

STUDIES IN EXPERIMENTAL EXTRACORPOREAL
THROMBOSIS.

III. EFFECTS OF CERTAIN ANTICOAGULANTS (HEPARIN AND HIRUDIN)
ON EXTRACORPOREAL THROMBOSIS AND ON THE MECHANISM
OF THROMBUS FORMATION.

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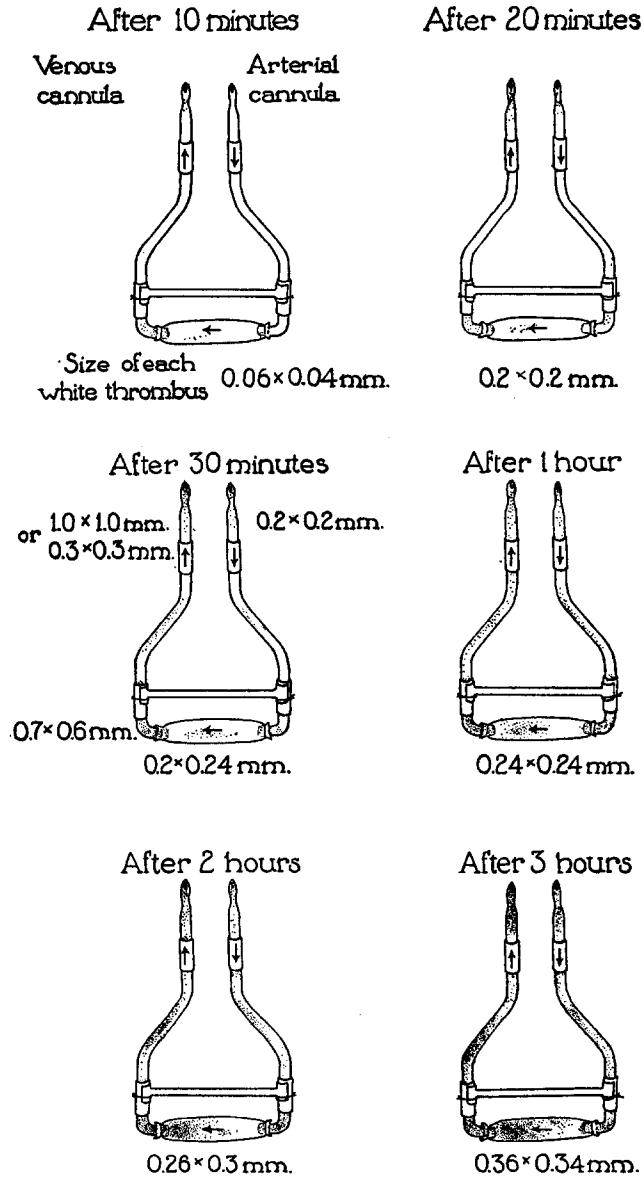
PLATES 2 AND 3.

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Welch (9) describes the steps in the formation of a thrombus after injury to the vessel wall, as follows: There is an accumulation of blood platelets adhering to the wall at the point of injury; leucocytes, which may at first be present in small numbers, increase rapidly, collecting at the margins of the platelet masses and between them. Not until the leucocytes have accumulated in considerable numbers, does the fibrin appear. Therefore, the formation of fibrin is secondary. Nevertheless, the fibrin formation plays a great part in the growth of red thrombi and the occurrence of obstruction and of intravascular clotting.

The effects of anticoagulants on the process of thrombosis with the new method of investigation of extracorporeal thrombosis was studied. Interest centers especially on the study of the mechanics of thrombosis and the preventive or retarding influence of anticoagulants which prohibit clotting for long periods. Here heparin, an antiproteolytic introduced by Howell (3), has been employed mainly. Howell (4) states that heparin is the anticoagulant always present in normal blood, and that it may be used intravascularly without injurious effects. Reed (7) found that repeated injections of this material always result in an increase of clotting time. In my experiments the heparin used (Hynson, Westcott and Dunning) was

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TEXT-FIG. 1. Progression in formation of white thrombi.

standardized, 1 mg.¹ rendering 5 cc. of cat blood incoagulable *in vitro* for 24 hours. It is dissolved in physiologic sodium chloride solution, 20 mg. to 1 cc., and is injected intravenously in doses of 20 to 25 mg. for each kilo of body weight.

Experimental Studies with Heparin.

