controls | Infused with normal saline | | | 4 |

**(2) 36 survival experiments.**

| | 3 day | 6 day | Total No. |
|---|---|---|---|
| Post-infusion thrombi | 6 | 6 | 12 |
| Untreated thrombi | 12 | 12 | 24 |

## Figure 8.

Minimum Infusion Dose Range.

This chart depicts the changes that occur in clot size, peripheral whole blood lysis and coagulation factors with infusion of streptokinase in a dose range from 1250$^{u}$/kg./hr. to 2,000$^{u}$/kg./hr.

The base line figures are the times at which specimens were taken, measurements recorded, the infusion started and finished.

With per cent size change of the clot 100% is the size at the beginning of the infusion, and 0% represents complete clot lysis.

# MINIMUM INFUSION DOSE RANGE.

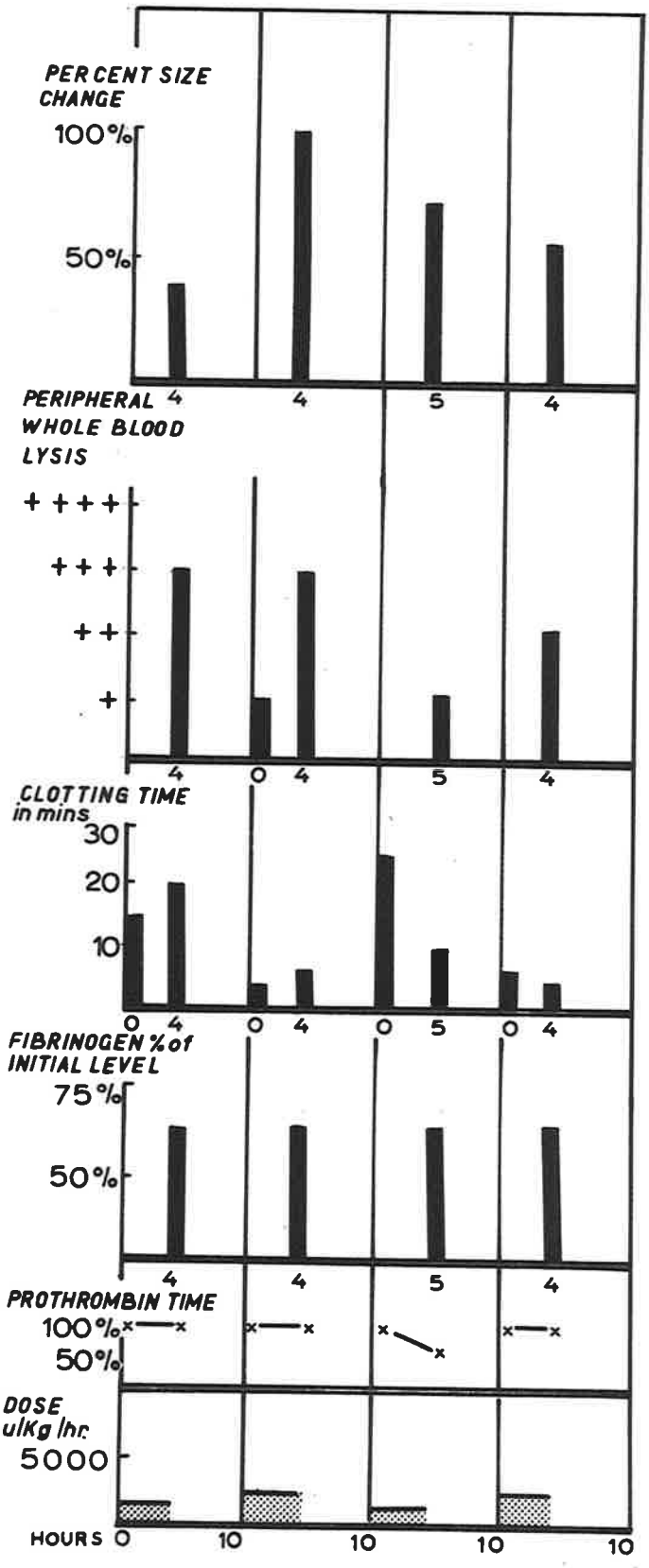


Figure 9.

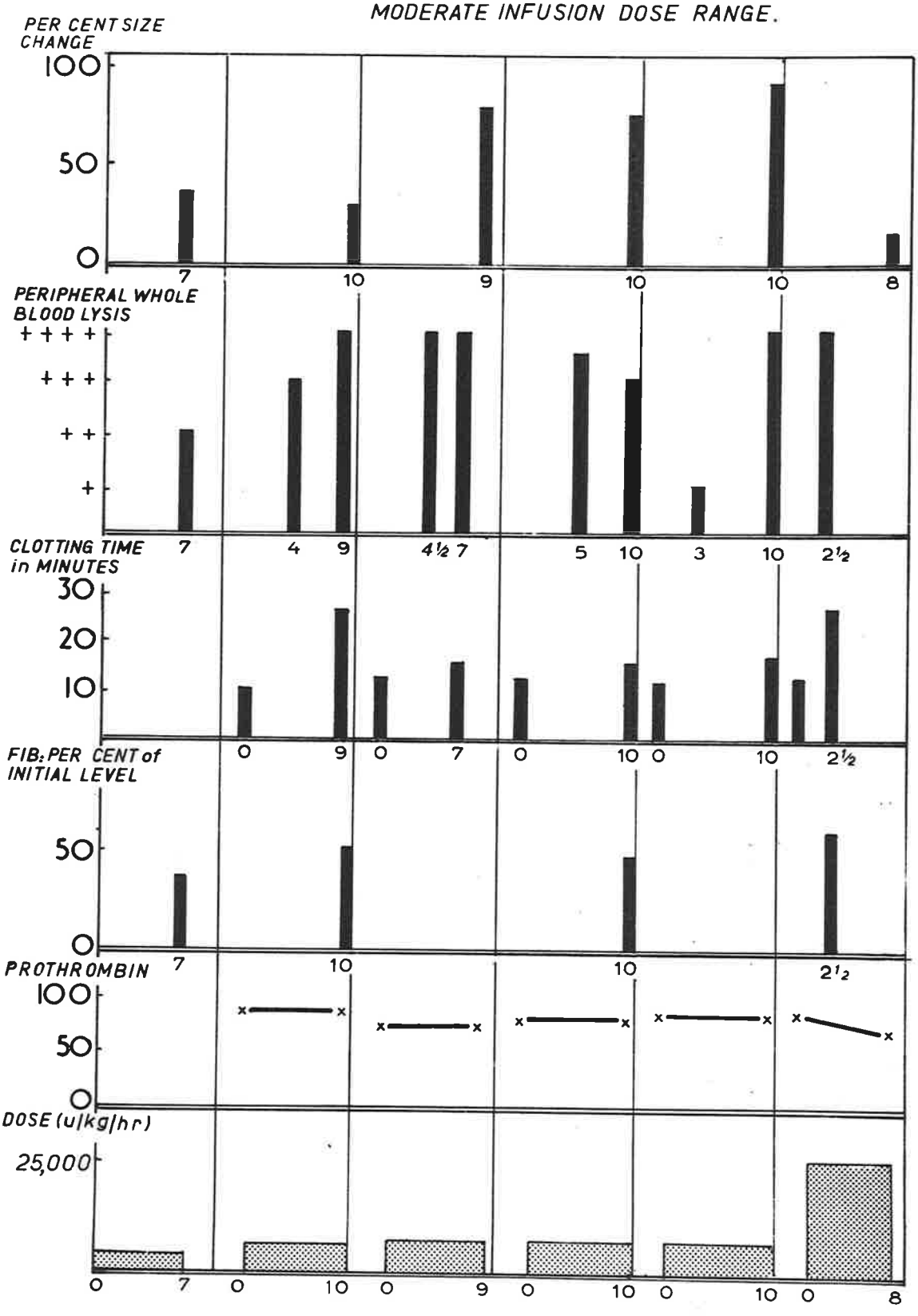
Moderate Infusion Dose Range.

This chart shows the changes in clot size, peripheral whole blood lysis, and coagulation factors with infusion of streptokinase in a dose range from 3,500$^{u}$/kg./hr. to 25,000$^{u}$/kg./hr.

The base line figures are the times at which specimens were taken, measurements recorded, the infusion started and finished.

With per cent size change of the clot 100% is the size at the beginning of the infusion, and 0% represents complete clot lysis.

MODERATE INFUSION DOSE RANGE.



remaining two.

(c) Massive dose range (Fig. 10).

Six of the eight thrombi showed a reduction in size greater than seventy-five per cent. Peripheral whole blood lysis was four plus in five animals from which specimens were taken, with diffuse and marked oozing at two hours in all eight wounds. Three plus lysis was evident in two remaining specimens with minimal clot in vitro and no specimen was taken from the third. Clotting time showed no significant alteration and no prothrombin or fibrinogen levels were determined since these had shown constant change in the previous groups.

(d) Control infusions (Fig. 11).

Sixty mls. of normal saline were infused into four rabbits over a four hour period. There was a twenty-five per cent decrease in size in two specimens with no change in one and a prolongation of the thrombus in another. The fibrinogen level dropped twenty-five per cent but no significant alteration occurred in the prothrombin levels, clotting times, or peripheral whole blood lysis.

Figure 10.

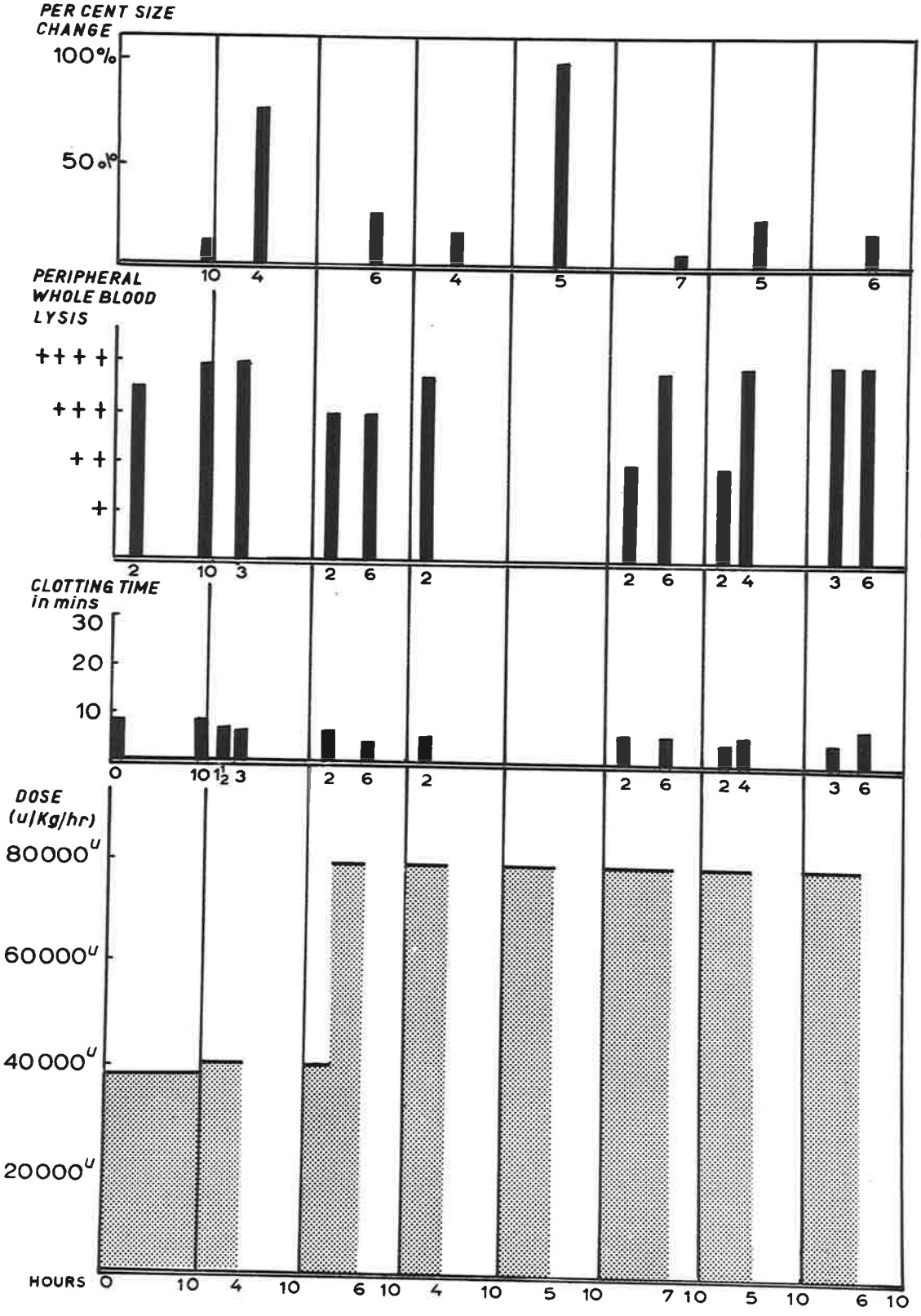
Massive Infusion Dose Range.

This chart shows the changes in clot size, peripheral whole blood lysis, and coagulation factors on infusion with streptokinase over a dose range from 38,000$^{u}$/kg./hr. to 80,000$^{u}$/kg./hr.

The base line figures are the times at which specimens were taken, measurements recorded, the infusion started and finished.

With per cent size change of the clot 100% is the size at the beginning of the infusion, and 0% represents complete clot lysis.

MASSIVE INFUSION DOSE RANGE.



## Figure 11.

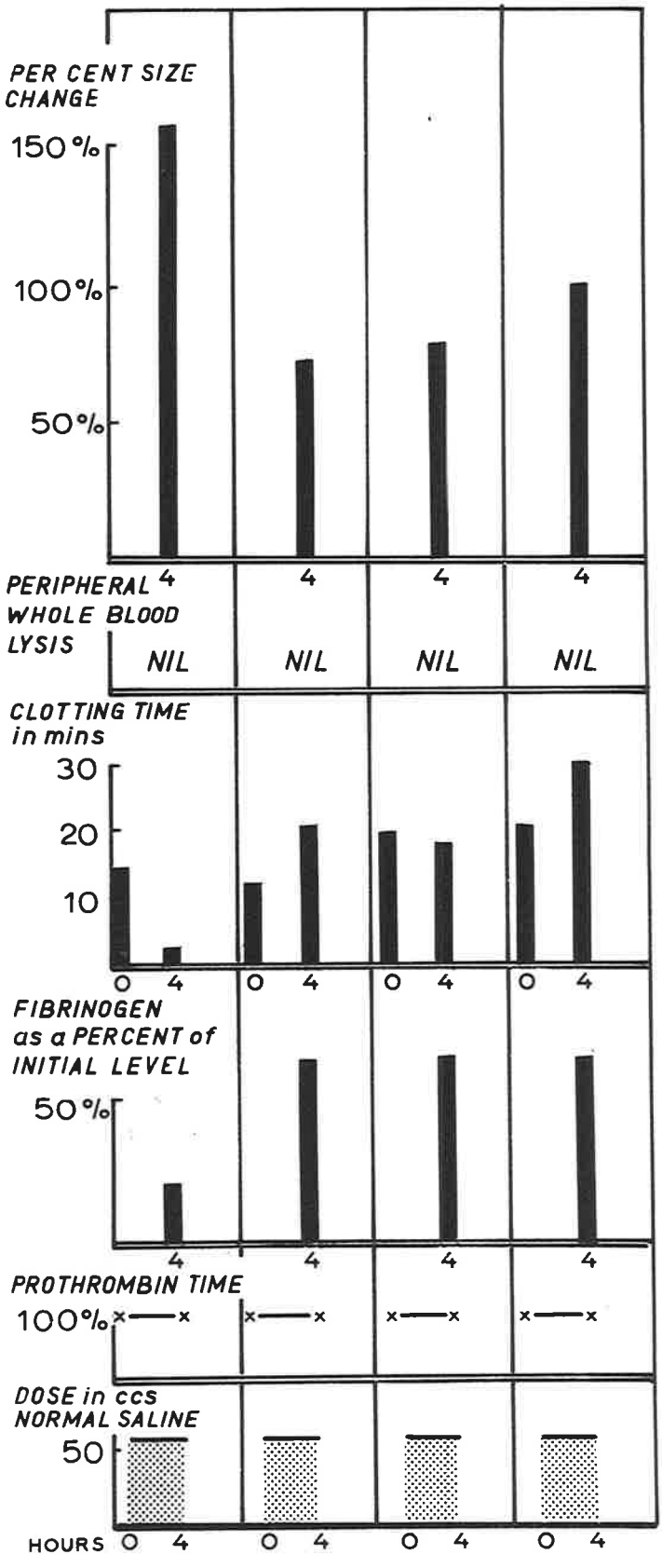
### Control Infusions.

In the control group normal saline was used at an infusion rate of 60 mls. over four hours. Records of change in size of the thrombus, peripheral whole blood lysis, and coagulation factors were made.

The base line figures are the times at which specimens were taken, measurements recorded, the infusion started and finished.

With per cent size change of the clot 100% is the size at the beginning of the infusion, and 0% represents complete clot lysis.

CONTROL INFUSIONS



Series 2. Survival Experiments.

(a) Thrombi in three day post-operative rabbits.

(Figs. 12 and 13.)

Streptokinase infusion was commenced soon after the thrombus had formed, at a dose rate of 40,000 u/kg./hour for four hours. At the end of the infusion five out of six thrombi were smaller in the range shown in Figure 12. At the end of three days, two thrombi had extended to give a large occlusive thrombus, and the remaining four were smaller, with definite evidence of blood flow through only one of these veins. Microscopy revealed red cell degeneration in the thrombi with no organization. The twelve three-day control thrombi (Figure 13) showed a reduction in size, in situ, in sixty per cent of cases; all appeared to block the vessel macroscopically, but there was evidence of through flow in two specimens. Another specimen appeared to block the vessel but flow was possible around the clot. On microscopy red cell degeneration had occurred in all thrombi with early cellular organization at the periphery.

(b) Thrombi in six day post-operative rabbits.

The six thrombi in this series were treated with streptokinase in the same dose range and over the same period as those in Group (a), (Figure 14). Five out of six showed a reduction in

## Figure 13.

THREE-DAY THROMBI FOLLOWING

STREPTOKINASE INFUSION.

This chart shows below the change in size of the thrombus at the end of infusion expressed as a per cent of its original size.

Above it shows the change in size of the thrombus after 3 days, expressed as a per cent of its size at the end of infusion. The columns in the same vertical line represent the same thrombus.

100% in each diagram represents the base line from which the change in clot size was estimated.

THREE DAY THROMBI FOLLOWING S.K. INFUSION.

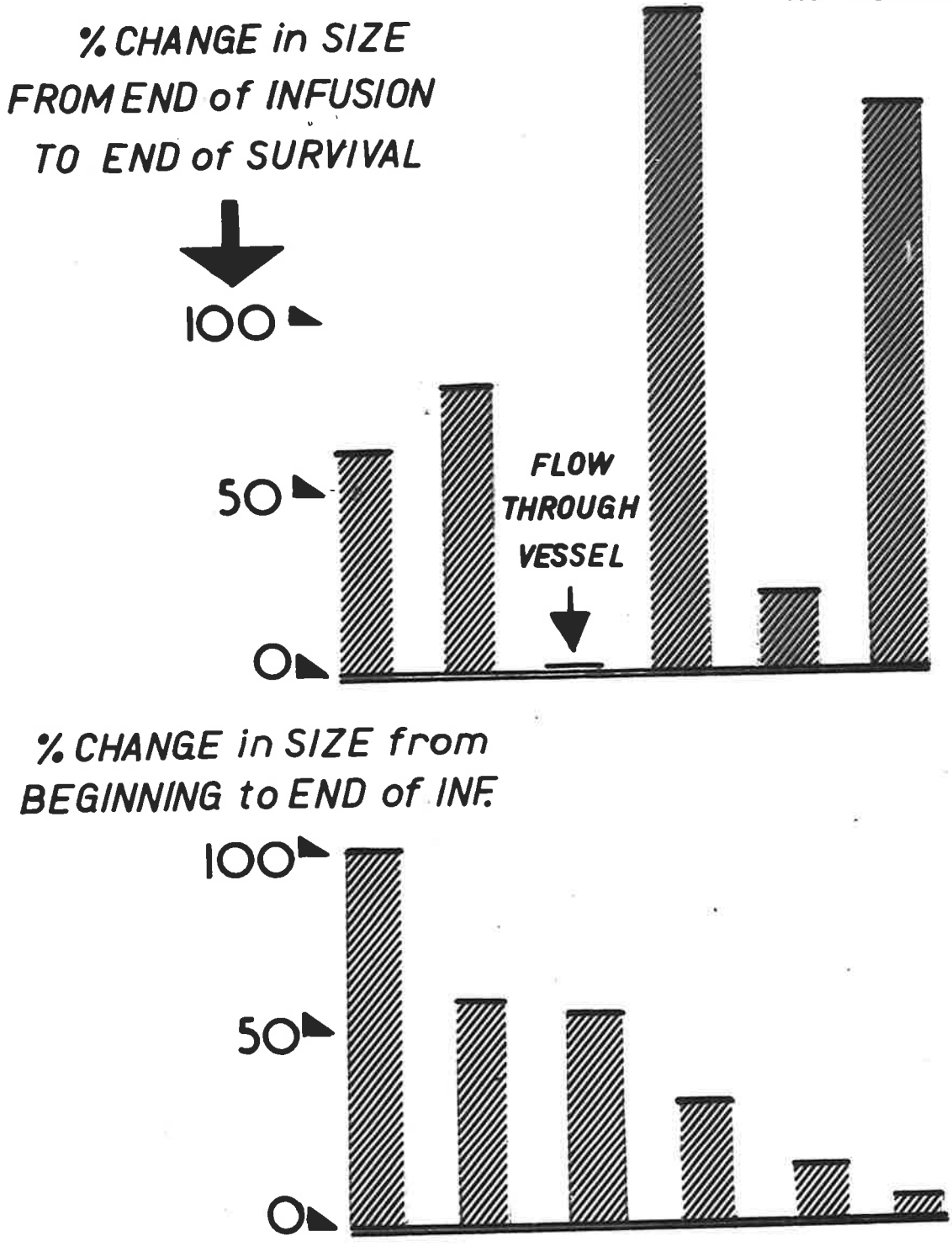


Figure 13.

