RECANALIZATION OF THROMBOSED ARTERIES UNDER ANTICOAGULANT THERAPY

BY

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[WITH SPECIAL PLATE]

Arterial occlusion, due entirely or partially to thrombosis, is a result of various pathological processes. In every case, however, it is a serious development which endangers the vitality of the organ or extremity concerned. It is evident that details of treatment must be modified according to the primary disease and the artery affected. Apart from promoting the collateral circulation and other measures designed to balance the disproportion between blood supply and local oxygen requirements, it is the dissolution or recanalization of the obstructing clot which should be the ultimate aim of treatment.

We have already presented evidence that the rate of recanalization of venous channels which had been thrombosed experimentally was greatly accelerated when the prothrombin activity was adequately lowered by the administration of suitable doses of a dicoumarol anticoagulant (ethyl biscoumacetate, "tromexan") (Wright, Kubik, and Hayden, 1952). Encouraged by the findings in a preliminary investigation upon recanalization of arteries (Kubik and Wright, 1950), a more complete experimental study has been undertaken to assess the value of dicoumarol treatment in thrombosis in these vessels.

Methods

Since it was found that blood prothrombin levels fluctuated considerably during anticoagulant treatment and that doe rabbits often died from uterine haemorrhage, full-grown young bucks were used throughout the following experiments.

Under pentobarbitone sodium anaesthesia the femoral artery was exposed for a length of 2 cm. on one or both sides through a longitudinal incision on the median aspect of the thigh. The stripped length of vessel was lifted on four ligatures spaced about 1 cm. apart, so that its lumen was able to be compressed in three sections. Just enough pressure was applied to the ligatures to prevent the blood flow in the artery while 0.02 ml. of thrombin solution (1,000 units per ml.) was injected through a fine intradermal hypodermic needle into the middle section and 0.02 ml. of 10% sodium morrhuate into the proximal and distal ones. In those animals in which mechanical interference to the vessel had caused arterial constriction, a solution of papaverine sulphate (1.25%) was applied to overcome the spasm (Kinmonth, 1952). The injected substances were maintained in the occluded sections for ten minutes, after which time the pressure on the ligatures was gradually released.

A small flow of blood was occasionally apparent immediately after the release of the pressure, but when fresh blood came in contact with the sclerosed areas and with the local thrombin coagulum, thrombosis occurred within a few moments and could be recognized from the outside of the vessel as a fusiform dark-red section, incompressible when gently pinched.

In our preliminary report (Kubik and Wright, 1950) peripheral gangrene was often found in the foot after thrombosis of the femoral artery. This was possibly due either to injury of the femoral nerve or to thrombosis of the arteries of the foot produced by morrhuate carried peripherally by the blood stream. This complication did not occur with the improved technique described here.

After arterial occlusion the animals were divided into two groups: (A) those which received ethyl biscoumacetate daily by mouth, starting 24 hours after the thrombus had been formed; and (B) those which, as controls, received no treatment. The ethyl biscoumacetate, as a suspension in methylcellulose ("cellofas") (Wright, Kubik, and Hayden, 1952), was given through a short rubber tube, which was passed to the back of the pharynx so that regurgitation could not occur. The dosage was controlled by prothrombin estimations carried out three times a week, using the Quick single-stage technique on blood drawn by ear-vein puncture. The prothrombin time aimed at for every animal was 30 seconds (rabbit normal, 9 seconds), and, to achieve this, doses of 100–300 mg. per kg. body weight were required daily. Prothrombin-level fluctuations in rabbits are, however, both rapid and profound, and in the course of the experiments several animals were lost through concealed haemorrhage into the caecum. Nevertheless, the prothrombin times in the majority were maintained between 25 and 35 seconds throughout the experimental period, and only these animals are included in this report.