Rabbits weighing about 2 kilos were anesthetized, and the carotid artery and jugular vein of the same or opposite sides cautiously exposed for more than 1 cm. All small vessels needing to be cut were first ligated to prevent subsequent loss of the blood rendered incoagulable by heparin injection. Then the anticoagulant was injected and the apparatus for extracorporeal thrombosis connected with the vessels of the animal. Since physiologic sodium chloride solution is usually regarded as an indifferent medium so far as blood is concerned, it was used in the container in which the collodion tube was immersed, as a control or standard experiment. The clotting time of the blood from the ear was tested often during the experiment by Mills' method; immediately after injection and persisting for about 4 hours the clotting time may be 30 minutes, or perhaps several hours. 4 and sometimes 5 hours after the injection of this amount of heparin the clotting time of the blood became shorter than 30 minutes, and tended to decrease rather quickly: for instance, in the next half hour, it became normal or sometimes less than normal.

There was considerable uniformity in the formation of white thrombi. From the results of thirty experiments, the sequence of events (Text-fig. 1) was as follows: After 5 minutes, no white thrombi were visible macroscopically, but a few small white thrombi were seen microscopically, which were chiefly composed of clumps of platelets, situated along the wrinkles of the bottom of the collodion tube. In size these clumps measured 0.06 by 0.04 or 0.04 by 0.04 mm. On and around the platelet clumps a few leucocytes were recognized. After 10 minutes numerous tiny white thrombi were seen macroscopically in the venous corner tube, or on one of the curved surfaces. In the collodion tube the number of white thrombi appeared increased, but they were still recognized only under the microscope. Also a few tiny ones were found in the venous cannula. After 15 or 20 minutes a few tiny white thrombi appeared in the arterial corner tube, while those in the venous cannula and corner

¹ 1 mg. of pure heparin renders 100 cc. of human blood incoagulable for 24 hours or more (Howell's personal communication).

tube increased in size and number. After 25 or 30 minutes numerous small white thrombi became macroscopically visible in the arterial cannula and in the curves of the side tubes, but were fewer on the arterial side. Those in the other parts increased in number and size. Sometimes the whole inner surface of the venous corner tube was coated with a number of white thrombi which were connected by threads of fibrin. The thrombi in the venous cannula and corner tube may be large (0.7 or 0.6 mm. in diameter); those in the cannula almost obstructed the constricted part. The blood stream in the apparatus gradually became slower, the laying down of the platelets was relatively decreased, and so the further growth of the white thrombi was relatively decreased. After 2 hours the thrombi had increased in size and number and the narrow part of the venous cannula was almost obstructed. In the collodion tube, many large white thrombi were obtainable (Fig. 1), often in the form of fern-like radiations, in the venous half or arterial half or sometimes in the center of the bottom of the collodion tube. These were propagated against the current. The site and growth of the white thrombi in the apparatus are shown in Text-fig. 1. The following protocol is typical of what was generally observed:

At 11.10 a.m. Rabbit 10, weighing 1400 gm., was injected intravenously with 40 mg. of heparin. At 12.00 the apparatus was connected and the collodion tube surrounded with physiologic sodium chloride solution of body temperature. The clotting time *in vitro* was more than 30 minutes. At 2.00 the apparatus was disconnected and the collodion tube detached. The blood remained totally incoagulable. All the surfaces of the apparatus were gently washed with physiologic sodium chloride solution. In the tubes of the venous side, numerous white thrombi were seen with a smaller number in the arterial tubes. The venous cannula seemed to be almost obstructed by a mass of white thrombi. In the collodion tube many white thrombi were observed in a fern-like radiating mass, which seen microscopically in side view appeared like a succession of hills and valleys. The size of the individual thrombus varies. Thus of thirteen thrombi seen under low power in one microscopic field, one thrombus measured 0.20 by 0.20 mm., four measured 0.16 by 0.12 mm., and eight 0.06 by 0.04 mm.

Experiments with Hirudin.

When hirudin was used instead of heparin, in a dosage of 10 mg. for each kilo of body weight in three experiments, the results were almost the same as when heparin was used (Fig. 2).

The Mechanism of Thrombosis and the Influence of Heparin.

These findings in the tubes should be discussed in connection with the mechanics of the blood flow. The blood in the carotid artery carries many platelets and leucocytes, which are hurled against the narrow part of the glass cannula. Platelets and leucocytes may adhere to the wall, but as the current is rapid, they usually become dislodged. The narrow part of the arterial corner tube is, as a rule, less often obstructed than that of the venous cannula, and then only at a later period. Wherever whirlpools, eddies and stagnation occur, platelets come in contact with foreign surfaces and they are agglutinated and white thrombi tend to form. At the spots where eddies form, relatively stagnant areas of blood are found; these favor agglutination probably through surface changes in the platelets.