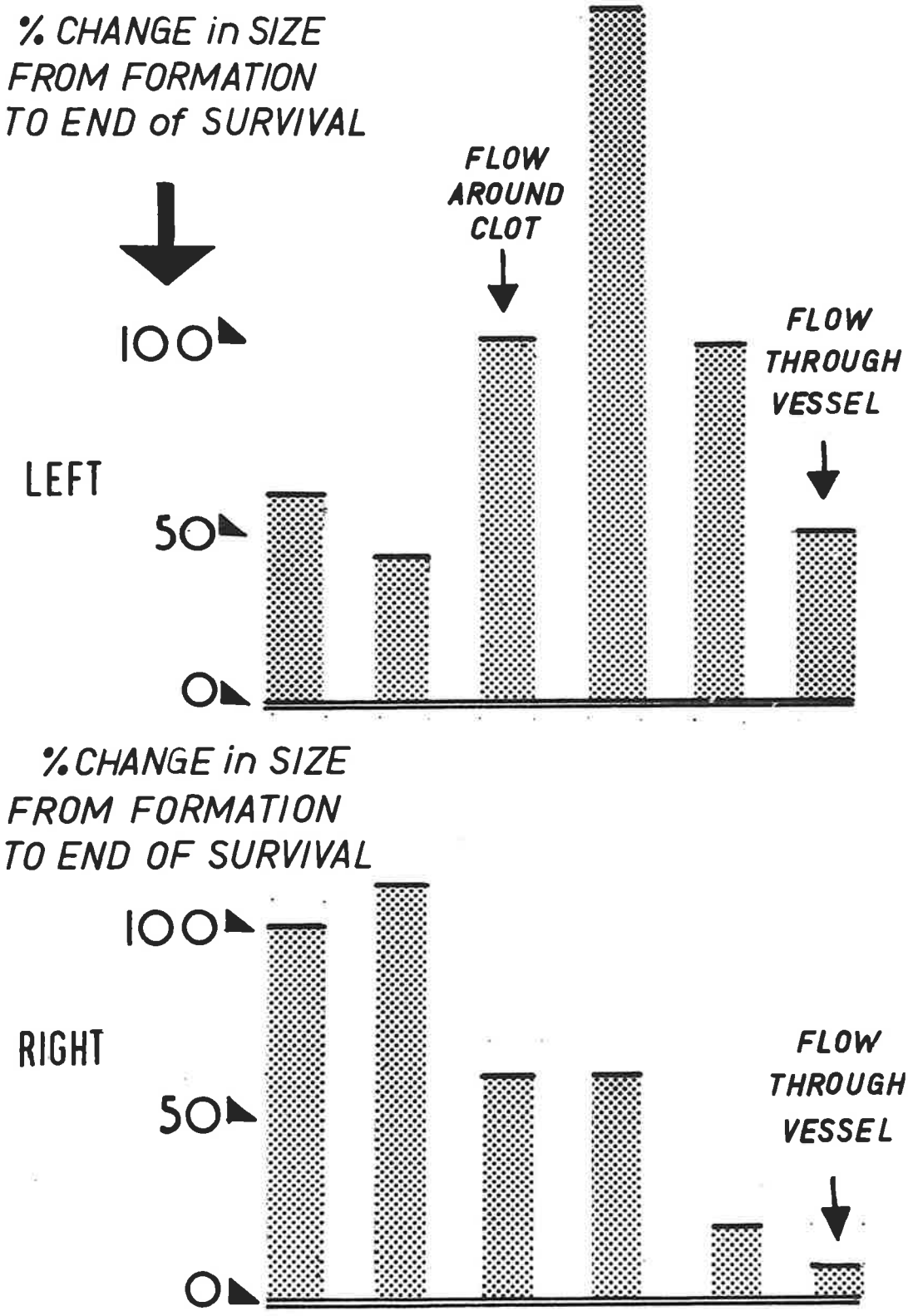
THREE DAY CONTROL THROMBI.

This chart shows changes in clot size at the end of the three days expressed as a per cent of their original size at the end of formation. where 100% represents the initial clot size.

The columns below represent thrombi created in the right jugular vein.

The columns above represent thrombi created in the left jugular vein.

# THREE DAY CONTROL THROMBI



size of fifty per cent or more at the end of infusion but only one diminished further over the next six days, with blood filling the segment on re-examination. The remainder increased in size or showed no change. These final thrombi were occlusive on serial section with minimal marginal retraction and no through flow.

Most sections showed a degenerated clot centre with secondary thrombosis at the periphery. Of the twelve control thrombi (Figure 15) only two were totally occlusive on removal; the remainder were patent macroscopically with blood filling the segment, but showed on microscopy, nubbins of clot with good channels, and blood flow through the vessel. These control thrombi showed white cell infiltration of the existing clot with well formed crevices and definite marginal organization.

Series 3. Human Thrombi. (Table III). (Page 71).

By the use of high doses of streptokinase per hour visible lysis of a venous thrombus was achieved in one of six patients (Figs. 16 and 17). Fig. 18 shows the spontaneous haematoma that developed at the same time as venous patency was restored. However, no peripheral whole blood lysis was ever obtained in this case and was attributed to the fact that the first specimen of peripheral blood was not taken until eight hours after the beginning of infusion and the

Figure 14.

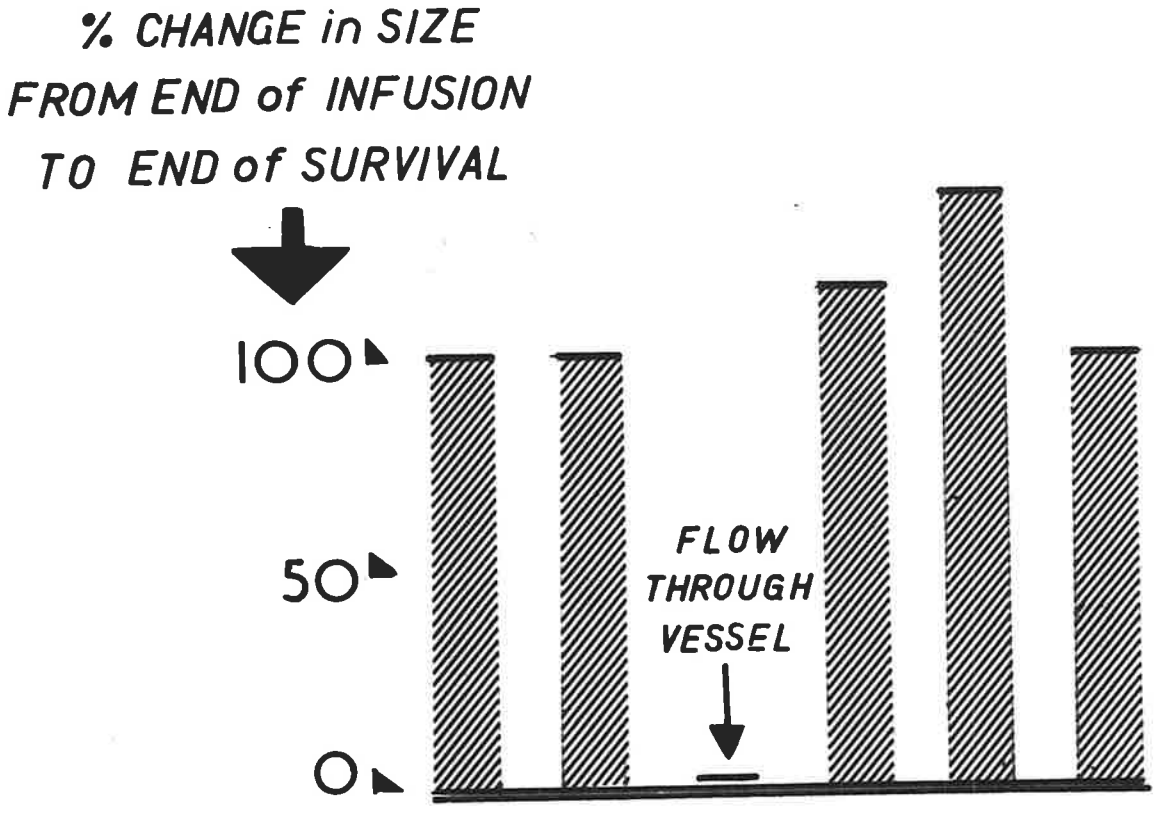
SIX-DAY THROMBI FOLLOWING STREPTOKINASE INFUSION.

This chart shows the change in clot size following infusion with streptokinase at a dose range of 40,000 u/kg. /hr. over four hours. The change is expressed as a per cent of its original size. Above, it shows the change in size after six days expressed as a per cent of its size at the end of infusion.

The columns in the same vertical line represent the same thrombus.

100% in each diagram represents the base line from which the change in clot size was estimated.

## SIX DAY THROMBI FOLLOWING S.K. INFUSION.



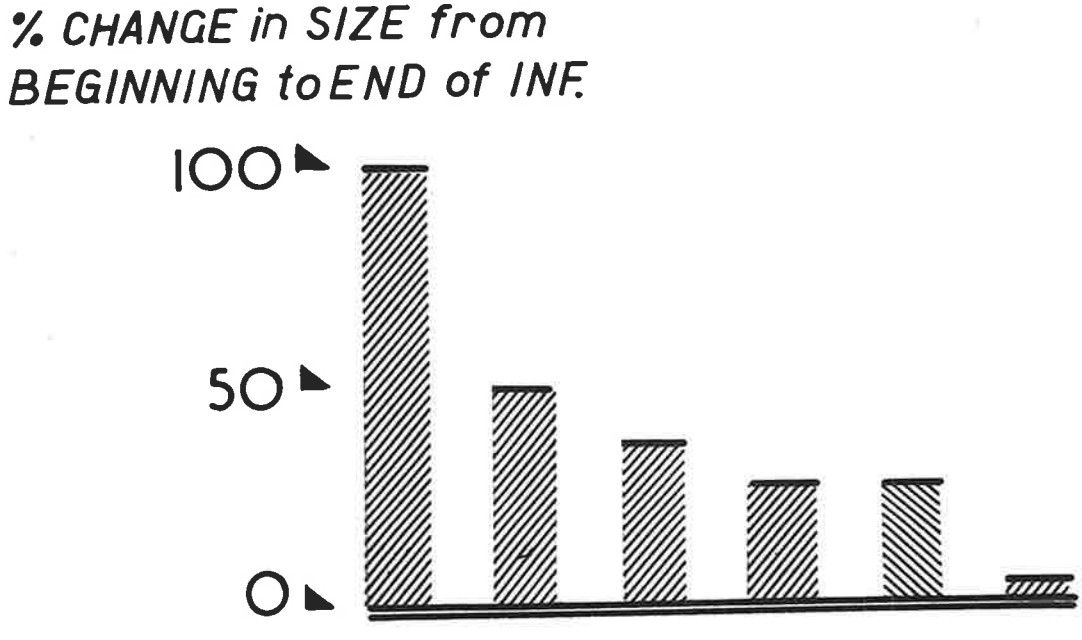
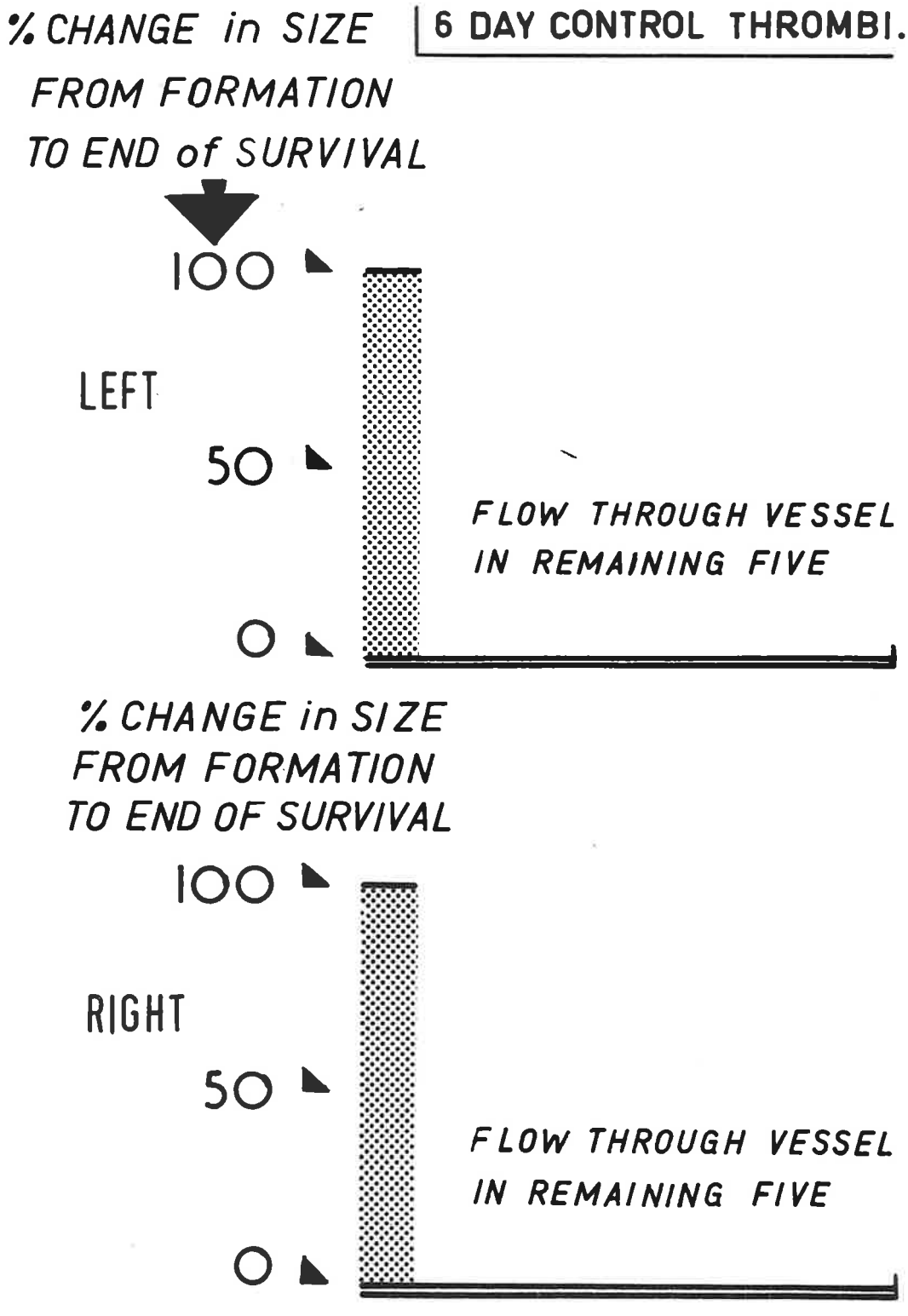



Figure 15.

SIX-DAY CONTROL THROMBI.

This chart shows the lack of change in two thrombi with complete absence of thrombus on inspection in any of the other vessels. There was blood flow through all these. Thrombi were formed in both the right and left jugular veins.


% CHANGE in SIZE
FROM FORMATION
TO END of SURVIVAL
6 DAY CONTROL THROMBI.
100
LEFT
50
0
FLOW THROUGH VESSEL
IN REMAINING FIVE
% CHANGE in SIZE
FROM FORMATION
TO END OF SURVIVAL
100
RIGHT
50
0
FLOW THROUGH VESSEL
IN REMAINING FIVE

## TABLE III.

## INFUSION OF STREPTOKINASE INTO SIX PATIENTS WITH VENOUS THROMBOSIS.

| Site | Change in Size | Dose/Hour | Dose Total | Peripheral Whole Blood Lysis | Clot Lysis |
|---|---|---|---|---|---|
| Lt. POPLITEAL VEIN THROMBOSIS | Calf circumference reduced 2.5 cms. | 13,750 u/hr. | 550,000 u over 24 hours | +++ in 24 hrs. after 12 hours infusion | Both legs of equal size |
| Lt. POPLITEAL VEIN THROMBOSIS | Calf circumference reduced 2.5 cms. | 14,130 u/hr. | 325,000 u/ 11½ hours | ++++ in 40 mins. after 6 hours infusion | Haematuria ? leg improvement |
| R. SAPHENOUS VEIN THROMBOSIS | Persistent firm cord over 12.5cms | 45,536 u/hr. | 2,550,000 u over 56 hrs. | ++++ in 7 hours after 4 hours infusion | Surrounding redness and pain reduced clot intact. |
| R. SUPERFICIAL VARICOSE VEIN THROMBOSIS | 3 cms. long no change in size | 100,000 u/ hr. | 3,000,000 u / 30 hours | ++++/24 hrs. after 2½ hrs. infusion | Persistent venous occlusion. |

TABLE III. (Contd.)

| Site | Change in Size | Dose/Hour | Dose Total | Peripheral Whole Blood Lysis | Clot Lysis |
|---|---|---|---|---|---|
| R. FOREARM VEIN THROMBOSIS | 13 cms. long, no change in size | 155,000 u/ hr. | 9,600,000 u / 62 hours | ++/24 hrs. after 4½ hours infusion | Redness and pain lost. Clot intact. |
| Lt. SAPHENOUS VEIN THROMBOSIS | Upper 7.5cms of fluid blood. 15 cms. below this a thickened vein but blood present. 10 cms. of further occlusion distally. | 177,000 u/ hr. | 8,850,000 u / 50½ hours | Nil in 24 hours. 1st specimen after 8 hrs. infusion. | Lysis complete. Improvement reformation. |

Figure 16.

THE GROSS APPEARANCE OF A SUPERFICIAL VEIN THROMBUS PRIOR TO STREPTOKINASE INFUSION.

The length enclosed by the pencil lines represents the length of superficial thrombosed vein in the leg prior to infusion with streptokinase.

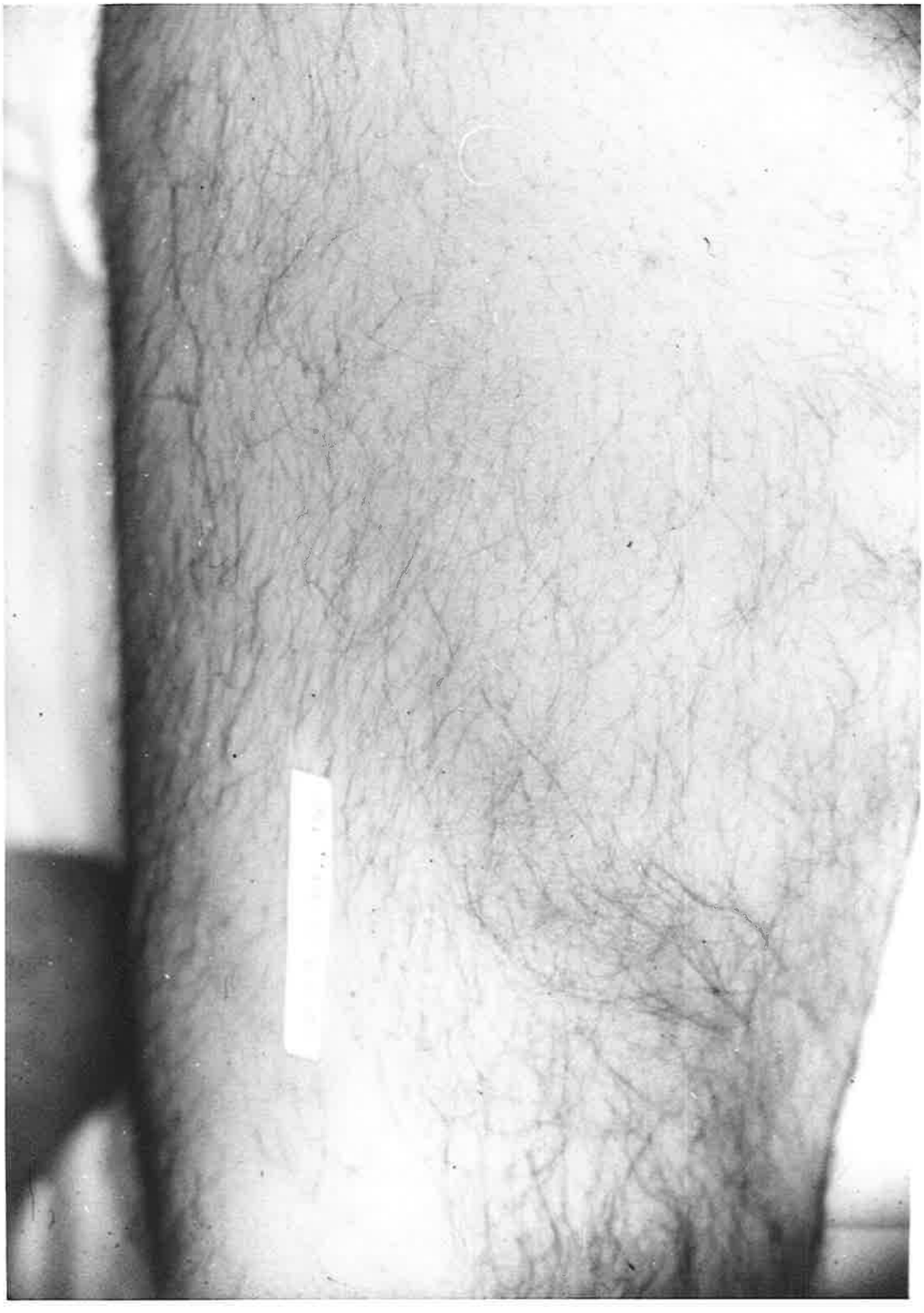


Figure 17.

THE LENGTH OF THROMBOSED VEIN IN WHICH PATENCY WAS RESTORED.

The dark area in the lower left corner of this picture is the length of vein over which clot lysis occurred after 50 hours of streptokinase infusion.