An arteriographic technique was used to follow any changes which might occur in the occluded vessel, the first *x*-ray film being taken 24 hours after thrombosing to show the extent of the obstruction. Under pentobarbitone sodium anaesthesia, a 3-cm. midline abdominal incision was made, the gut was held aside within the body cavity, and the aorta was exposed for a length of about 1 cm. A fine hypodermic needle (No. 16), inserted into a polythene tube about 20 cm. long and with a syringe attachment at the distal end, was introduced into the exposed aorta. Through this, 2 ml. of thorotrast, as contrast medium, was injected during a period of 4 seconds and the *x*-ray film was taken while it was still flowing in. Double-wrapped 10 by 12 in. (25 by 30 cm.) "improved fast Ilfex film" with exposure factors of 53 kV, 50 mA, and 0.5 second was used throughout.

Further *x*-ray films were made with the same technique at two-weekly intervals for Group A and at four-weekly intervals for Group B.

Results

It was early apparent that a considerable difference in the time required for the recanalization of the femoral arteries occurred between those animals which received adequate anticoagulant treatment and the untreated controls. In the eight animals in Group A, the average time taken for one or both vessels to become patent was only 3.25 weeks, while in Group B none of the six controls were patent by 8 weeks, and further *x*-ray films taken at various later dates showed no evidence of recanalization at any time. The details of both groups are set out in the Table. Typical arteriograms of animals (Nos. 6 and 10) from each group are shown in the Plate, Figs. 1 and 2.

The criterion of "occluded" or "patent" is, moreover, a stringent one. In Group A, when one artery has become patent, the thrombus in the remaining occluded vessel generally shows a considerable reduction in its length, even though complete patency has not been established. A longer period of treatment would presumably further reduce the size of the occluding mass, if not allow of its entire removal. In the control group, on the other hand, no such changes were evident, the size of the occlusion showing no material diminution; indeed, in one animal (No. 12) which was

Time Taken (Weeks) for Recanalization of Thrombosed Arteries

	Week of X-ray Film	Right Femoral	Left Femoral
Group A (treated):			
Rabbit 1 ..	2½ (died)	O	O
,, 2 ..	4	P	P
,, 3 ..	4	O	P
,, 4 ..	3	O	P
,, 5 ..	2	P	P
,, 6 ..	3	O	P
,, 7 ..	8	—	P
,, 8 ..	2	—	P
Group B (control):			
Rabbit 9 ..	8	O	O
,, 10 ..	13	O	O
,, 11 ..	22	O	—
,, 12 ..	46	—	O
,, 13 ..	12	O	—
,, 14 ..	15	O	—

O=Occluded. P=Patent.

specially kept, the artery was still fully occluded after eleven months. Other animals of this group were destroyed at varying intervals.

Histological sections of arteries from animals of both groups were examined at the end of the experimental period. In the treated group the animal was sacrificed after the patency of the femoral artery had been established by arteriogram. Fig. 3 shows a photomicrograph of the artery from rabbit No. 6, which was open after three weeks of anticoagulant treatment. The intima appears normal except at one point where there is a small residual mural thrombus. The lumen is otherwise free from obstruction.

In the control group histological sections were taken through the vessel at the level of the obstruction. Fig. 4 shows a typical organizing thrombus with fibroblasts and haemosiderin granules in the granulation tissue matrix three months after arterial obstruction had been produced (rabbit No. 10).

Discussion

In comparison with the interest shown in the influence of anticoagulants on experimental venous thrombosis, little attention, so far as we know, has been paid to corresponding changes in arteries. Experimental work on coronary occlusion has been undertaken, but even here the arterial block was produced by ligation of a branch of the coronary vessel. The result of subsequent anticoagulant therapy on the myocardial infarction so produced was described by Beattie and his colleagues (1948). Le Roy and Nalefski (1948) investigated the possible ill effects of dicoumarol on the myocardium, but it is obvious that neither of these groups of workers were interested in the effects of their therapy on the primary occlusion, or the technique of ligation would not have been used.