The investigations show that in every part of the apparatus, even on the paraffined surface, the early stages of thrombosis can be demonstrated; though they appear earlier and are more marked, on the rough, irregular surface, for example, on the wrinkles of the collodion membrane. In the widest part of the apparatus, eddies occur on the surface in contact with the collodion and the platelets settle down.

The fact that clumps often assume a radiate or fern-like form in the lower part of the venous half of the collodion tube where whirlpools are seen through the collodion membrane, supports the von Recklinghausen theory (6). Eberth and Schimmelbusch's view (2, 8) in this regard commands attention but does not sufficiently explain the formation of platelet thrombi. Von Recklinghausen's theory appears more acceptable in explaining the mechanics of thrombosis. But in these experiments in the ampulla-like widening of the vessel, the thrombus forms mainly in the distal part of it and rarely in the proximal part. Platelets accumulate more in the distal part of the collodion tube than in the proximal end of it. Therefore, the explanation of the formation of the platelet clumps is to be found in the movement of the blood stream, rich in platelets and adequately slow, washing against the pathologic inner wall of the vessels, stagnating there and breaking into eddy currents against the irregular rough surface. If the stream is much slower, the white thrombi are chiefly formed in the arterial part of the apparatus; for instance in the collodion

tube it takes place in the arterial half of the lower part near the mouth of the corner tube. This is exactly in accord with the results of the experiments of Aschoff (1) and his coworkers with sand, showing that if the sand stream from the smaller vessel flows very slowly, a sand bank is formed in the bottom of its mouth.

The Use of Anticoagulant as an Antithrombotic Substance.

As Mason (5) has already stated, heparin might be effective against certain kinds of thrombosis, considering the thrombosis as a kind of intravascular clotting of the blood. But, since the first stage of autochthonous thrombosis is the deposition of platelets and not the deposition of fibrin, it does not suffice. At least single doses of the heparin used (Hynson, Westcott and Dunning) did not prevent the formation of white thrombi on foreign surfaces. In spite of the administration of a sufficient dose of this anticoagulant, and in spite of the incoagulability of the blood *in vitro*, white thrombi appear at the site where the conditions were conducive to clotting. The white thrombi gradually grew and sooner or later obstructed the lumen of the apparatus, and still later the lumen of the vessel. Therefore it appears that anticoagulants such as heparin² and hirudin in their form and in single doses do not afford complete protection against thrombosis. Experiments involving the continuous administration of heparin are contemplated.

In order to examine the structure of thrombi formed during the period of effective action of the anticoagulant, the apparatus itself may be removed and the ligatures holding the collodion tube in place may be cut at any desired time during the experiment. Thus the collodion tube with its contents is removed, its upper wall is carefully slit with a pair of delicate scissors and its inner surface cautiously washed with physiologic sodium chloride solution. Like a glass slide the collodion membrane is translucent enough to be examined directly under the microscope.

It is not desirable, perhaps, to dwell further on the character of the structure of the thrombus because that would but repeat Welch's

² The heparin used was supplied by Hynson, Westcott and Dunning. Professor Howell believes that pure heparin may entirely prevent the formation of white thrombi.

description (Figs. 3 and 4). Suffice it to say that his findings as to the structure of the white thrombi were verified by the method used in these experiments as well in serial sections as in the fresh state. But there is one respect in which my results appear to differ from his: the formation of fibrin is postponed on account of the anticoagulant action.

CONCLUSIONS.

1. Effects of anticoagulants, heparin and hirudin, on extracorporeal thrombosis were studied in thirty-three experiments by means of the extracorporeal vascular loop. In spite of adequate single doses of the anticoagulants white thrombi are formed and obstruction to flow may follow in the course of time, but the formation of red thrombi is markedly retarded.

2. The new method throws some light on the mechanics of thrombosis. The influences of foreign surfaces, of irregularities on the surface of the vessels, of whirlpools and of eddy motion of the blood current and of slowing and of stagnation with consequent prolonged contact with foreign surfaces are demonstrated, and these are analyzed in respect to the laying down of platelets and leucocytes.

3. The structure of white thrombi formed after anticoagulant injection is almost identical with that described by Welch. In one respect, however, the present results appear to differ from his: the formation of fibrin is postponed and retarded, and hence the collections of platelets are deposited in greater amount.