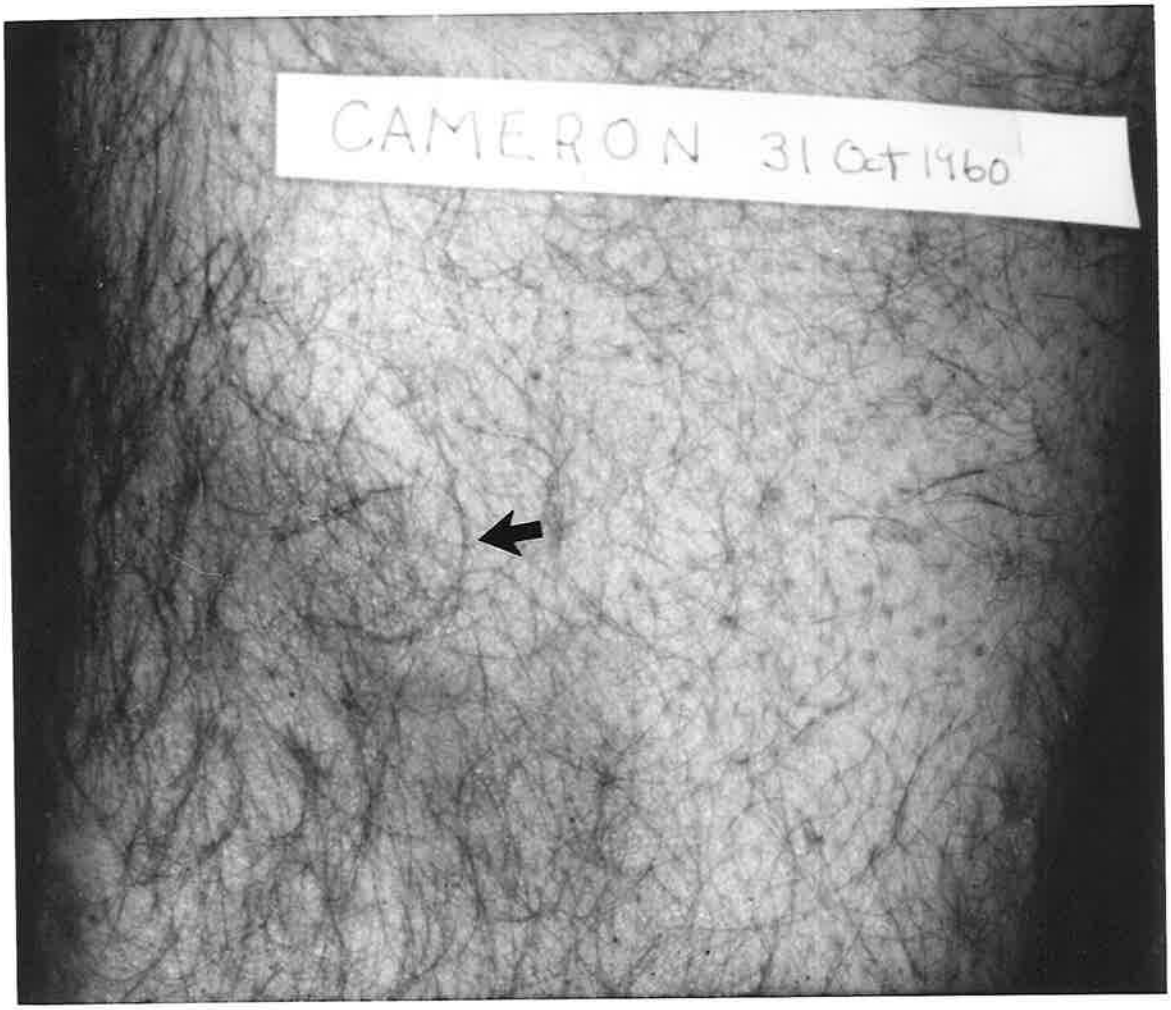
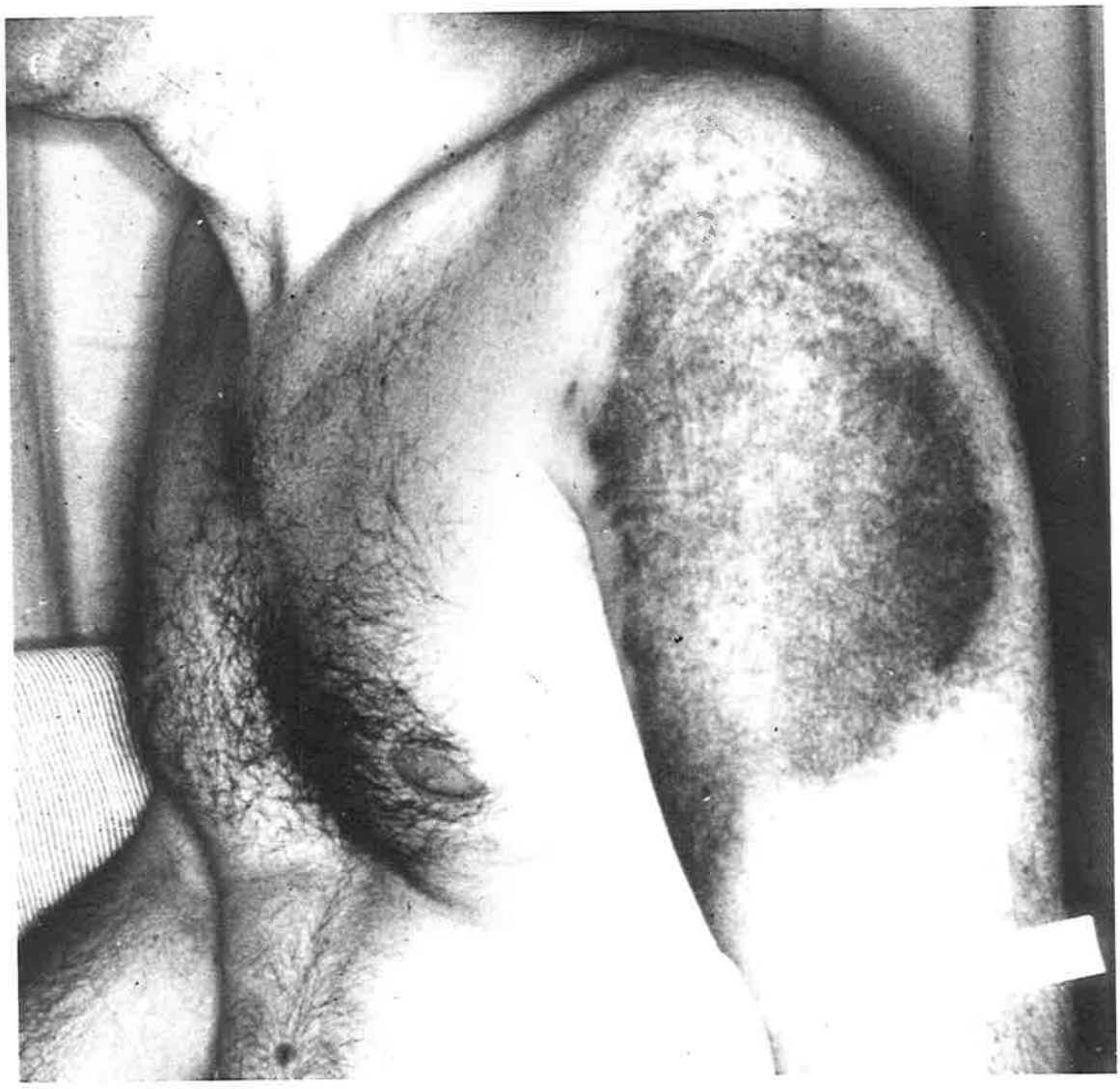

CAMERON 31 Oct 1960

Figure 18.

SPONTANEOUS HAEMATOMA DURING STREPTOKINASE INFUSION.

This picture shows the spontaneous haematoma that developed at the same time as lysis of the superficial vein thrombus in Fig. 16 was achieved by prolonged infusion of streptokinase.



circulating plasminogen had been converted to plasmin.

Two cases showed four plus whole blood lysis in specimens taken within the first eight hours of the infusion and one case was three plus in twenty-four hours. The remaining two showed no peripheral whole blood lysis in twenty-four hours. All the fibrinogen levels decreased and the prothrombin times increased, with the clotting time showing no significant change in any case. The earlier patients developed a fever of 104° - 105°F during the infusion, but the most recent cases have not been higher than 99° - 100°F with minimal general reaction to the infusion. It has not been necessary to use cortisone to counteract any side-effects produced by the streptokinase.

Phase II. Non-infusion experiments.

Series 4. Analysis of Canine Thrombi (Table IV).

(1) MACROSCOPIC EXAMINATION.

The gross specimens of all thrombi, in vivo, showed some variation in themselves, and on comparison with each other, at the selected time for removal. In both arteries and veins the diameter of the segment containing a thrombus increased for the first week and then contracted gradually to normal size over the following two weeks. This shrinkage continued, being most marked in the long term intima-stripped arteries and veins, and vein grafts. The final dimension of these vessels was approximately two-thirds their original size. The veins and arteries thrombosed by reinjection only, were less markedly narrowed with a through circulation being evident in the late venous thrombi; no pulsation was present in the arteries thrombosed in the same way.

(2) MICROSCOPIC EXAMINATION.

This will be presented under the two headings of organization and canalization. Tables relating the two processes are shown in appendices I and II.

(a) Organization of Thrombi.

1. Reinjection venous and arterial thrombi.

Organization of the thrombus had already commenced

## TABLE IV.

### ANALYSIS OF 139 EXPERIMENTAL CANINE THROMBI.

Total number of thrombi studied.

(a) REINJECTION THROMBI.

| | Early[1] | Late[2] | Total. |
|---|---|---|---|
| Venous | 9 | 23 | 32 |
| Arterial | 12 | 10 | 22 |

(b) THROMBI IN INTIMA-STRIPPED VESSELS.

| | Early | Late | Total. |
|---|---|---|---|
| Venous | 10 | 11 | 21 |
| Arterial | 8 | 14 | 22 |

(c) REINJECTION THROMBI IN VENOUS GRAFTS IN AN ARTERIAL SEGMENT.

| | Early | Late | Total. |
|---|---|---|---|
| Venous | 14 | 28 | 42 |

(1) 'Early' is the term applied to thrombi up to the end of the twentieth day from formation.

(2) 'Late' is the term applied to all thrombi at twenty-one days or over.

in specimens removed at twenty-four hours. In the veins there was a discontinuous endothelium-like layer at the retracted clot margin, whilst at the arterial clot margin this layer was continuous. These cells appeared to be circulatory in origin (Fig. 19). As retraction and contraction occurred crevices appeared within and at the margin of the clot (around 3 days), and became lined with flattened cells. (Fig. 20).
By the end of the first week intimal proliferation was evident and cellular infiltration from the vessel wall to the clot, at the site of attachment, was marked. Pigment containing macrophages, degenerated red cells and mononuclears were present within the clot mass at fourteen days (Figures 21, 22) with definite fibrous organization evident at the margins. But not until twenty-one days could the final organized picture be assumed. At this time the venous thrombi varied in their form, (Fig. 23 diagram) being totally occlusive; the lumen crossed by a bridge of organized thrombus; concentric thickening of the vessel wall by organized thrombus, or an organized nubbin attached to one area of the wall. The majority showed this pattern of organization around wide through channels. (Fig. 24).

Figure 19.

Photomicrograph to illustrate cell deposition from the circulation onto the clot surface.

The photomicrograph shows the ease with which cells can be deposited from the circulation in the early stages to help in the initial phases of organization, and before the vessel wall plays any part. Between the retracted clot margin and vessel wall is fresh blood.

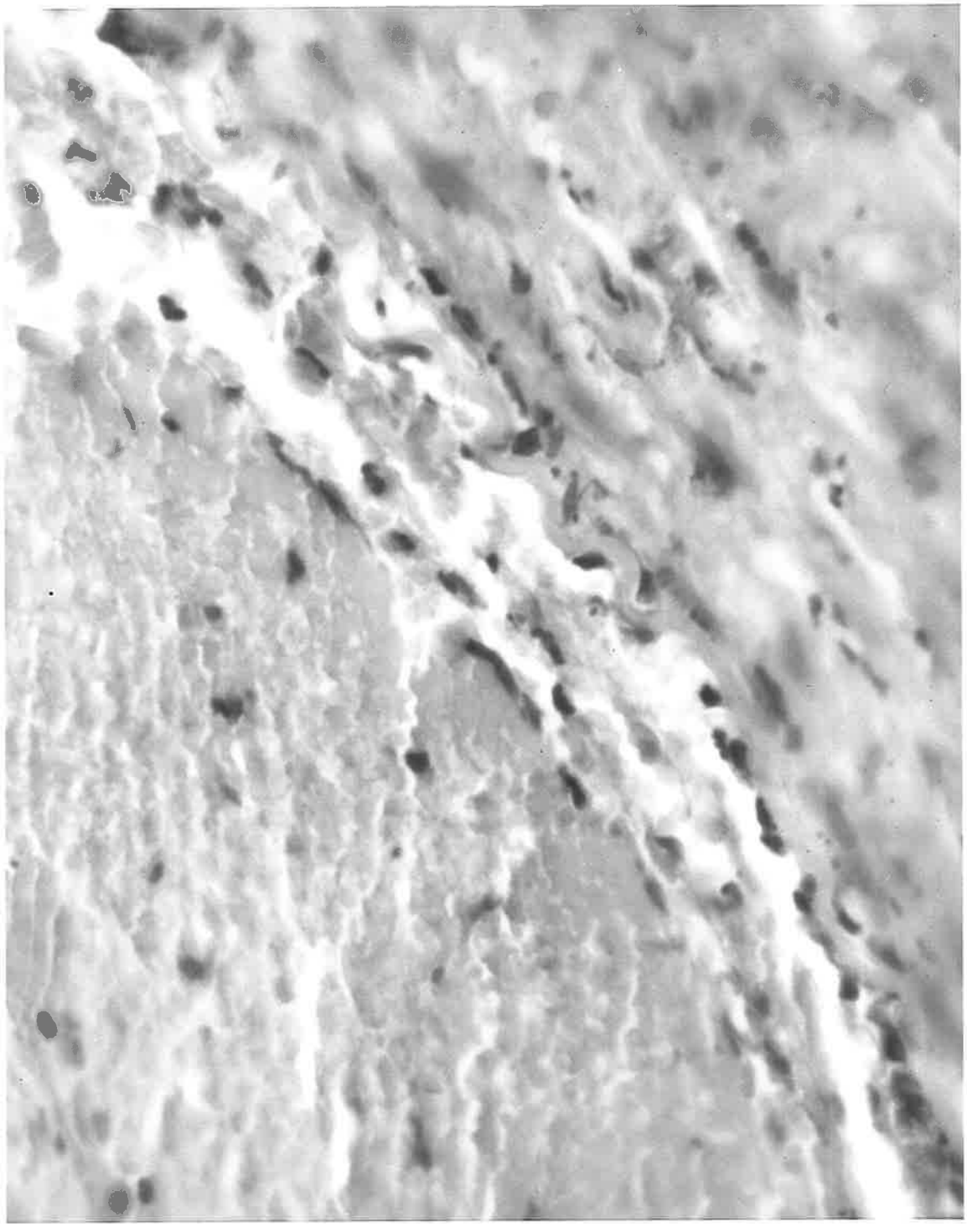


Figure 20.

Photomicrograph of a reinjection venous thrombus at 72 hours.

At 72 hours an endothelial covering to the clot is evident with some marginal retraction, red cell clumping, and degeneration.

The appearance of a similar thrombus in an artery at this stage is the same.

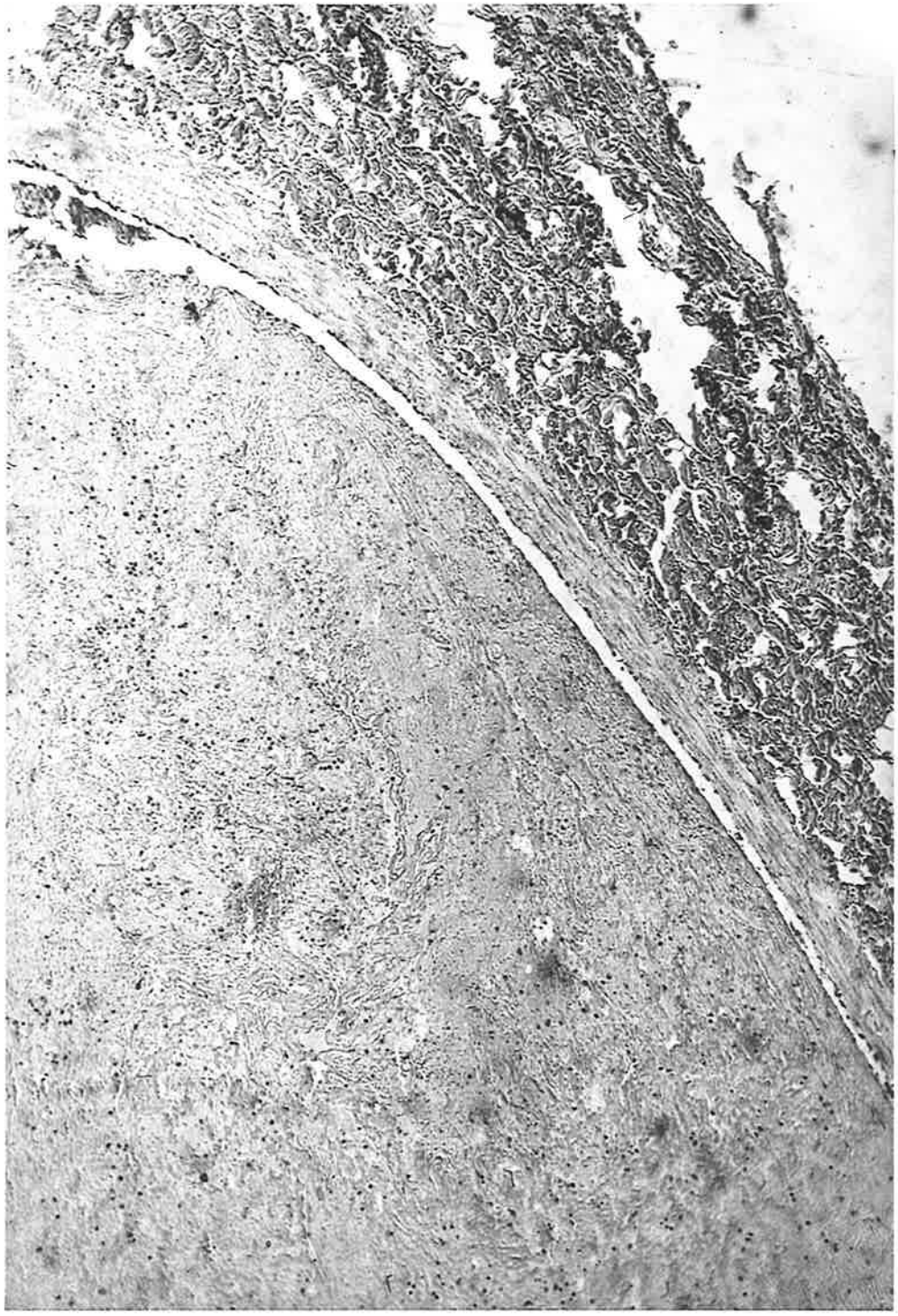


Figure 21.

Photomicrograph of a reinjection venous thrombus at 14 days.

Surface organization is evident here with degeneration and disintegration of the central red cell mass.

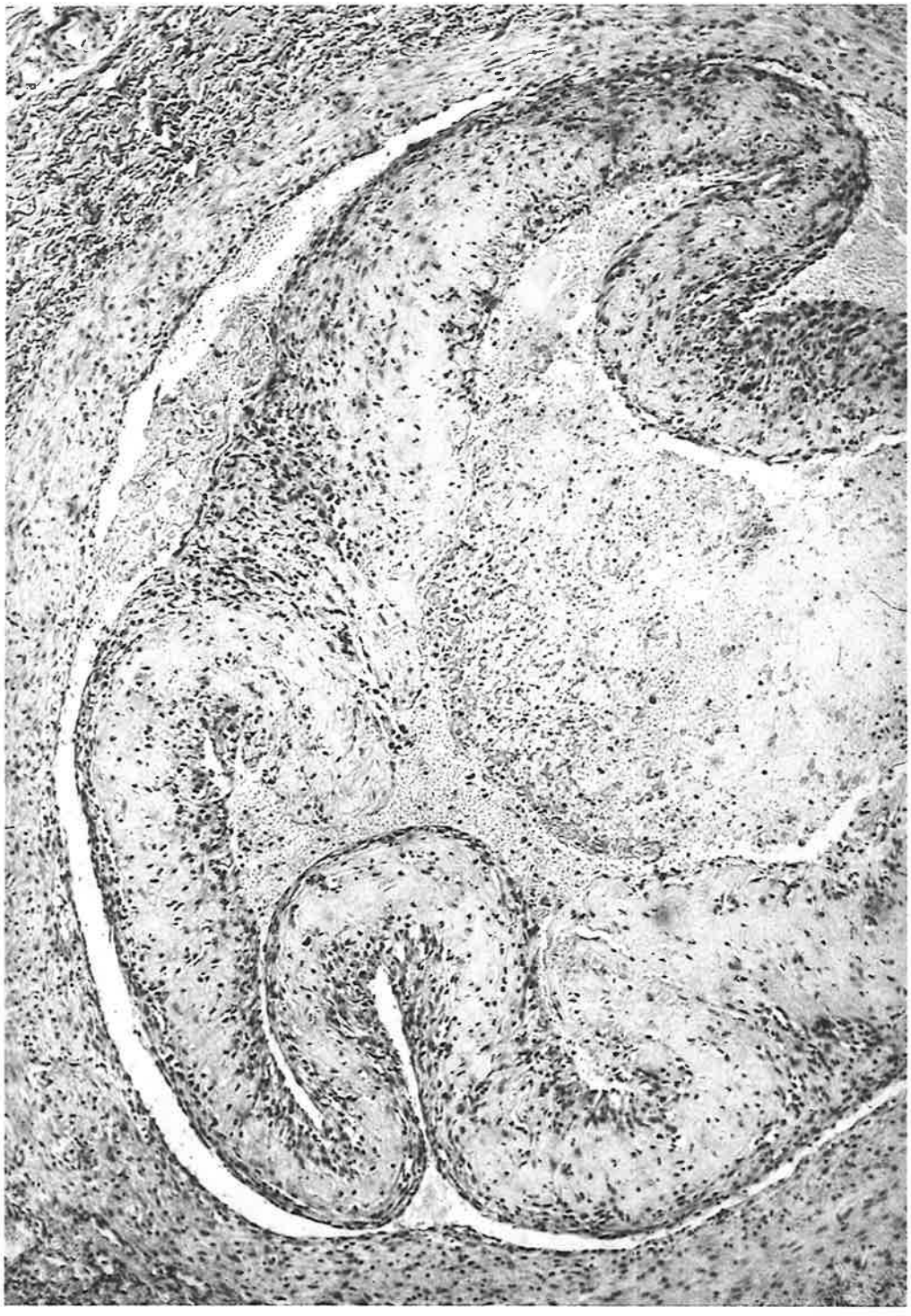


Figure 22.