It is remarkable that, though a considerable number of experiments have established the fact that the administration of dicoumarol or its derivatives reduces the formation of intravascular coagulation (Marple and Wright, 1950), the experimental investigation of the influence of these drugs on preformed thrombi *in situ* has been omitted. Loewe (1947) and Loewe and Hirsch (1947) suggested that the administration of heparin facilitated the reopening of veins recently occluded by fresh unorganized thrombus, but a study of thrombosed arteries has been largely neglected, though it obviously has a wide clinical application.

Experimentally it is difficult to produce conditions similar to those occurring in the majority of occlusive arterial diseases. First, in the naturally occurring process the endothelium undergoes changes which cannot be reproduced in the experimental animal; the actual thrombosis occurs in a vessel previously diseased. Secondly, in clinical practice, except in cases of embolism, the obstruction develops gradually and there is, as yet, no experimental method for producing such a progressive occlusion. In the third place, the inflammatory character of certain of the arterial diseases cannot be fully imitated, though recently Rabinovitch and Pines (1949) have claimed satisfactory results in reproducing this change in veins by injecting staphylococci into the wall of the blood vessel. In our method, using both thrombin, which produces a red coagulum, and morrhuate, which damages the endothelium, we believe that many of the features of normally occurring arterial occlusion are paralleled.

In assessing the results presented here it must be borne in mind that rabbits are relatively resistant to the coumarin group of anticoagulants. Their plasma possesses a considerably greater prothrombin activity than that of human blood (Quick, 1936). Larger doses and more prolonged treatment are consequently needed to influence their prothrombin levels to correspond with the human equivalent. In these animals, moreover, wide fluctuations of prothrombin activity while receiving coumarin treatment make it difficult to maintain them at an adequate level without the risk of haemorrhage. The results which we have obtained, however, make it clear that the administration of the anticoagulant has, under the conditions of our experiments, the effect of promoting the recanalization of the thrombosed lengths of the arteries.

How this effect is brought about is still uncertain, but in a previous paper (Wright, Kubik, and Hayden, 1952) we described our findings and suggested a possible mode of action of ethyl biscoumacetate on venous thrombi. The increased rate of recanalization observed is possibly due to the normally continuous action of fibrinolysin, which is usually masked by the continual deposition of small quantities of fresh fibrin on the thrombus surface, and which is disclosed in the presence of anticoagulant when fibrin deposition is prevented. The action of the anticoagulant would therefore appear to be an indirect one.

From preliminary experiments it was found more difficult to thrombose an artery than a vein, but once occluded it remained so. We did not observe any spontaneous recanalization in our control group even after many weeks. In the light of this it is difficult to explain the disappearance of the arterial thrombus with ethyl biscoumacetate treatment wholly by its indirect action. Schmid and Stockinger (1951) observed that dicoumarol, and still more ethyl biscoumacetate, significantly depressed the growth and dividing power of fibroblasts in tissue culture. This effect was present even in the low concentrations found in the blood during anticoagulant therapy. It is therefore possible that the organization of the thrombus is modified by these drugs, and this plays a leading part in promoting the recanalization of the intra-arterial thrombus.

Application of experimental data to human pathology is always somewhat hazardous. The conclusion seems justified, however, that adequate prolonged treatment with dicoumarol in general and with ethyl biscoumacetate in particular has a favourable influence on the reabsorption of arterial thrombi, provided therapy is started soon after occlusion has occurred. Such treatment may result in reopening of the occluded vessel when the obstruction is due to thrombotic and not to arteriosclerotic changes.

Summary

A method for producing thrombi in rabbits' femoral arteries is described.

The time taken for recanalization to take place in control and anticoagulant-treated animals was recorded by arteriography.

The treated group showed an average recanalization period of 3½ weeks, while the controls were still occluded after several months.

The influence of anticoagulant treatment is dependent upon the maintenance of a low prothrombin level.

A discussion of the possible mode of action is offered.

We wish to express our thanks to Dr. C. J. Hodson and to members of the x-ray diagnostic department for their help with this work.