Photomicrograph of a reinjection arterial thrombus at 14 days.

This shows cellular infiltration and fibrosis. Organization has occurred mainly in the superficial areas, with fresh red cells in the centre of the degenerating red cell mass seen at the left margin of the vessel lumen (arrow).

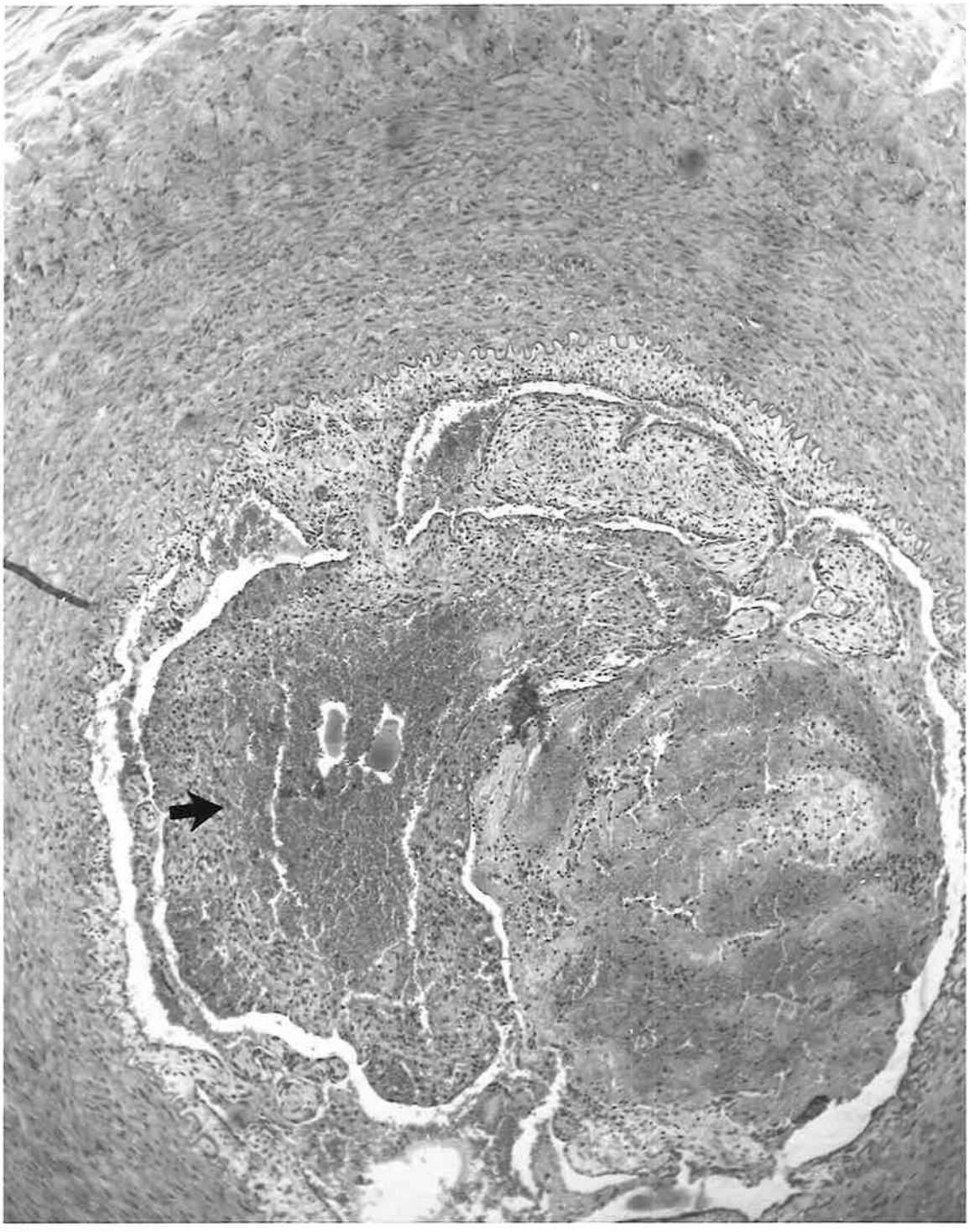


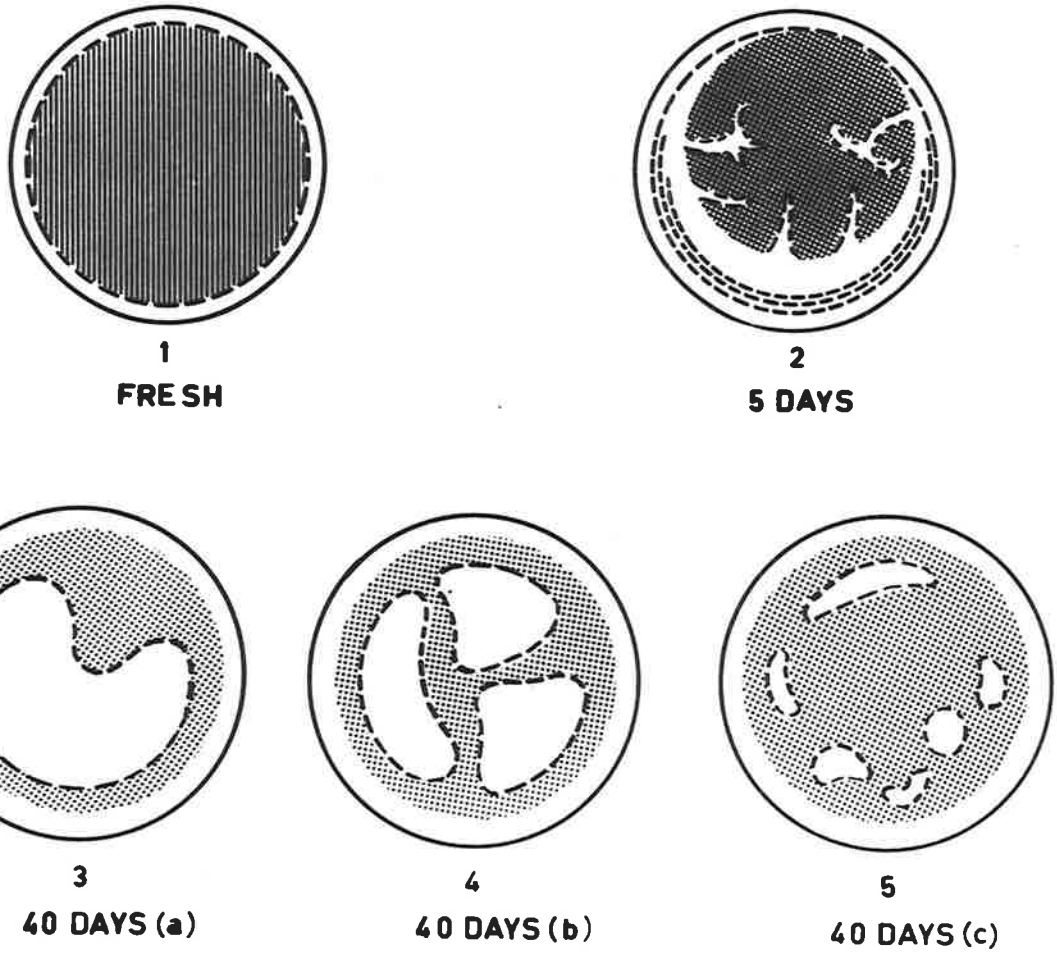
**Figure 23.**

Diagrammatic representation of the ageing of reinjection thrombi.

This diagram shows the essential changes in a thrombus during organisation up to the final residuum which may be occlusive, concentric, bridge-like or a nubbin.

Venous thrombi organize mainly as (a) and (b), arterial thrombi as (c).

# REINJECTION THROMBUS



In contrast, the arterial thrombi were predominately occlusive. The cell changes at any time were the same as those seen in the venous thrombi but the cell mass was penetrated by canals whose area was less than fifty per cent of the total lumen - 40 (c) Figure 23. As the thrombi aged beyond twenty-one days there was no change in this occlusive form, but there was a progressive loss of inflammatory cells and an increase of fibrous tissue and pigment cells. (Figure 24).

Intimal proliferation was a more marked feature of arterial organization varying in its intensity from marginal involvement to almost complete replacement of the thrombus (Figure 25). Some muscle cells were seen in the walls of the small channels penetrating the thrombus giving the appearance of miniature blood vessels.

**Figure 24.**

**Photomicrograph of a reinjection venous thrombus after 40 days.**

This shows a fibrous nubbin attached to the vessel wall with a wide canal allowing recirculation. Pigment cells are evident in the clot centre with a small channel showing muscle in its wall on microscopy.

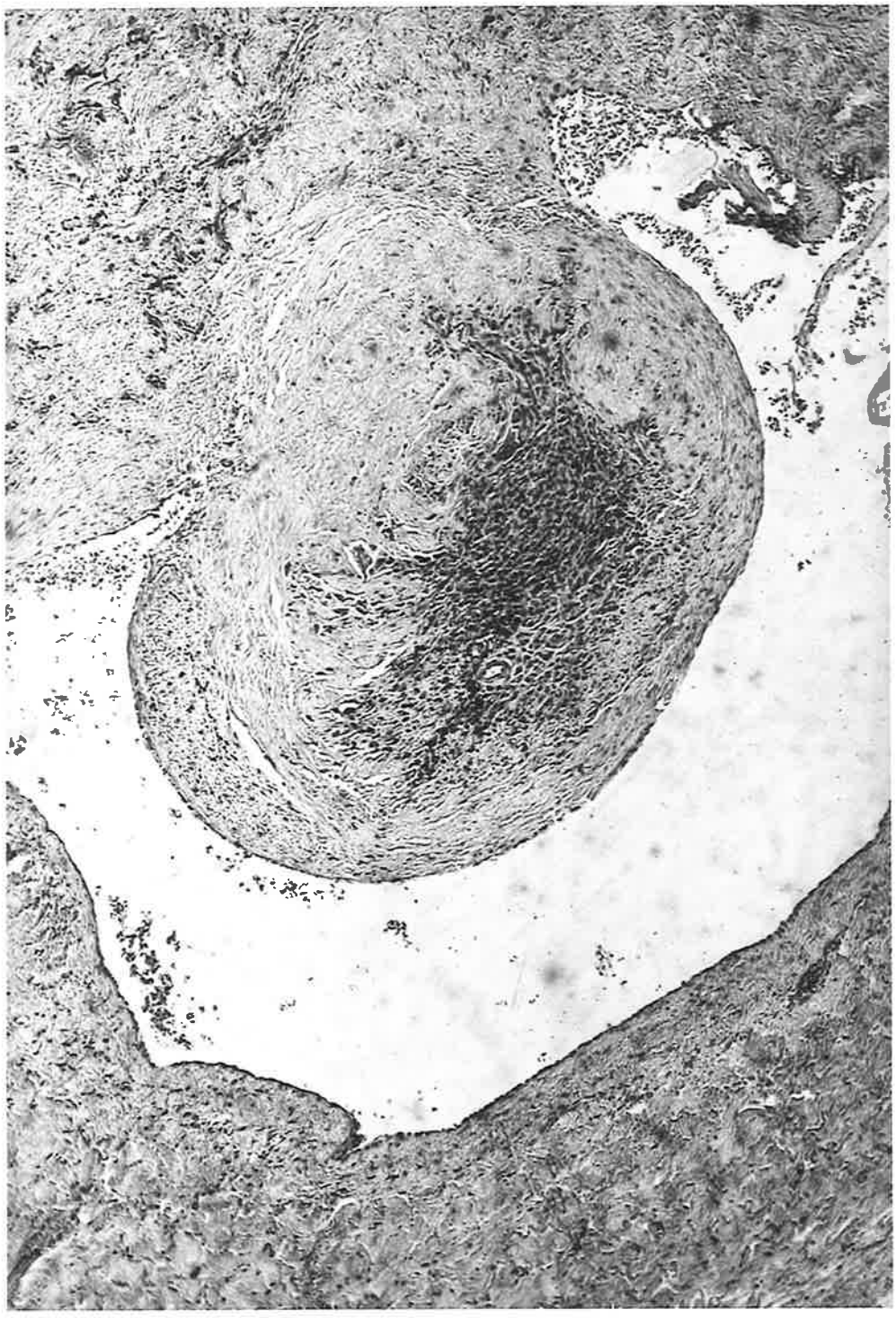
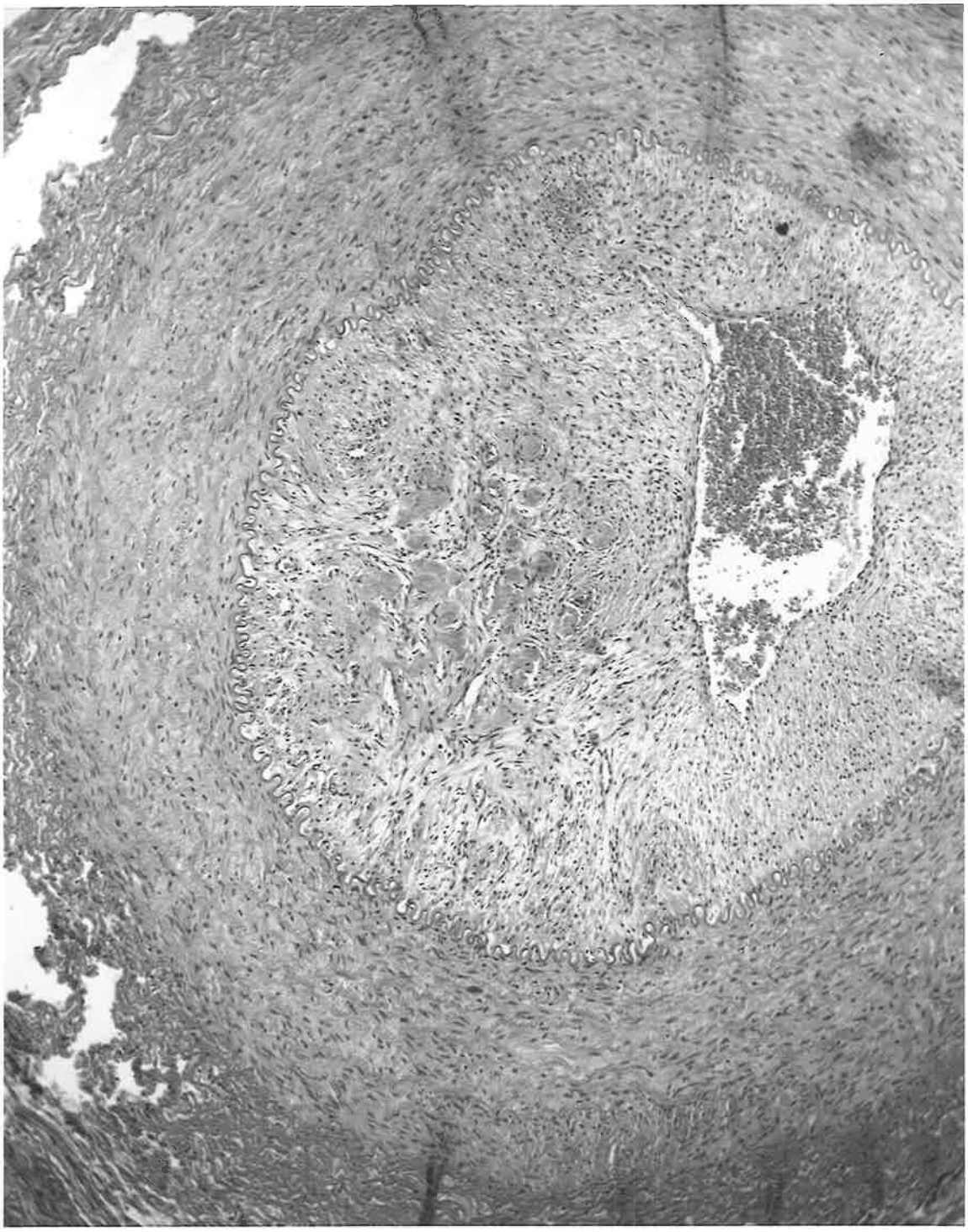


Figure 25.

Photomicrograph of a reinjection arterial thrombus after 40 days.

This shows a medium sized canal allowing a through circulation, but not of the same calibre as the venous channels.

The cellular replacement is due predominantly to intimal proliferation. Some islands of degenerated red cells can be seen at the left margin of the thrombus.



2. Thrombi in intima-stripped veins and arteries.

Fresh thrombi were totally occlusive and made up of a red cell mass with more fibrin formation than was seen in the reinjection thrombi. The fibrin strands fixed the clot to the vessel wall. Red cell degeneration, cellular lining of the crevices, fibrin strand condensation and loose fibrous tissue invading from the periphery, were evident at a week. Fibrous tissue replacement continued to increase, being very evident at the end of fourteen days (Figures 26 and 27). Progressive fibrosis of the thrombus continued with obstruction of the vessel lumen in most cases and penetration by small canals only (Figure 28 Diagram). Most venous specimens showed this pattern of organization but some specimens of forty days and over showed a medium or large channel penetrating the thrombus with through flow (Figure 29).

Any artery, however, was invariably occluded (Figure 30). The thrombus was adherent to the vessel wall forming a cord-like structure similar to human arteries that have occluded after thromboendarterectomy performed months previously. It was penetrated by only small muscular walled channels with no significant through flow.

**Figure 26.**

Photomicrograph of a fourteen day thrombus in an intima-stripped vein.

Marked fibrous tissue replacement of the thrombus is evident in this section. Pigment cells are the predominant cells present (arrow) with smaller inflammatory cells in the upper and lower fields.

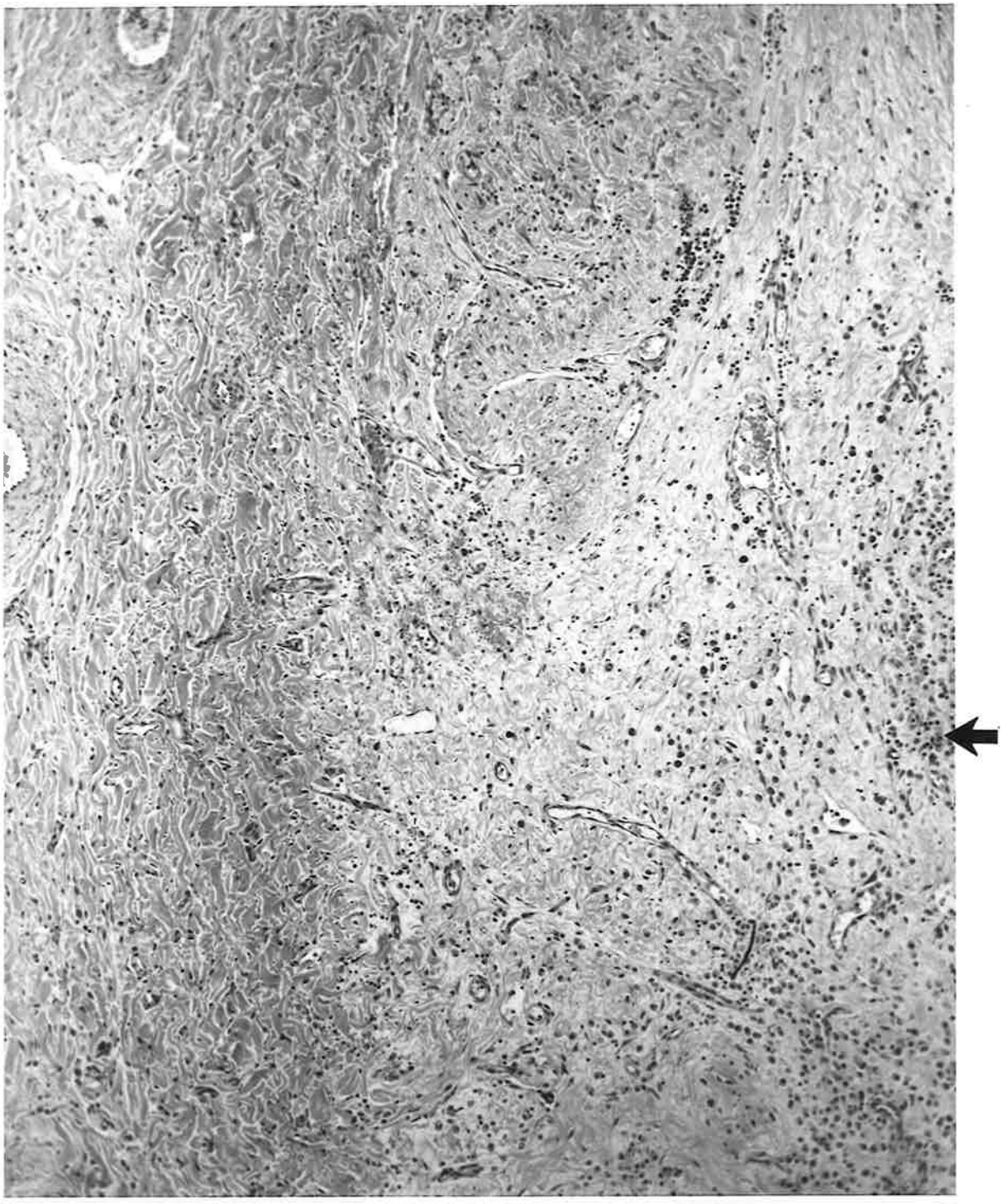


Figure 27.

Photomicrograph of a fourteen day thrombus in an intima-stripped artery.

This section shows early, intense, fibrous change with crevices in the cell mass.

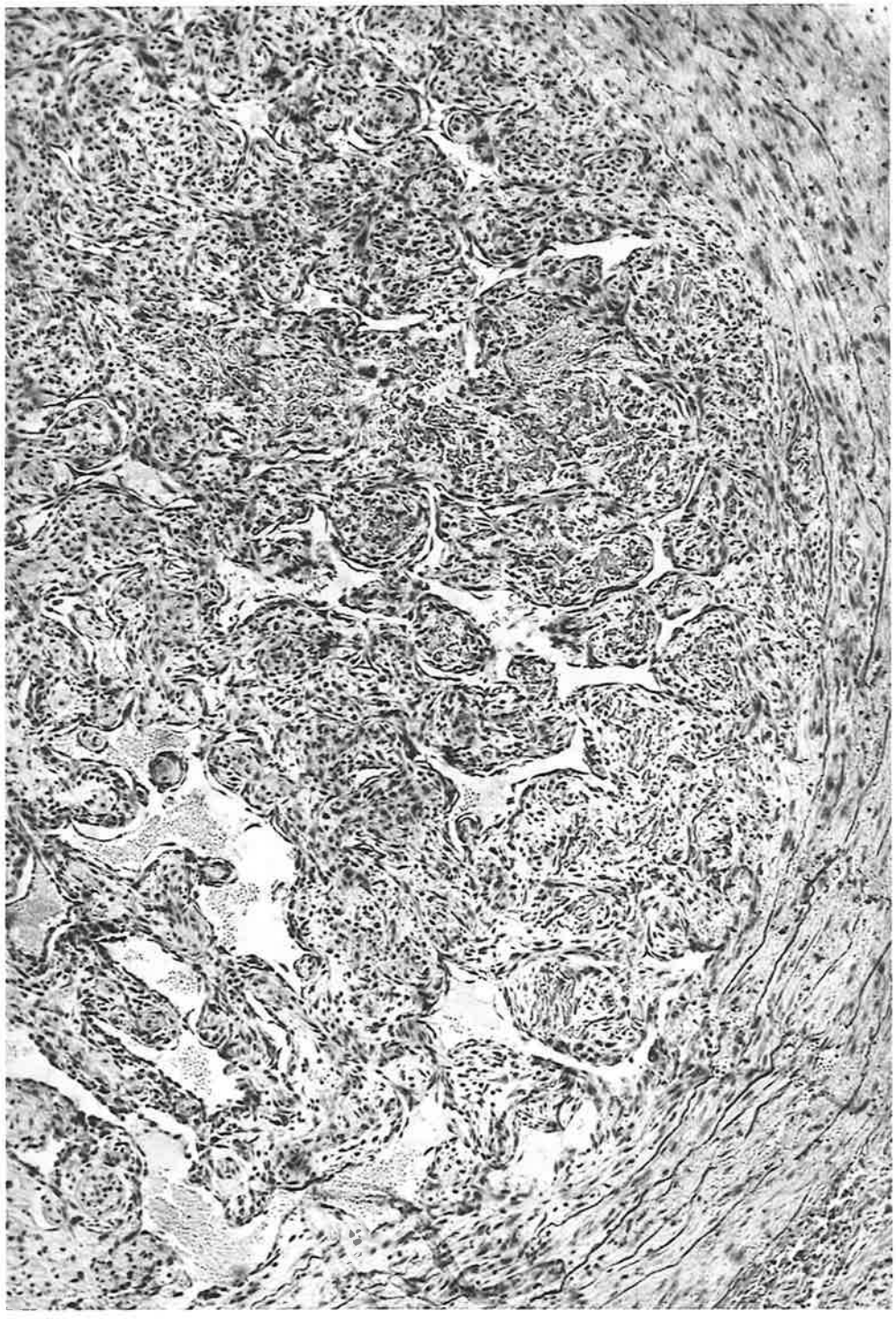
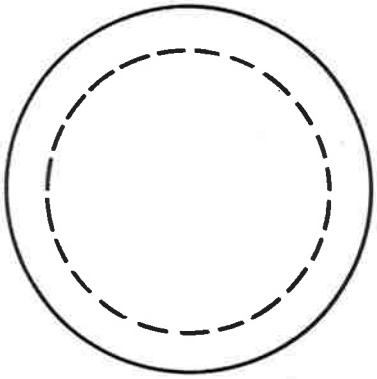


**Figure 28.**

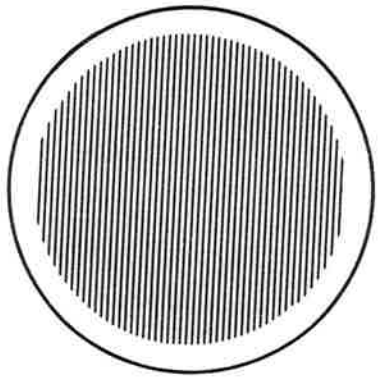
**Diagrammatic representation of the ageing of a thrombus in an intima-stripped vessel.**

This is a diagrammatic representation of the course of organization and the final appearance of a thrombus and its vessel produced by this method. The shrinkage of the vessel can be seen, and the small canals penetrating the thrombus.

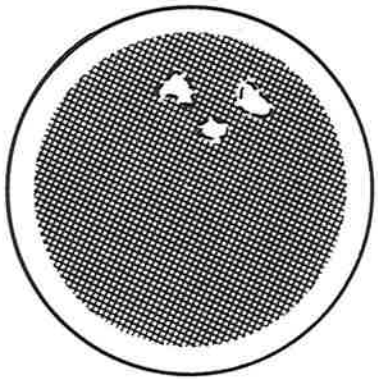
# THROMBUS IN AN INTIMA STRIPPED VESSEL



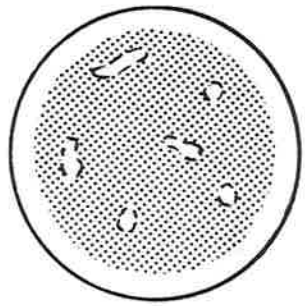
1
**Normal Artery**



2
**Fresh Thrombus**



3
**5 Days**



4
**40 Days**

Fresh red blood cells

Degenerated red blood cells

Fibrous tissue

Endothelium

Figure 29.

Photomicrograph of a thrombus in an intima-stripped vein after 40 days.

This section shows a medium sized channel between the wall (arrow) and the organized thrombus in the centre of the picture. In a few thrombi the channels were wide, but, as in Figure 30, most showed smaller channels.

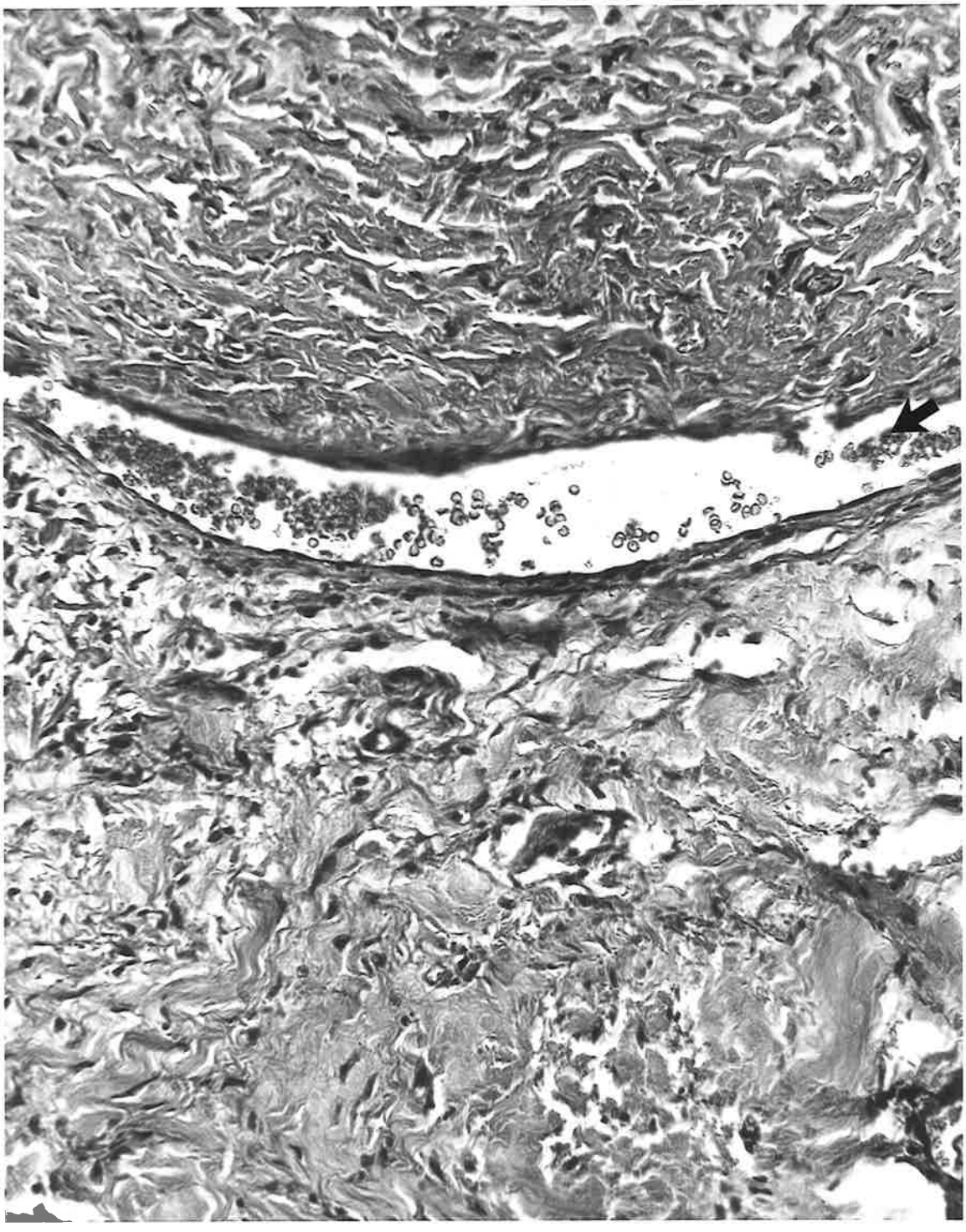


Figure 39.

Photomicrograph of an intima-stripped artery with a thrombus after forty days.

This section is occlusive and fibrous. Small channels can be seen, some with muscle in their walls, and this is representative of all specimens at this stage. In the intima-stripped arteries many sections showed thick muscle in the walls of small channels penetrating the organized thrombus. Reinjection thrombi in normal arteries showed a less well formed muscle layer around the new small channels.

3. Reinjection Thrombi in venous grafts incorporated in the arterial circulation (Figure 31, Diagram).

The thrombi in vein grafts showed the same cellular infiltration and degenerative changes at corresponding periods as those of the reinjection venous thrombi. The late thrombi, however, showed a marked difference in the degree of canalization.

The thrombus, although in a vein, was occlusive, and penetrated by medium or small canals. These were more evident in the earlier specimens than in the late (Figure 32).

There was neither evidence of wide channels nor adequate through flow in the late specimens (Figures 33, 34).

In this respect they were similar to the reinjection arterial thrombi. The veno-arterial suture line showed dense formation of the fibrous tissue and this seemed to prevent entry of the proximal blood stream into the vein graft segment.

(b) Canalization. (Table V). (Page 118).

Injection studies using a solution of India ink in 1% agar revealed channels traversing the thrombus to a varying degree in all specimens.

The simple reinjection venous and arterial thrombi showed good dye penetration through serial sections cut at varying intervals from proximal to distal end. The occluded veins showed wide channels with through flow (Figures 35 and 36), whereas the arterial thrombi were penetrated by mostly small and medium canals. There was no evident

Figure 31.

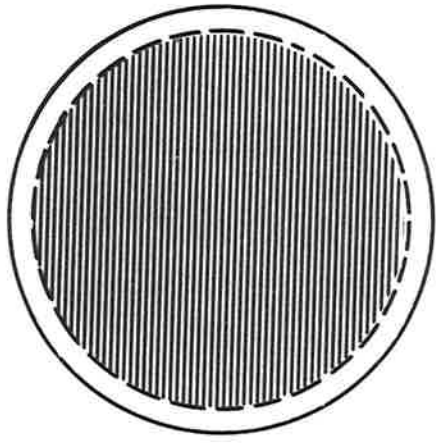
Reinjection Thrombus in Vein Graft.

This diagram shows the final appearance of thrombi in a vein graft.

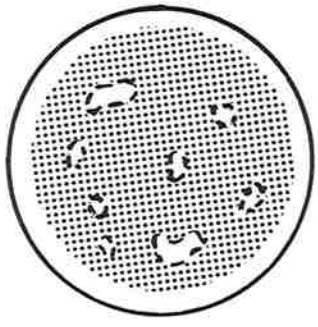
Small canals and dense fibrosis are seen at the suture line, with slightly larger canals and the same dense fibrosis centrally.

The vessel has shrunk with no through flow evident in any specimen.

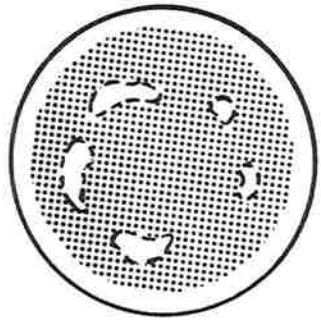
# REINJECTION THROMBUS IN A VEIN GRAFT.



Fresh Thrombus



40 Day Thrombus
at Venoarterial line



40 Day Thrombus
at Vein Graft centre

Figure 32.

Photomicrograph of a twenty-eight day reinjection thrombus in a vein graft in the arterial system.

This picture shows marginal canals only with fresh blood in the lumen.

The remainder of the clot had been organised and showed no other canals of this calibre, or with fresh blood in the lumen.

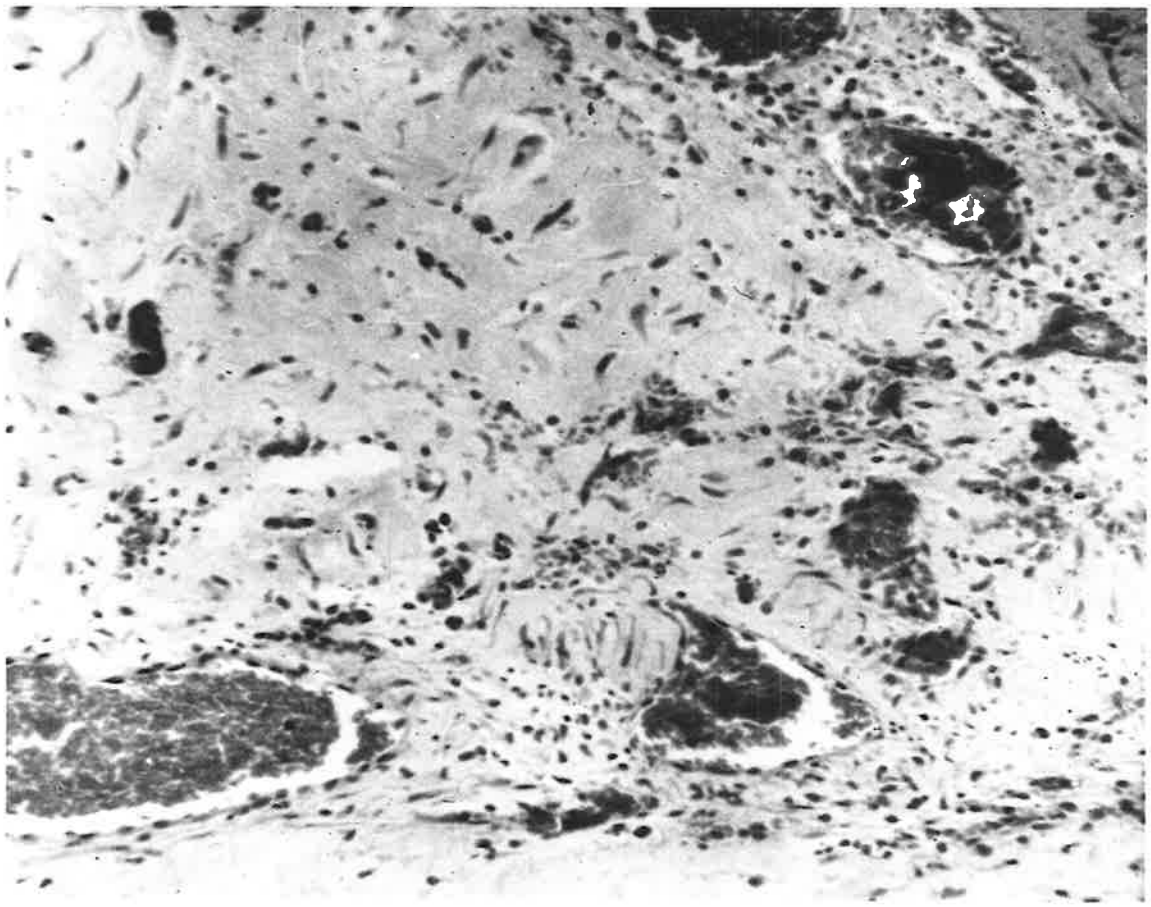


Figure 33.

Photomicrograph of a sixty day reinjection thrombus in a vein graft incorporated in the arterial system.

This overall picture shows fibrous organisation of the thrombus with little canalisation, and no through circulation.

This is the typical picture in these specimens at this age.

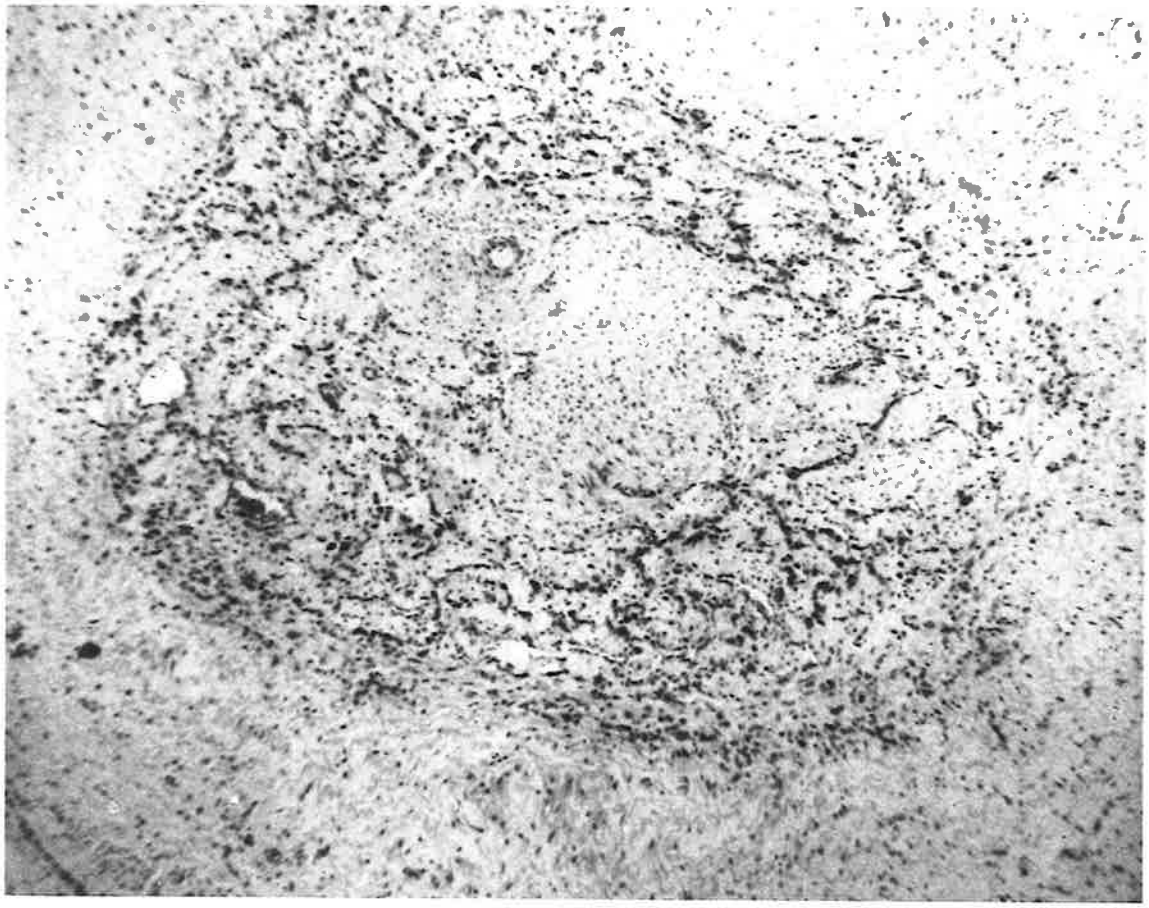
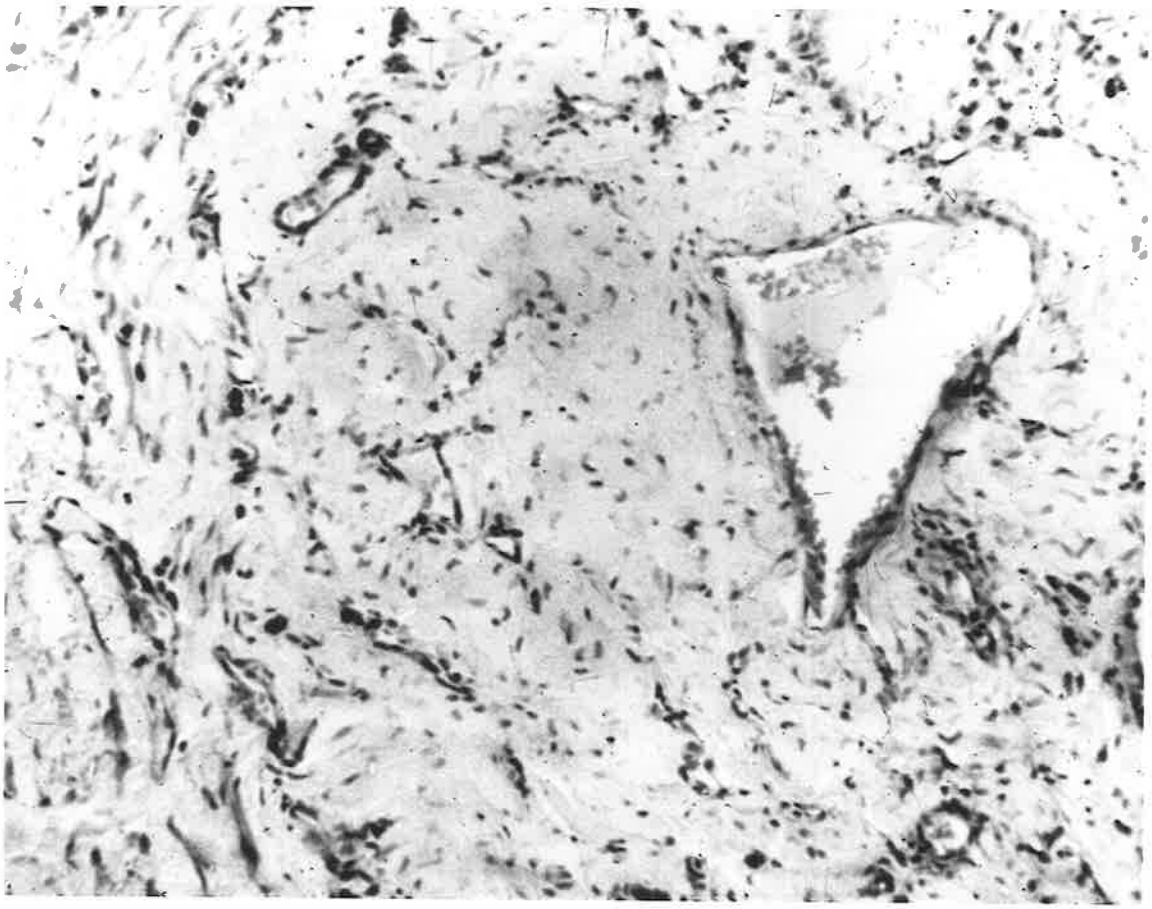


Figure 34.