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METABOLIC CHANGES ASSOCIATED WITH OPERATION*

BY

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That the recovery from injury follows a recognized pattern has been known from very early times. This was clearly appreciated by John Hunter (1794). In 1872 Bauer observed that an increased elimination of nitrogen followed upon haemorrhage, and Hawk and Gies (1904) showed that the actual operation of venesection without withdrawal of blood was enough to cause increased urinary output of nitrogen and sulphur. This increased nitrogen loss was also noticed by Benedict (1915) during prolonged starvation. Since that time many observers have noted this increased nitrogen catabolism as a result of injury, associated with increased phosphorus, sulphur, and potassium loss (Cuthbertson *et al.*, 1939 ; Cuthbertson, 1942 ; Wilkinson *et al.*, 1949). The varying nature of the injuries causing this has tended to emphasize that something inherent in the trauma, or some underlying response, might be common to many and various injuries done to the body, and deprivations to which it might be exposed.

Wilson and Stewart (1939) noted that, in burns, sodium was lost from the extracellular fluids into the cells of the damaged tissues, and also into the erythrocytes of the general circulation. The observations of Cuthbertson *et al.* (1939) on the urinary excretion of potassium and nitrogen led them to believe that the increased excretion of these substances was due to muscle catabolism following operative injury, but they noted that potassium was lost in greater proportion than nitrogen when compared with their relative concentrations in muscle protoplasm. Howard *et al.* (1946) have also shown that the nitrogen, potassium, sulphur, and phosphorus loss is not proportional to their content in muscle, and that the greatest potassium loss occurs in the first 24 hours. Blixenkrone-Møller (1949) demonstrated that the maximal potassium loss after operation precedes the maximal nitrogen excretion.

Wilkinson *et al.* (1950) demonstrated that after surgical operation potassium is lost in the urine in high concentration when the potassium intake is low, that the loss is greatest in the first three days after operation, and that it precedes in time the nitrogen loss, which persists throughout the first post-operative week. In a previous paper Wilkinson *et al.* (1949) had drawn attention to the marked retention of sodium and chloride in the early post-operative period, occurring at the same time as the phase of increased potassium loss. Annersten and Norinder (1946) had previously observed a slight reduction in serum sodium and chloride after operation. They did not believe that these changes were significant. Similarly Wilkinson *et al.* (1951) noted such slight changes, but again did not believe them to be an explanation of the retention of sodium and chloride after operation.

I first became interested in this problem in 1948, when I observed the retention of chloride and of sodium after operation. The effect was thought to be very similar to the results of the administration of 11-deoxycorticosterone acetate (D.C.A.). In view of the observations of Hans Selye, it was believed that these electrolyte changes might well be due to increased adrenocortical function following the stimulus of operation. It was decided to observe these changes again and, at the same time, to measure adrenocortical function.

Results

Electrolyte Changes

Fig. 1 shows the typical features of the electrolyte changes in the plasma and urine before and after operation. In all cases a great reduction in the excretion of sodium and of chloride in the urine was observed immediately following operation ; this persisted for about seven days. After this, the sodium and chloride excretion in the urine tended to rise above the pre-operative level. Associated with these changes, a constant but much diminished fall in serum sodium and plasma chloride was detected during the same period. A return to the pre-operative level occurred about the end of the first week.

During the same period the serum potassium concentration appeared to be variable and showed no pronounced change. In general, the concentration of potassium in the