Photomicrograph of a sixty day reinjection thrombus in a vein graft incorporated in the arterial circulation.

This photomicrograph is a magnified view of a solitary channel penetrating an organized thrombus.

The appearance of the whole vessel and thrombus was not unlike that seen in the reinjection arterial thrombus of Figure 25.



## TABLE V.

## CANALIZATION OF THROMBI.

| | Total | No canals Occlusive | Canaliculi | Medium channels | Wide Canalization |
|---|---|---|---|---|---|
| Reinjection Venous Thrombi | 32 | 6 | 4 | 7 | 15 |
| Reinjection Arterial Thrombi | 22 | 9 | 4 | 7 | 2 |
| Thrombi in intima-stripped veins | 21 | 4 | 10 | 3 | 4 |
| Thrombi in intima-stripped arteries | 22 | 4 | 14 | 3 | 1 |
| Thrombi in venous grafts incorporated into an arterial segment | 42 | 10 | 12 | 19 | 1 |

Figure 35.

Photomicrograph of a recanalized venous reinjection thrombus.

This section shows the wide lumen for blood flow and the presence of dye has been verified in all sections.

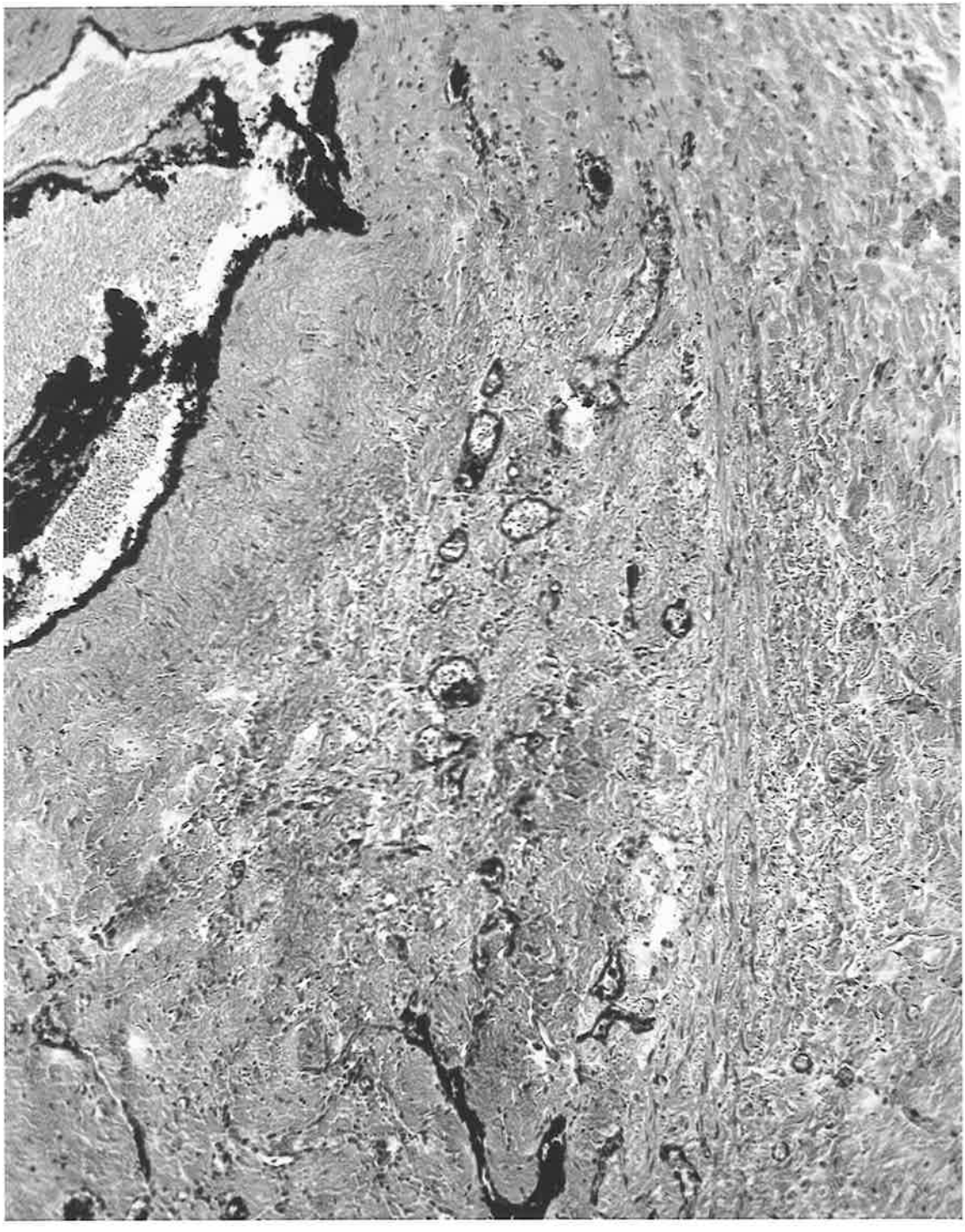
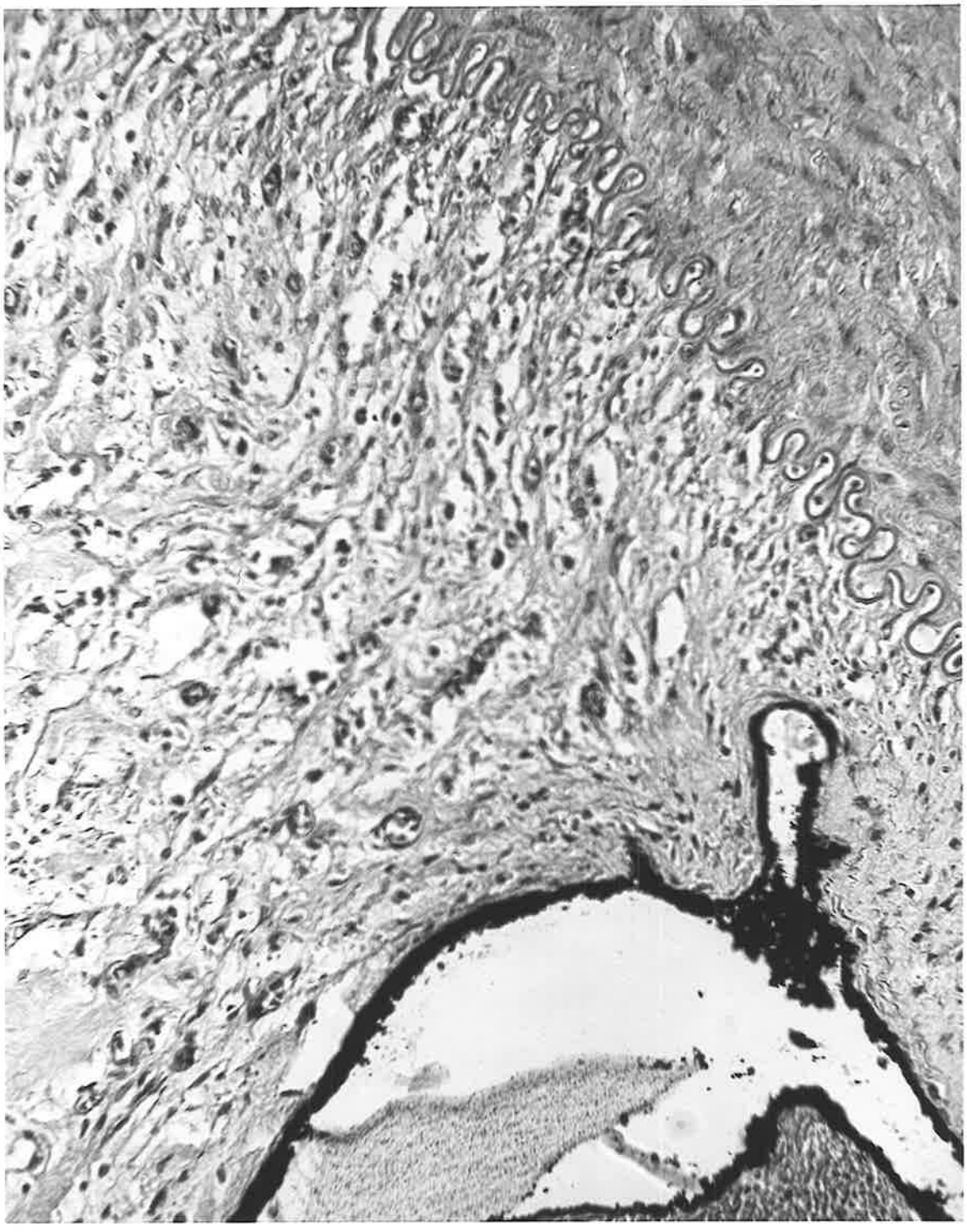


Figure 38.

Photomicrograph of a recanalized arterial reinjection thrombus.

This shows dye in a canal of medium size in contrast to the small canals of the thrombus in Figure 38.



pulsation in these vessels.

Thrombi in the intima-stripped veins showed through channels in all (Figure 37). Four late specimens showed wide canalization and good through flow with the remainder showing less satisfactory channels, and no blood flow on naked-eye inspection. The majority resembled the thrombi in intima-stripped arteries. The thrombi formed in the intima-stripped arteries (Figure 38) were densely fibrous, occlusive, and penetrated by small canals which showed less dye distally, one specimen having no dye in either a central or distal section. The reinjection thrombi in venous grafts showed no through flow of dye and this was attributed to the intense fibrous reaction and shrinkage which occurred at the proximal veno-arterial suture line. However, the channels seen were of the same calibre and number as those seen in the arteries occluded by reinjection thrombi resembling them in all respects. There was no hint of the wide canalization or through flow seen in similar thrombi in the venous circulation.

Figure 37.

Photomicrograph of a thrombus in an intima-stripped vein after 40 days with dye penetrating the canaliculi.

These canaliculi are the size of most channels in these thrombi. A few did show wider canals and a through circulation.

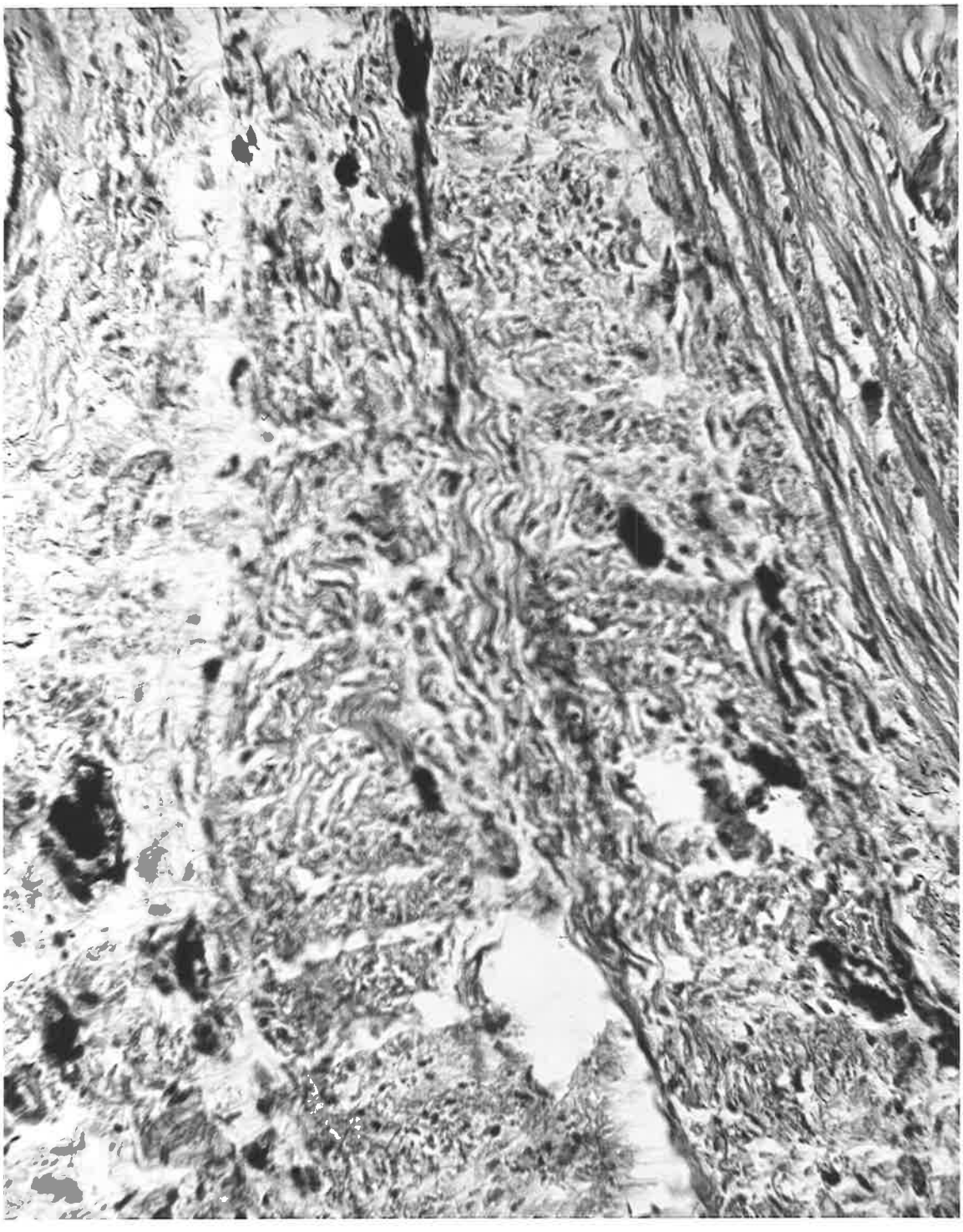
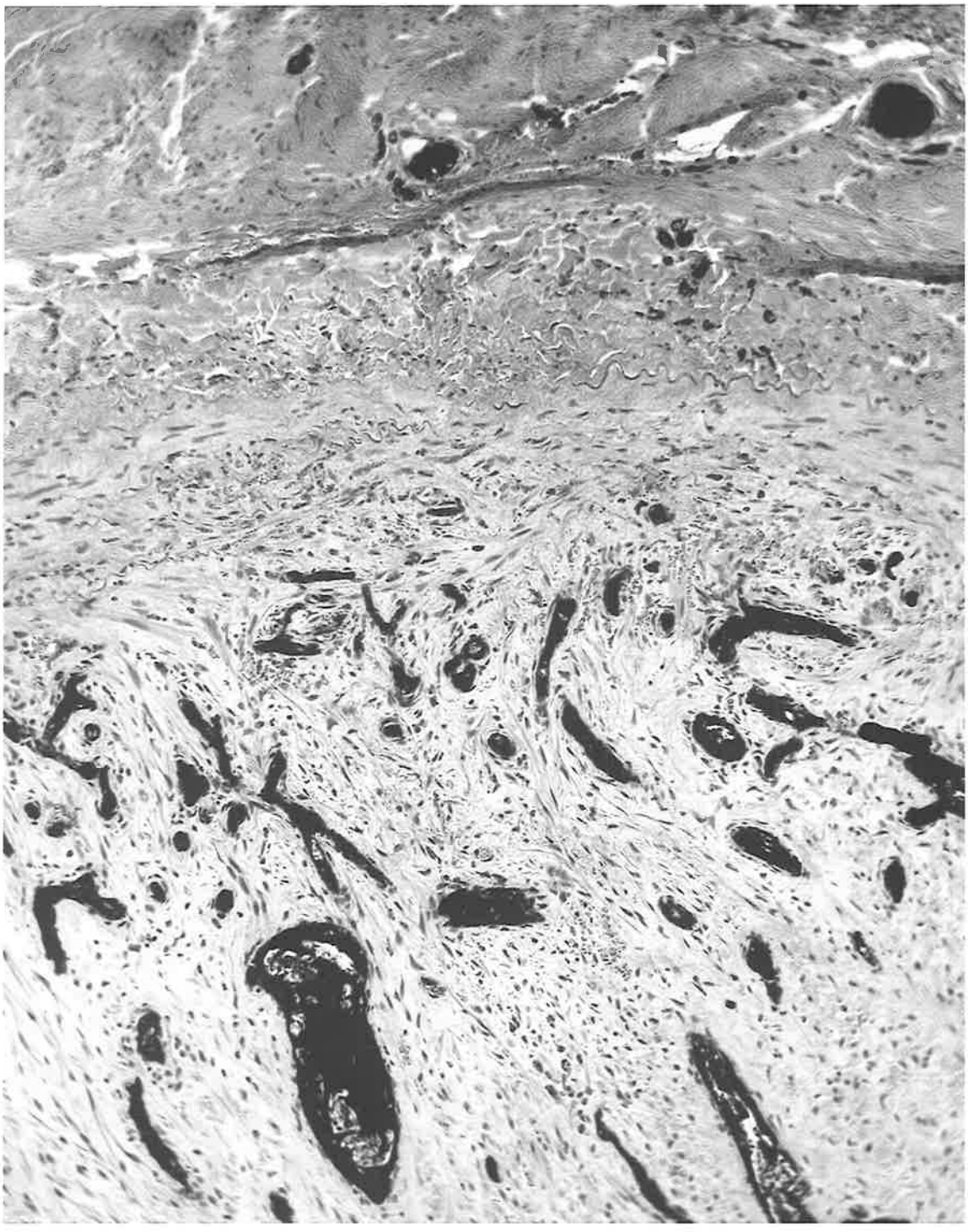


Figure 38.

Photomicrograph of a thrombus produced in an artery by intimectomy with dye in the canaliculi.

Dye has penetrated all sections of this vessel and its thrombus. There is communication through these canaliculi with the proximal and distal lumen; but their size was not large enough to allow any significant through flow.



Series 5. Human Thrombi.

(1) Arterial.

From Table VI it can be seen that little organization of thrombi in these large and medium human atherosclerotic vessels occurred. The vessel wall was distorted by the atherosclerotic plaque and showed no satisfactory division of its intima, media, or adventitia. The thrombi were a mass of degenerated red cells, eosinophilic staining, with an occasional white cell lining the crevices at the margin.

This is in contrast to the canalization and organization of thrombi described by Duguid in atherosclerotic coronary arteries. It is also in marked contrast to the organization and canalization seen in various types of thrombi produced experimentally with or without damage to the vessel wall. It would seem that the wall is unable to react to the intraluminal foreign body, and the nature of the circulation prevents its canalization.

The changes in the limb, the arteriographic picture, and the gross and microscopic view of the occlusive lesions are seen in Figures 39, 40, 41, 42.

Table VI.

This table reveals in eight out of ten cases no such correlation but rather complete lack of organization and canalization irrespective of the age of the thrombus.

Case 3 was a specimen from the coronary artery. The remainder were from the aorta, carotid, femoral and popliteal arteries.

Case 10 was sufficiently early to attribute the cellular changes to the circulation, and the greater part of the vessel wall was normal.

## TABLE VI.

**Occlusive thrombi in human atherosclerotic arteries.**

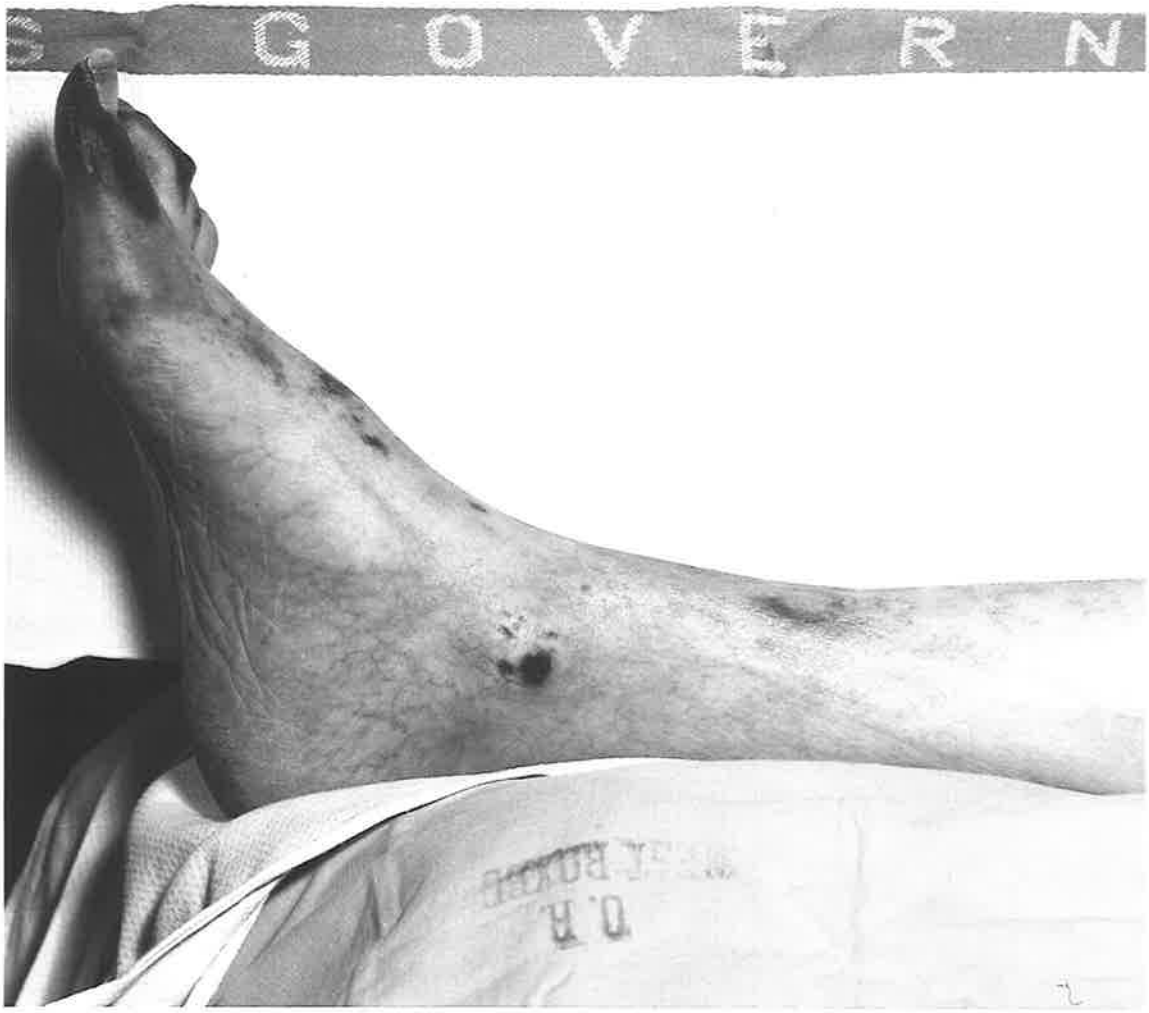
**Correlation of chronological and histological age.**

| Case | Minimum Age | Maximum Age | Correlation of age and microscopy | Correlation of age and symptoms | Organization | Recanalization |
|---|---|---|---|---|---|---|
| 1. Prunty | 14 days | 12 wks. | No | No | No | No |
| 2. Swift | 35 days | 5 wks. | No | No | No | No |
| 3. Lincoln | 12 days | 10 wks. | Yes | No | Yes | No |
| 4. Parshely | 7 days | 36 wks. | No | No | No | No |
| 5. Lawry | 17 days | 8 wks. | No | No | No | No |
| 6. Godzik | 21 days | 8 wks. | No | No | No | No |
| 7. Laracy | 10 days | 16 wks. | No | No | No | No |
| 8. Foley | 13 days | 7 wks. | No | No | No | No |
| 9. Parelli | 5 days | 26 wks. | No | No | No | No |
| 10. Schreiter | 5 days | 5 days | Yes | Yes | Yes | No |

Figure 39.

Gross appearance of a limb with severe arterial occlusion.

The foot and leg were cool with blue and white mottling, diminished sensation and movement. Distally, these changes have progressed to overt gangrene.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## Figure 40.

Arteriographic appearance of the limb in Figure 39.

This reveals a long popliteal block with collaterals around the block, and patency below to its bifurcation. The patent vessel shows a ragged outline.

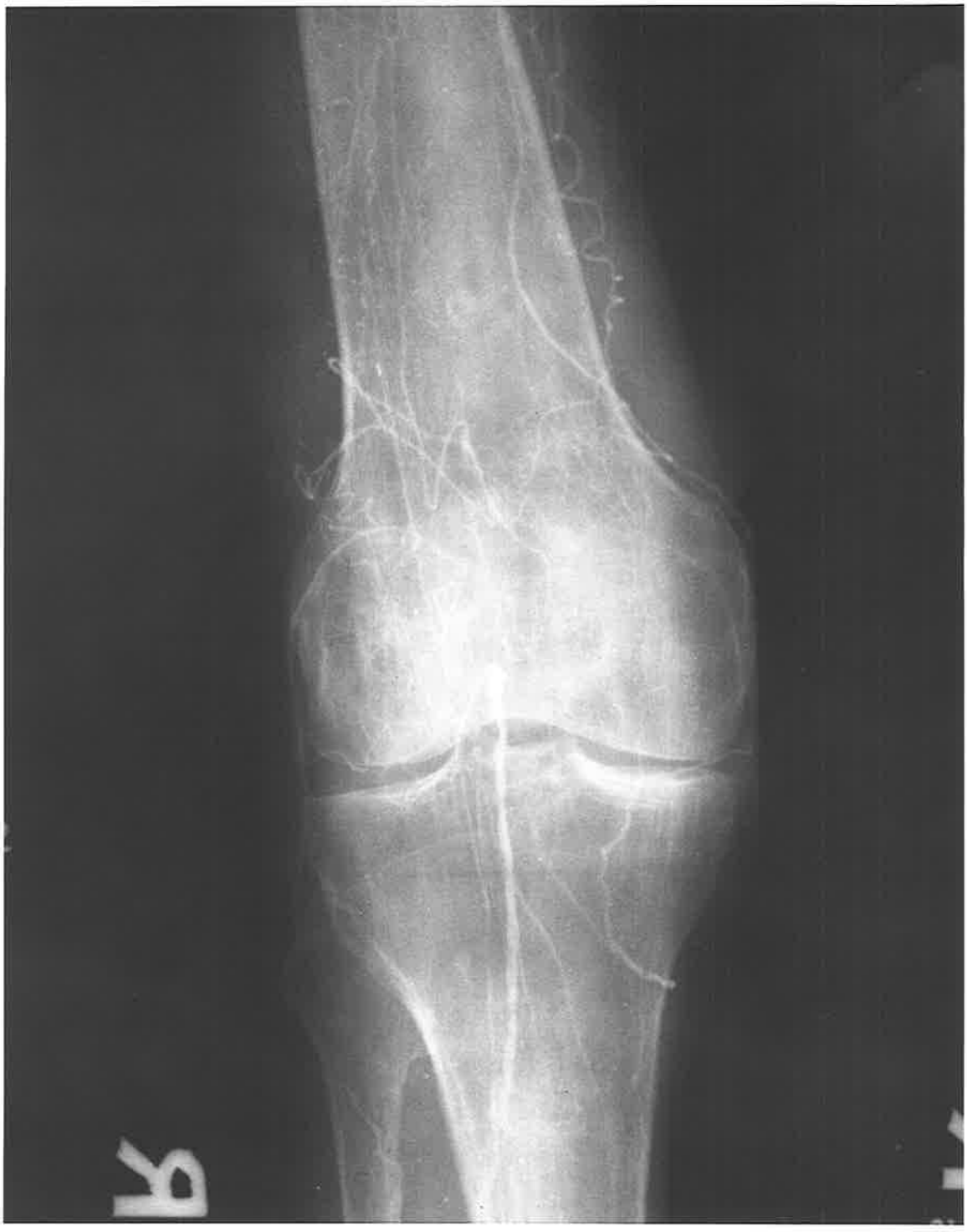
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Figure 41.

Macroscopic appearance of an occlusive human thrombus in an atherosclerotic vessel removed from an amputated stump.

This occluded artery was removed from the limb seen in Figure 39, after amputation. The lesion was red, moist, occlusive, and had been present in the artery for sixty days. This section is representative of the thrombus occluding the full length of the politeal artery. It was removed from the amputated leg.

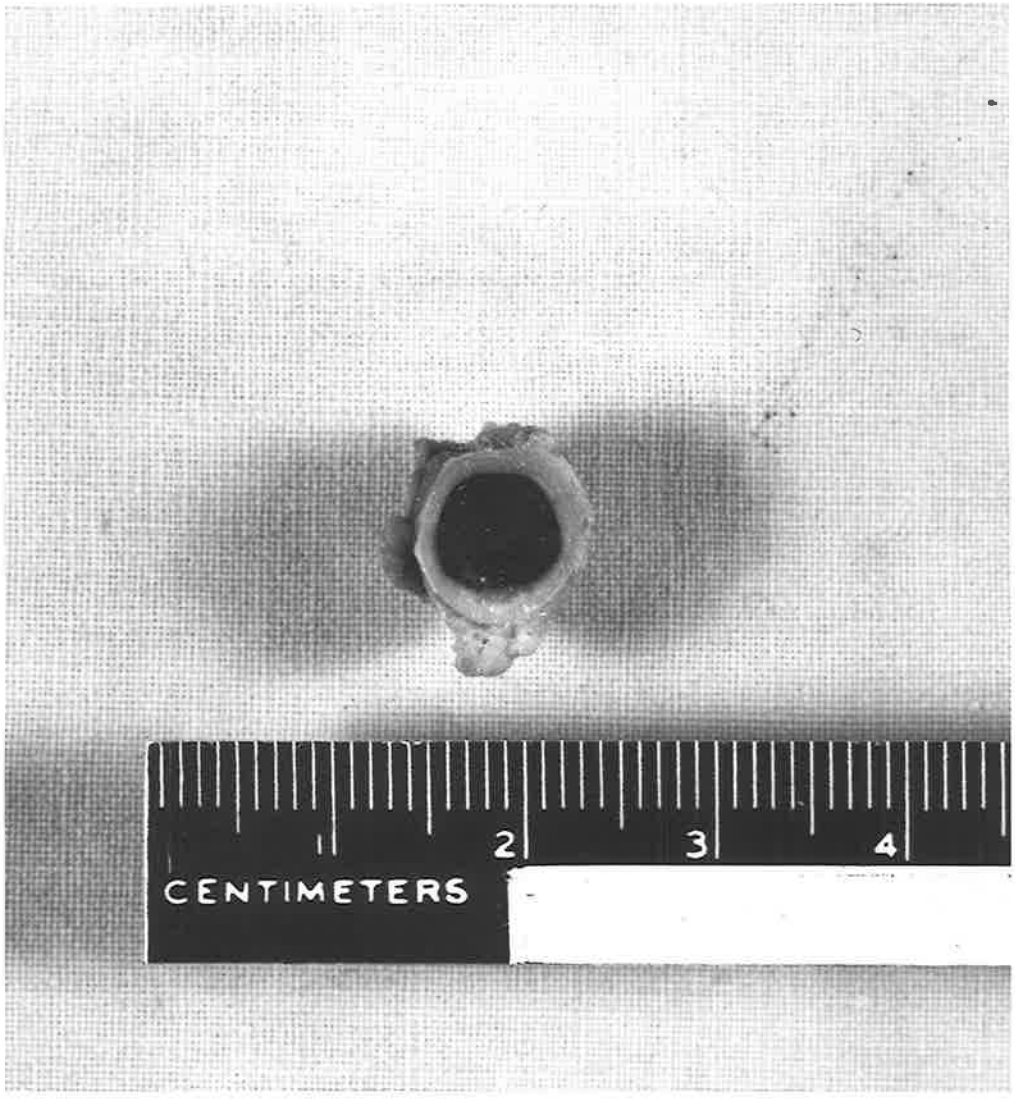
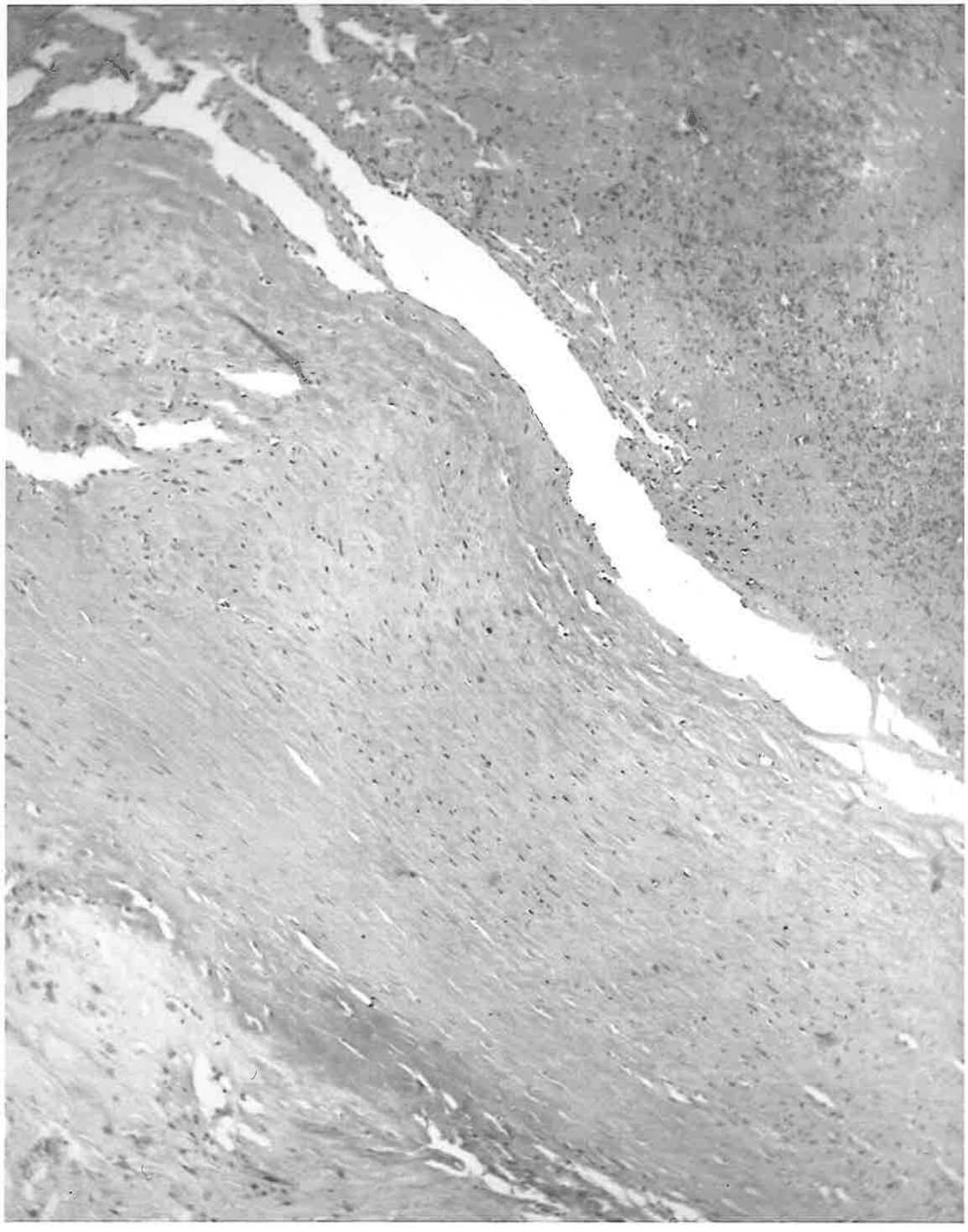

1
2
3
4
CENTIMETERS

Figure 42.

Photomicrograph of an occlusive arterial thrombus.

This photomicrograph shows part of the atherosclerotic vessel wall with adjacent thrombus. The thrombus was unorganized but a few crevices lined with flattened endothelium-like cells are evident at the margin. There was no canalization.



(2) Venous Thrombi (Table VII).

The vessels in which these thrombi occurred were white and scarred at operation but blood flow could be seen through all of them. Macroscopic observation revealed channels in all, varying from small to wide lumens.

Microscopy showed that the thrombi were organized, and replaced by fibrous tissue, with a few pigment carrying macrophages still present. This organization had occurred, like the venous reinjection thrombi, in a mural, occlusive or bridge-like form and surrounded in seven instances wide channels which allowed a good through circulation. The vein walls were thickened by fibrous tissue.

Organization of the thrombus resulted in destruction of the vein valves, scarring of the vein wall, and the development of the post-phlebitic syndrome. The appearance of such a limb can be seen in Figure 43. The patent venous system is seen in the phlebograms of the leg, Figures, 44, 45, and around the knee joint with no evidence of occlusion.

The perforatore of the calf present clinically are not seen in the first picture. In the vein the relatively normal wall and the nature of the circulation have allowed organization and canalization of the thrombus.

## Table VII.

This Table shows the organization and canalization of aged thrombi in human veins.

There is fibrous replacement of the red cell mass with canalization sufficient to allow a through circulation.

## TABLE VII.

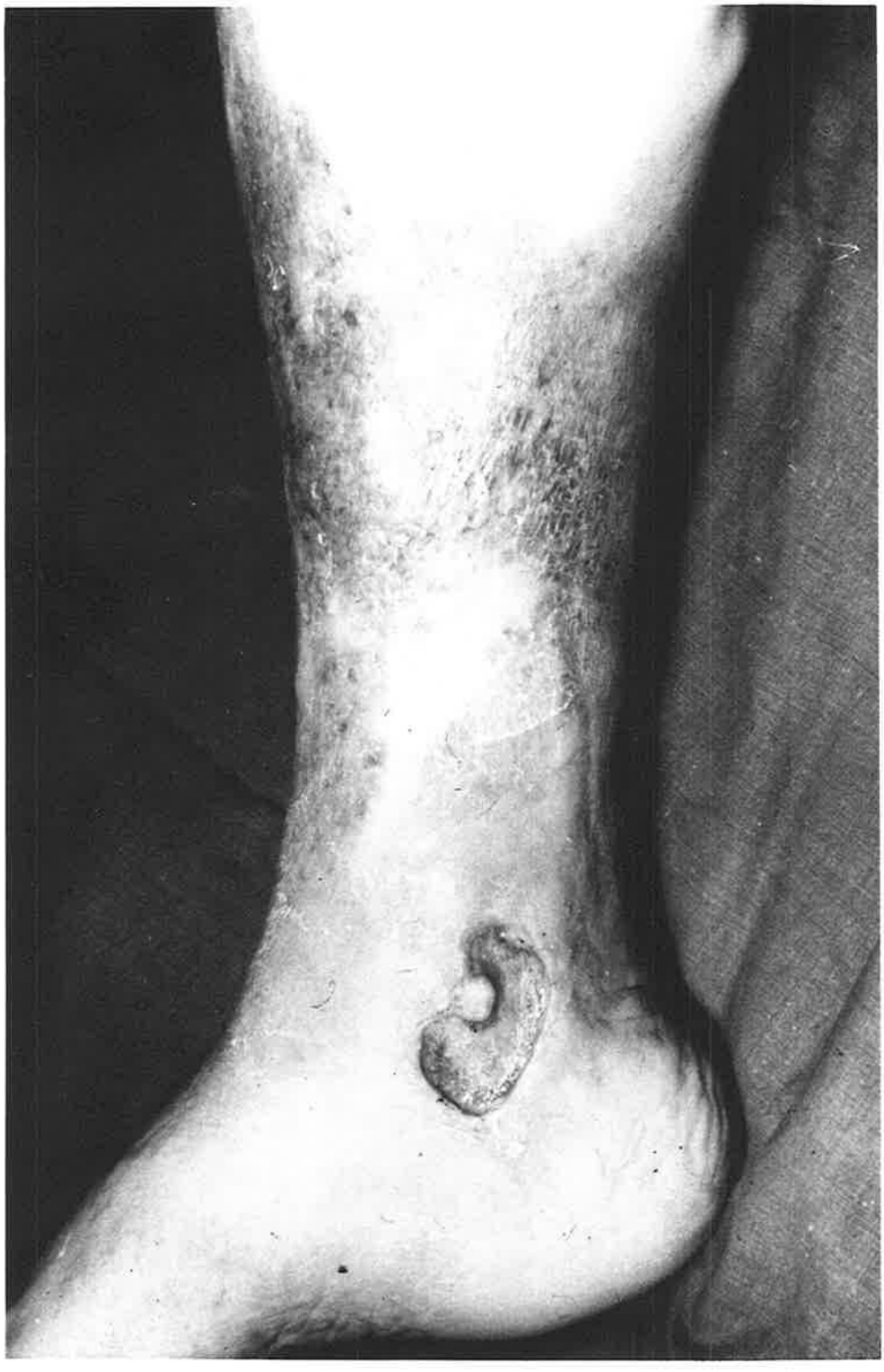
Chronological and histological age of human venous thrombi.

| Cases | Age | Organization to fibrous tissue | Canalization |
|---|---|---|---|
| 1. Lecord | 3 years | Yes | Small channels |
| 2. Morris | 4 years | Yes | Wide channels |
| 3. Vaillancourt | 20 years | Yes | Wide channels |
| 4. McAuliffe | 1 year | Yes | Wide channels |
| 5. Villieux | 15 years | Yes | Wide channels |
| 6. Crombie | Uncertain | Yes | Wide channels |
| 7. Demetre | 8 years | Yes | Medium channels |
| 8. Lehde | 3 years | Yes | Wide channels |
| 9. Sheehan | 4 weeks | Yes | Small and medium channels |

Figure 43.

The gross appearance of a limb following thrombosis of the deep veins of the leg.