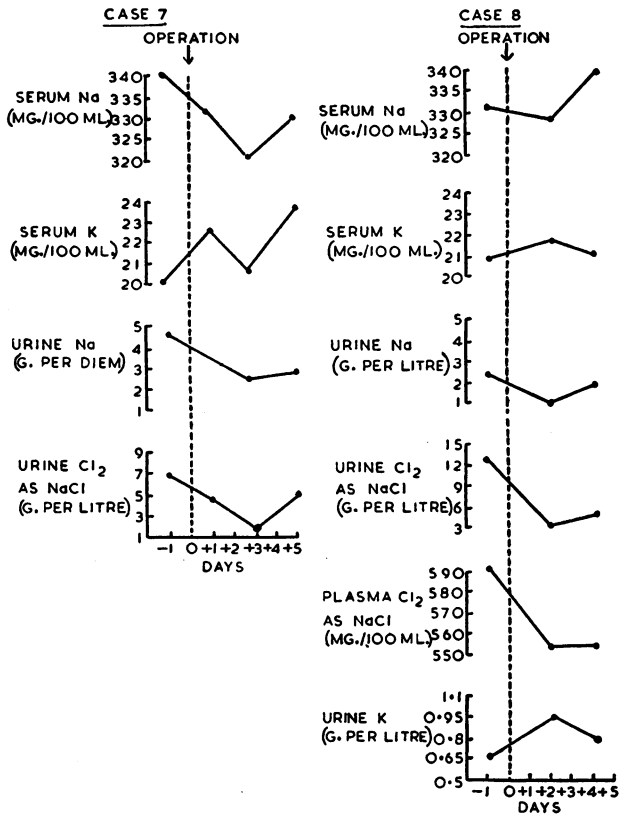

FIG. 1.—Typical examples of electrolyte changes in blood and urine.

*Part of a thesis submitted for the degree of M.D. of the University of Glasgow.

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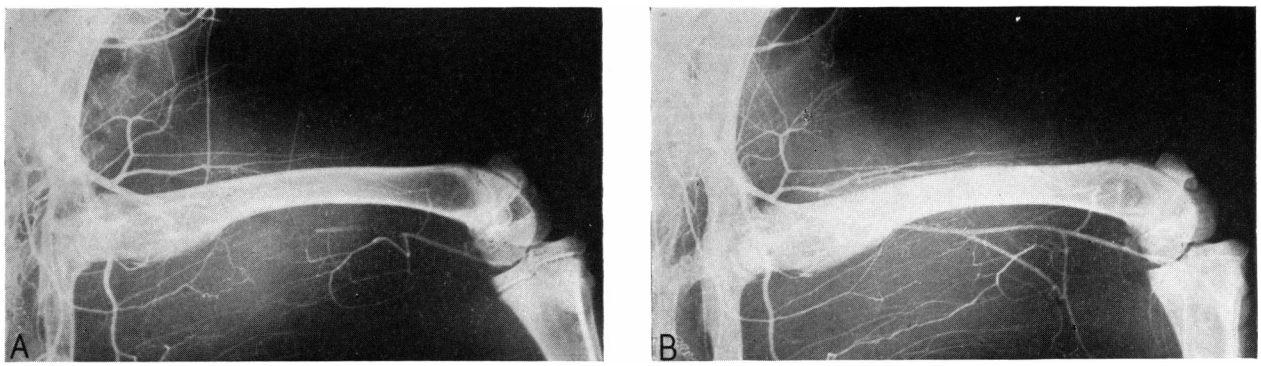


FIG. 1.—Femoral arteriograms from rabbit treated with anticoagulant. A, Complete occlusion 24 hours after thrombosing. B, Three weeks later, artery again patent.

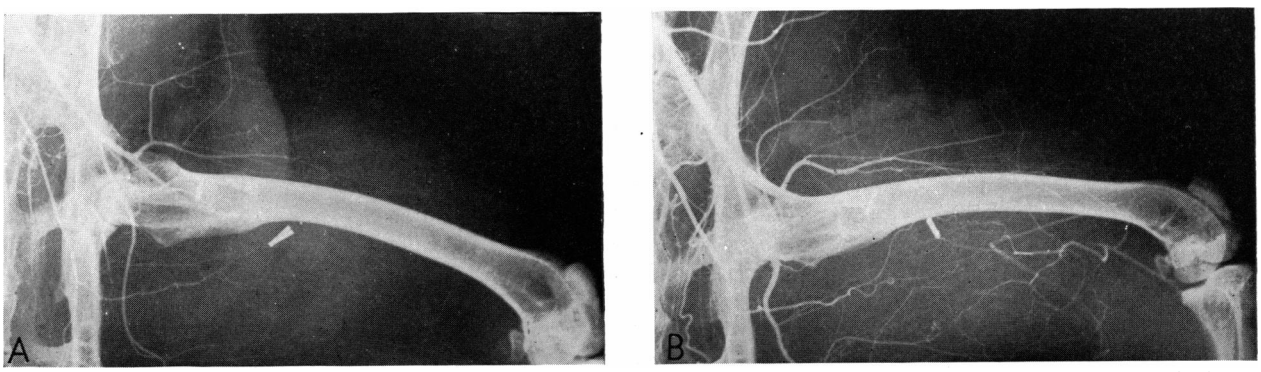


FIG. 2.—Femoral arteriograms from control rabbit. A, Complete occlusion 24 hours after thrombosing. B, Thirteen weeks later, artery still occluded. Development of collateral vessels is marked.

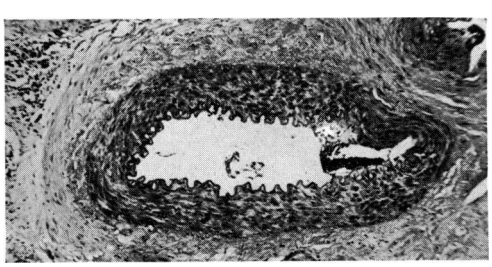


FIG. 3.—Section through femoral artery of treated rabbit at level of thrombus after patency has been restored. (×64.)

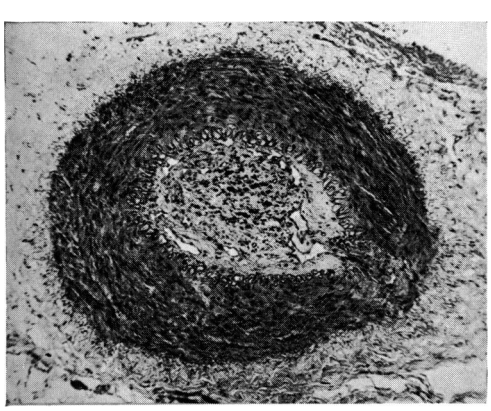
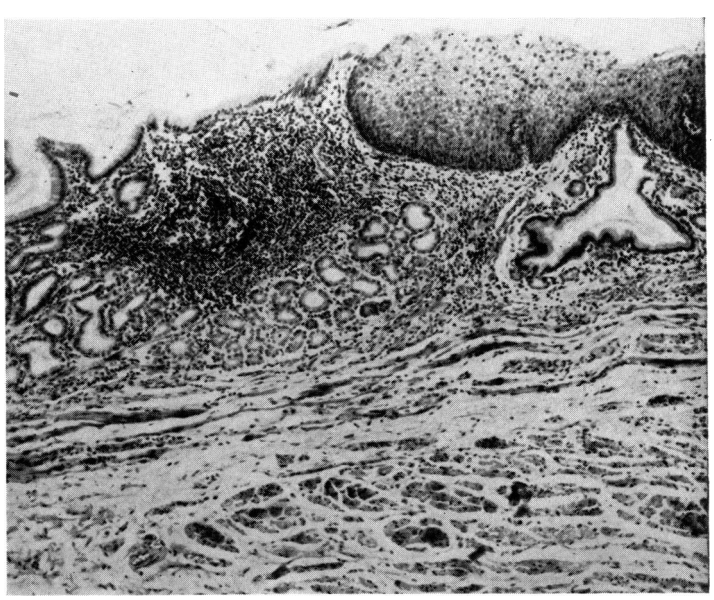


FIG. 4.—Section through femoral artery of control rabbit at level of occlusion. (×64.)

G. RIOS-SOLANS: SLIDING HERNIAS THROUGH THE OESOPHAGEAL HIATUS



Section showing junction of stratified squamous lining of oesophagus and mucous membrane of stomach. (×33.)