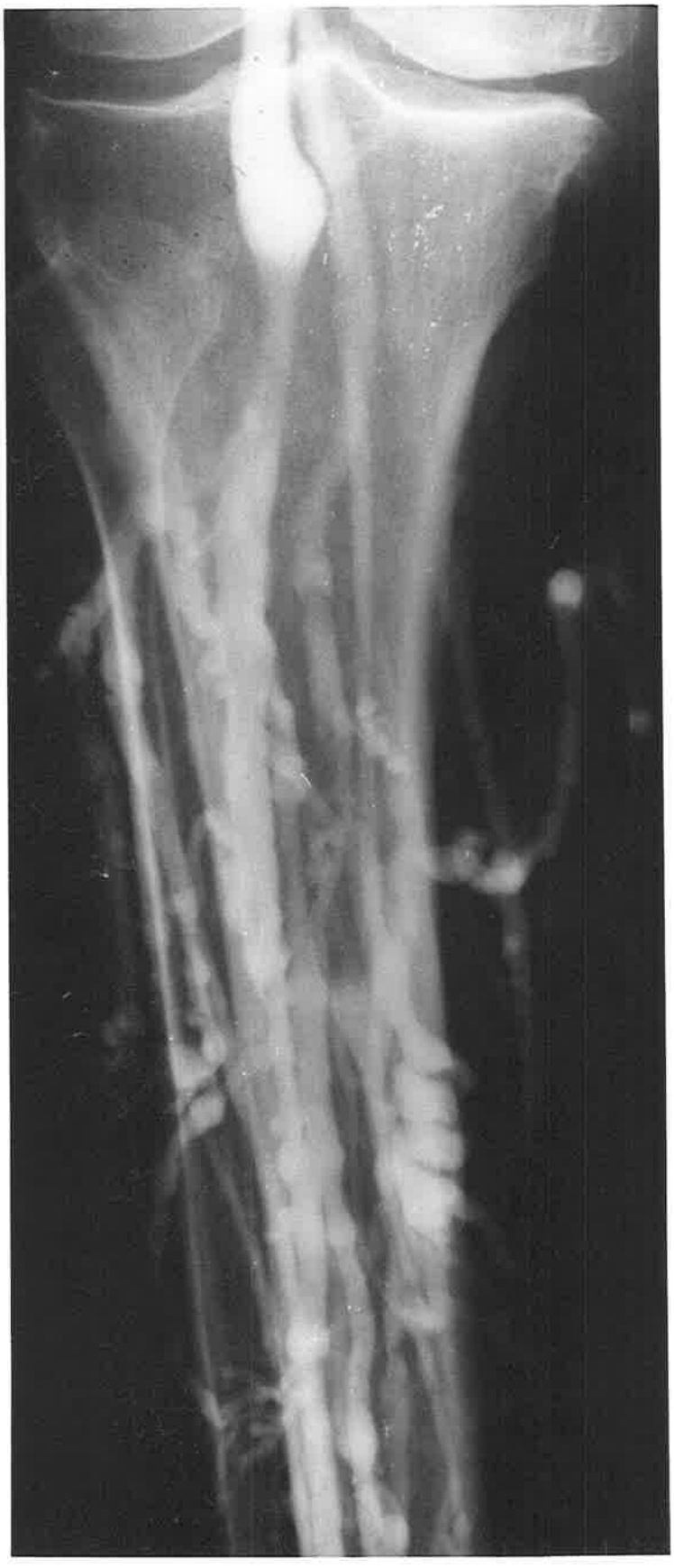
The ulceration occurs typically in this area and can cause considerable distress, as well as being very resistant to permanent healing.

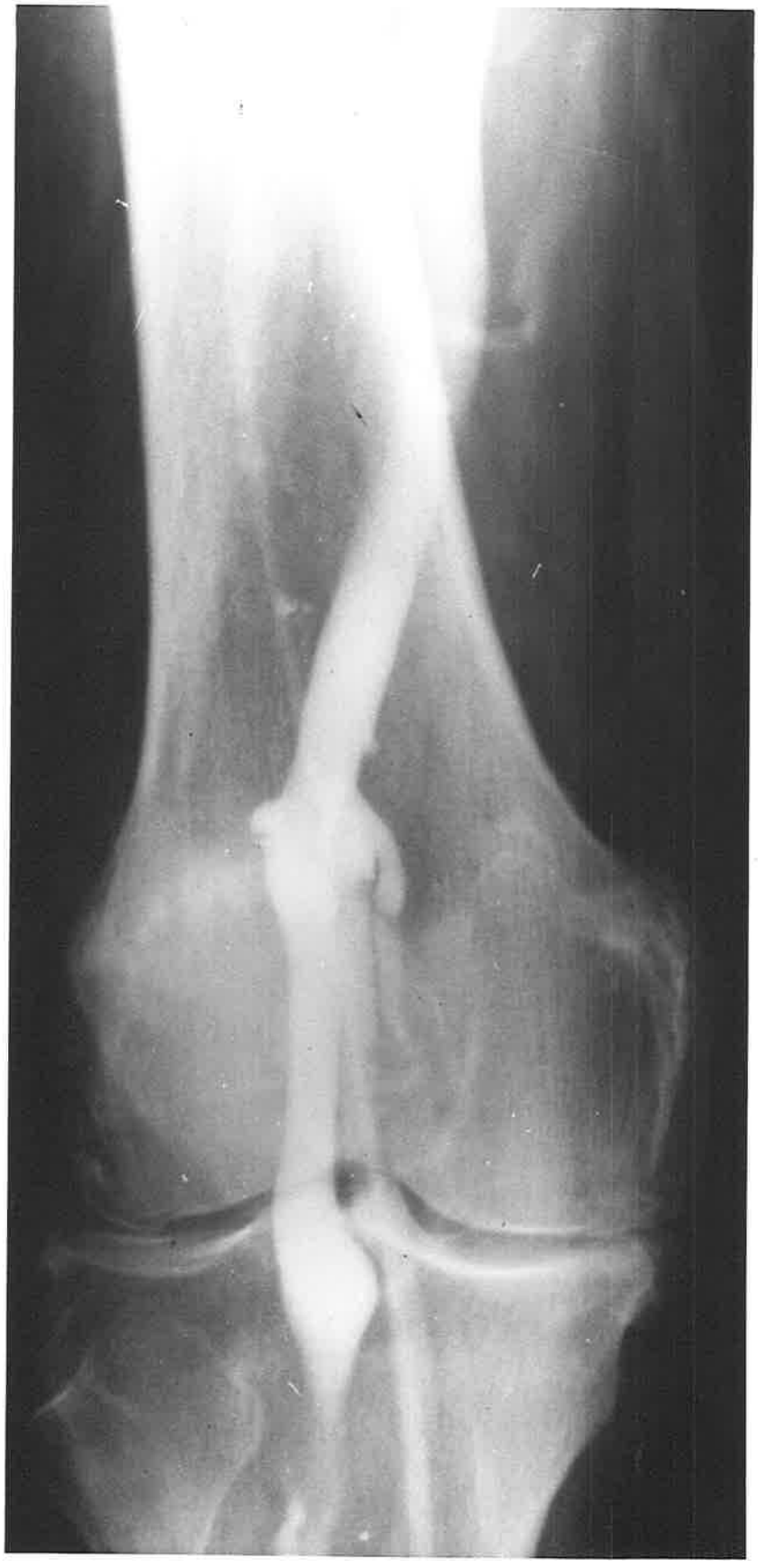


**Figures 44 and 45.**

Phlebogram of a patient with the post-phlebitic syndrome.

The deep venous system is patent, with ballooning of the vessel lumen at the valves.





# CHAPTER V.

## DISCUSSION.

Spontaneous whole clot lysis has been an accepted fact for over a century. The lysis of human plasma clots by filtrates of haemolytic streptococci was first observed by Tillett and Garner (1933). From Cohn's Fraction III of plasma the inactive lytic compound, plasminogen, was isolated in 1946. The necessary ingredients for the production of a lytic substance in vitro had been found, and with it developed a new approach to the thrombo-embolic disorders.

It seemed logical to attempt to lyse human clots, in vivo, by the active principle plasmin. This was produced by activation of plasminogen in vitro either spontaneously (Alkjaersig and Sherry, 1956) with chloroform (Denys 1889, Tagnon 1942 - 1943, Ambrus 1957) or with streptokinase (Clifton et al 1953, Sherry and Titchener 1954, Grossi et al 1955, Ruegsegger et al 1958).

Whilst this method was under test, it was decided to try in vivo activation of plasminogen by the use of various enzymes. This, it was hoped, would eliminate the problem of obtaining and purifying plasminogen, then combining it with an enzyme to produce a stable dry substance, active in solution. Crude pancreatic protease, trypsin, chymotrypsin, carboxypeptidase, varidase, streptokinase and urokinase have all been tried. In animals crude pancreatic protease, trypsin, chymotrypsin and papain produced no fibrinolytic activity in maximum acutely tolerated doses. The effect of infusion into man was

not pursued to any extent (Ambrus 1957) as the toxic side effects of chills, fever, and general malaise made them unsuitable. It seemed that streptokinase would be the most readily available least toxic drug, and its ability to produce whole clot lysis on addition to human plasma was an established fact.

It remained to find out whether in vivo clot lysis could be produced in man. Apart from the toxic effects of streptokinase there were the streptokinase antibodies and inhibitors to the streptokinase-plasmin system to take into account. This meant that before the infused streptokinase could take effect the total inhibition had to be overcome.

Tillett and Garner (1952) with arbitrary dose levels of streptokinase had dissolved thrombi in rabbit ear veins by continuous infusion.

Sherry (1954) showed that a single massive injection of streptokinase into dogs would result in a fifty per cent disappearance rate of thrombi produced in femoral arteries by thromboplastin. The transient peripheral whole blood lysis that occurred did not persist.

Then Johnson (1957) developed a technique whereby the relationship of log streptokinase concentration per ml. of whole blood and the reciprocal of the lysis time in minutes - provided it was greater than twenty minutes - could be represented by a straight line. From this it was possible to calculate the dose of streptokinase to overcome the total inhibition.

Sherry and Alkjaersig (1957) used a simpler method with 1 ml. aliquots of patients' plasma mixed with serial dilutions of streptokinase and then clotted with thrombin. The dose of streptokinase to give lysis in ten minutes was then multiplied arbitrarily by the plasma volume and infused over the initial two to three hours of the treatment. They reported that approximately fifty per cent of patients showed peripheral whole blood lysis during the latter part of the infusion.

This was the method at first employed in this series, with the loading dose given in twenty minutes, and then a proportion of this each hour until the end of infusion, (a modification of Johnson's technique as used by Fletcher et al 1959). This simpler method was not satisfactory in the animal or human cases of the series and was attributed to an insufficient dose of streptokinase to overcome the total inhibition level. The infusion dose was raised arbitrarily until satisfactory peripheral whole blood lysis was produced within the first two hours, and then the dose maintained at that level for the length of the infusion. The presence of peripheral whole blood lysis was a sure indication of streptokinase activity and the likelihood that the clot was being affected. Even so, no complete clot lysis occurred. The suggested mechanism of clot dissolution is by diffusion or adsorption of plasminogen activator to the thrombus, activation of the intrinsic clot plasminogen, and thrombolysis. Extrinsic plasmin action appears to be of little importance (Alkjaersig et al 1959).

In animals, the marked reduction in size of most of the thrombi over a limited period, especially in the massive dose range series, seemed to indicate that total lysis depended on the length of time of infusion, as well as the dose administered. It has not been possible because of practical difficulties to prove this in the rabbit and it has not occurred consistently in man even though large doses were infused for long periods - in vivo clot lysis occurring in one case only. With both animal and human thrombi whole blood lysis was difficult to produce and this may be due to streptokinase alteration or neutralization in vivo, since its effect in vitro is certain. The clot, too, may be a factor.

The effect of the streptokinase on blood coagulation factors was mainly on the fibrinogen. This showed a significant drop in all animal and human infusions where the dose administered was high. The clotting time and prothrombin showed some alteration but was significant only in two clinical cases who had previously been on anticoagulant therapy. In these two cases infusion was stopped after a short period because of haematuria. It seemed that the anticoagulants predisposed to a bleeding phenomenon and were better avoided prior to thrombolytic therapy. The earlier cases showed a marked rise in temperature with rigors but the most recent cases have been free of signs or symptoms, and needed no analgesics or antipyretics. This is considered to be due to improved purification of the powdered streptokinase.

The fact that clot lysis did not continue in vivo after stopping the infusion was not a surprise. However, the interesting aspect was that the control thrombi showed spontaneously a marked reduction in size. The absence of visible thrombus in 80% of veins at the end of six days would suggest not only retraction, contraction, and cellular invasion of the thrombus, but also clot disintegration. Whether this is the result of spontaneous lysis cannot be answered, but in view of the observations of Harrison 1948, Wessler and Freiman 1961, it seems most likely. The secondary thrombosis that occurred in veins with thrombi treated by infusion of streptokinase has been attributed to reduced activity of the lytic system. It is suggested that there was a reduction in total plasminogen following streptokinase infusion. Whether the enzyme also inhibited the vessel wall reaction to the thrombus, or other unrelated factors are involved has not been determined.

Because the action of streptokinase in vivo in man was so uncertain, attention was turned to the clot. Organization and canalisation have been noted, and Dible (1958) has gone to the extent of labelling them distinct and independent processes. But the factors involved in the canalization of thrombi in human veins, and their absence in the thrombi of large and medium arteries, have not been explained.

Ageing of a thrombus is progressive, passing steadily from a red cell mass to occlusive or canalized fibrous tissue with a thickened

vessel wall. This has been most recently recorded by Williams (1955) and such is the steady rate that the age of a thrombus can be told from its microscopic appearance except in the very early red cell stages and in the late stage when fibrosis is final and loss of cellularity marked. In the early stages organization is circulatory in origin, and these experiments have proved conclusively that organization in the late stages is from the vessel wall. The degree of cellular infiltration varies. It depends on whether the internal elastic lamina is intact or not. Cellular invasion from the wall to thrombi formed in the intima-stripped vessels is more marked than to the reinjection thrombi in normal vessels. It is impossible to determine whether these cells differentiate to other types but it seems probable that although the invading cells mature they do not change their type. This steady process depends on a reactive vessel wall. It is on this fact that the organization of human venous thrombi, and lack of it in arterial thrombi, is based.

A venous thrombus extends from the site of injury, inflammation, or pressure, into a length of normal vessel. The wall reacts as to a foreign body and organization occurs. This is not so in the arteries. The arterial thrombi viewed have been removed from atherosclerotic vessels that have shown calcified plaques macroscopically, and on microscopy, at any site of section. The vessel wall shows marked distortion of the normal pattern, and thrombi are

unorganized. Yet, if a vessel be endarterectomized, and a new thrombus forms, this undergoes ready organization. The activity of the vessel wall is destroyed by the plaque with the result that an eosinophilic staining mass of degenerated red cells persists.

If the wall is reactive organization will continue whether canalization occurs or not. This supports Dible's theory that the two processes are independent.

There remained the query as to what influenced canalization. Neither organized nor unorganized arterial thrombi showed wide canalization in any specimen. It may have been due to the vessel itself or a result of the blood flow to which it was subjected. To determine this a vein graft was incorporated in the arterial circulation, a thrombus similar to those that had canalized widely in the veins was made, and the clot changes noted at various stages. Organization was the same in all specimens, progressing from a red cell thrombus to a fibrous mass. However, no wide canalization was seen in any specimen. Only a few canals were present and these were small to medium in diameter. In this respect canalization resembled that seen in arterial thrombi, and not venous.

In unorganized occlusive human arterial thrombi there were no visible canals, and the densely organized thrombi in intima-stripped vessels showed small canals only. Yet the venous reinjection thrombi showed wide and medium canalization, the thrombi in the intima-

stripped veins predominantly medium canals with a few wide channels and the veins with old human thrombi were patent with visible blood flow.

It is suggested that the arterial pulsatile flow packs the thrombus into the vessel lumen with further agglutination of red cells occurring at either end. This allows little chance for contraction and retraction to give any crevices for through flow, and no opportunity for degenerated cells to be swept into the circulation and provide free access. This is in marked contrast to the venous thrombi that are only sponge-like and loosely attached to the vessel wall, can readily contract and retract, giving flow around the thrombus as well as through it, and the degenerated cells, as seen so vividly in Figure 17, that can readily escape into the circulation.

However, should this disintegration or clot separation occur prematurely at one section of the thrombus, the likelihood of pulmonary embolus is greatly increased. It may be that this is another underlying factor in embolism rather than a free clot tip being swept off by an eddying venous current.

If one considers restoration of the circulation following intravascular occlusion, then, whether treatment is given or not, intravenous thrombi will canalize and organization will occur. The vein walls become thickened and the valves destroyed. This is the underlying pathology of the post-phlebitic syndrome with its swelling

of the leg, poor skin nutrition, and slowly healing ulcers. It is better in idiopathic venous thrombosis that artificial lysis rather than the natural processes restore vessel patency. There is the advantage, also, that the initiating cause does not persist and the likelihood of clot reformation is much less.

In large and medium atherosclerotic arteries, as distinct from the coronary arteries with a diameter of less than 4 mms., no organization and canalization will occur and one must aim either to prevent extension of the thrombus, or remove thrombus plus the offending intramural lesion. Lytic agents on a thrombus in a diseased vessel will tide over the acute phase but the offending cause must be removed to prevent its recurrence.

Therapeutic implications:

There is no doubt that if streptokinase will produce peripheral whole blood lysis in vitro clot lysis in vivo can be expected. But until the drug can be manufactured with this property assured at a determinable dose level it must be considered an ineffective lytic agent. This ineffective lysis is perhaps more dangerous than the original occlusive state, as partial clot lysis predisposes to secondary extension and there appears, experimentally, to be a delay in clot organization.

The studies on intravascular thrombi have provided some further insight into the factors concerned in organization and canalization. It has also supported the fact that venous canalization will occur in the

absence of any therapy. The disadvantage of the destroyed valves and the post-phlebitic syndrome remain to be treated.

The venous thrombi, at the moment, are best treated by time alone, operation, or anticoagulants. The latter should be continued for three weeks until organization and vessel wall adherence are assured, and the critical time for pulmonary emboli has passed. Arterial thrombi in atherosclerotic vessels will neither organize nor canalize, and these can be treated by time, operation, or anticoagulants. The anticoagulants in these cases act by prevention of secondary thrombosis and occlusion of the collateral supply which is then able to open up and play the major part in maintaining peripheral vitality.

It may be that further work on the lytic agents will produce an effective drug - stable, active and readily administered. But prior to issue it will demand extensive investigation regarding dosage, and the type of lesion to be treated. This will result in marked improvement in the results of treatment in the thrombo-embolic disorders.

## APPENDIX I.

(Tables 1, 2, 3, 4, 5).

These Tables represent the variety of changes that may be seen in a thrombus on section at a stated time. The figures in Table III of the text are for the dominant channel present in the oldest part of the thrombus.

The symbol '+' in the first four columns indicates vascular occlusion with or without canalization.

Under 'fibrous change' the same symbol has been used to indicate the degree of fibrosis as well as its presence.

## APPENDIX I.

## TABLE 1.

### 32. REINJECTION VENOUS THROMBI.

**Canalization and fibroplasia related to time.**

| Age | Occlusion No channels | Occlusion Small channels | Occlusion Medium channels | Wide Canalization | Fibrous Change | Vessel wall changes |
|---|---|---|---|---|---|---|
| 1 hr. | fresh thrombus | | | | | Inflammation |
| 24 hrs. | early changes | | | | | muscle nuclei less |
| 3 days | marginal flow | marginal crevices | | | | |
| 5 days | | marginal retraction | | | | |
| 14 days | | small intra-clot channel | | | early | normal muscle cells |
| 21 days | | + | + | + | + | |
| 28 days | | + | + | + | ++ | |
| 40 days | | + | + | + | +++ | |
| older than 40 days | | + | + | + | ++++ | Fusion of clot + wall |

## APPENDIX I.

## TABLE 2.

### 22. REINJECTION ARTERIAL THROMBI.

| Age | Occlusion No channels | Occlusion Small channels | Occlusion Medium channels | Wide Canalization | Fibrous Change | Vessel wall change |
|---|---|---|---|---|---|---|
| 1 hr. | + fresh thrombus | | | | | Normal |
| 24 hrs. | minimal marginal retraction | | | | | |
| 3 days | | marginal retraction | | | | Pale Media |
| 5 days | + | | | | | |
| 7 days | + | | | | | |
| 14 days | + | + | | | + | |
| 21 days | | + | + | | ++ | Necrosis in spots |
| 28 days | | | + | | ++++ | |
| 40 days | | | + | + | ++++ | |
| older than 40 days | | + | + | + | ++++ | Normal wall |

## APPENDIX I.

### TABLE 3.

### 21. THROMBI IN INTIMA-STRIPPED VEINS.

| Age | Occlusion No channels | Occlusion Small channels | Occlusion Medium channels | Wide Canalization | Fibrous Change | Vessel wall change |
|---|---|---|---|---|---|---|
| 1 hr. | + | | | | | White cells |
| 24 hrs. | + | | | | | |
| 3 days | marginal retraction | | | | | |
| 5 days | | marginal crevices and flow | | | | |
| 7 days | | + | | | loose fibrous tissue | |
| 14 days | | + | | | + | |
| 21 days | | | + | + | ++ | |
| 28 days | | + | + | | ++ | blends with adventitia |
| 40 days | | | + | + | +++ | |
| older than 40 days | | + | + | | ++++ | Fusion of thrombus and vein wall |

## APPENDIX I.

## TABLE 4.

### 22. THROMBI IN INTIMA-STRIPPED ARTERIES.

| Age | Occlusion No channels | Occlusion Small channels | Occlusion Medium channels | Wide Canalization | Fibrous Change | Vessel wall change |
|---|---|---|---|---|---|---|
| 1 hr. | + | | | | | |
| 24 hrs. | + | | | | | white cells |
| 3 days | + | | | | | medial necrosis |
| 5 days | | + | | | | |
| 14 days | | + | | | + | |
| 21 days | | + | | | + | |
| 28 days | | + | | | ++ | thrombus and wall fusion |
| 40 days | | + | + | | +++ | |
| older than 40 days | | + | + | + | +++ | fusion of media and thrombus |

## APPENDIX I.

## TABLE 5.

### 42. REINJECTION THROMBI IN VEIN GRAFTS INCORPORATED IN THE ARTERIAL CIRCULATION.

| Age | Occlusion No channels | Occlusion Small channels | Occlusion Medium channels | Wide Canalization | Fibrous Change | Vessel wall change |
|---|---|---|---|---|---|---|
| 3 days | + | | | | | |
| 7 days | | marginal crevices. minimal retraction. | | | | |
| 14 days | | + | + | | early | |
| 21 days | | + | | | | |
| 28 days | | + | + | | + + | fibrosis |
| 40 days | | + | + | | | loss of definite wall outline |
| older than 40 days | | + | + | + | | |

## APPENDIX II.

### TABLE 1.

### CANALIZATION IN REINJECTION THROMBI.

| | Veins | Arteries | Vein Grafts |
|---|---|---|---|
| Total Number | 5 | 4 | 5 |
| Canalized | 5 | 4 | No dye penetration |
| Non-canalized | nil | nil | |

## APPENDIX II.

### TABLE 2.

### CANALIZATION IN THROMBI OF INTIMA-STRIPPED VESSELS.

| | Veins | Arteries |
|---|---|---|
| Total Number | 6 | 5 |
| Canalized | 6 | 4 |
| Non-canalized | nil | 1 |

Four arteries although showing some dye in the most distal sections showed much less both in the amount present and the number of canaliculi penetrated.

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## LIST OF ILLUSTRATIONS.

Figure 44. Phlebogram of a patient with a long-standing post-phlebitic syndrome - lower leg.

Figure 45. Phlebogram of a patient with a long-standing post-phlebitic syndrome - lower thigh and knee.