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EXPLANATION OF PLATES.

PLATE 2.

FIG. 1. Growths of white thrombi after heparin injection. *a*, after 10 minutes white thrombi appeared as a white line on the wrinkle of the center of the collodion tube. *b*, after 30 minutes the white thrombi grow up more remarkably. *c*, after 2 hours white thrombi multiply and are laid down in radiating form. *d*, after 3 hours.

FIG. 2. White thrombi after hirudin injection. *a*, after 2 hours. *b*, after 1 hour.

PLATE 3.

FIG. 3. Hillocks of platelet thrombi 1 hour after anticoagulant injection. *a*, clumps of platelets. *b*, fibrin with white cells.

FIG. 4. Hillocks of platelet thrombi 3 hours after anticoagulant injection. *a*, clumps of platelets. *b*, fibrin with white cells.

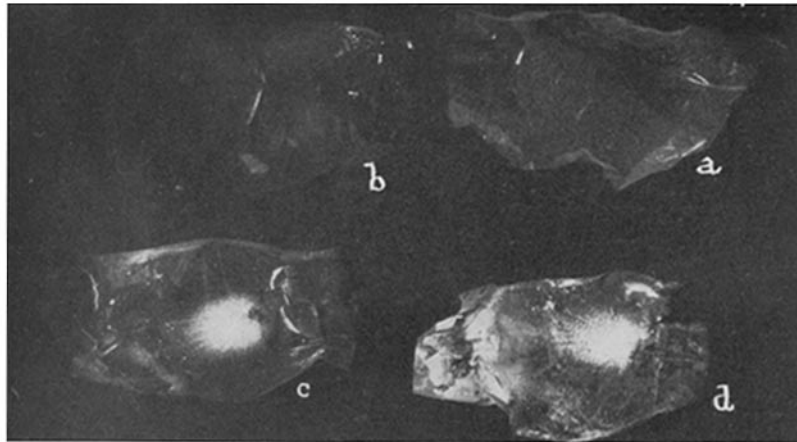


FIG. 1.

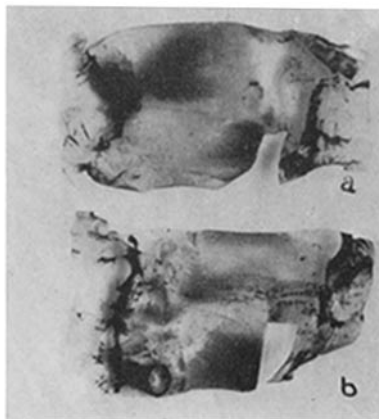


FIG. 2.

(Shionoya: Extracorporeal thrombosis. III.)

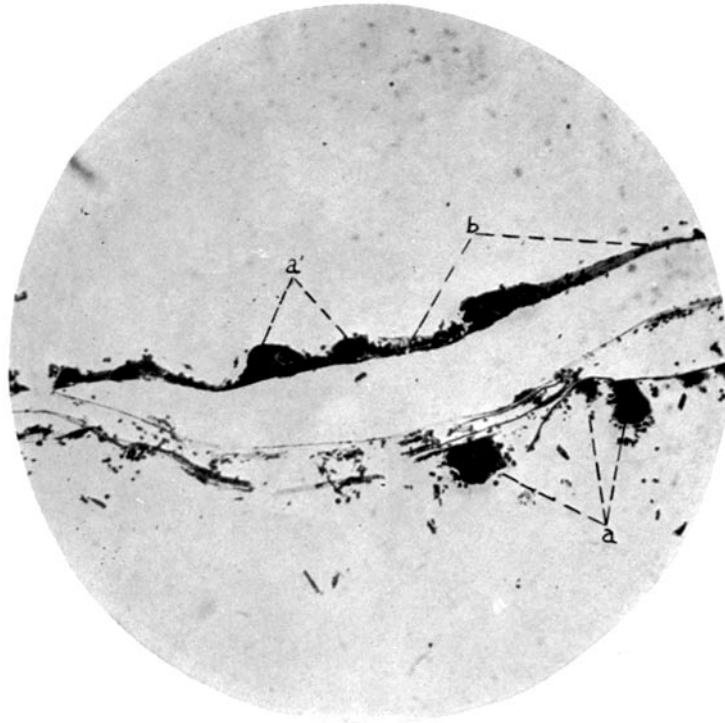


FIG. 3.

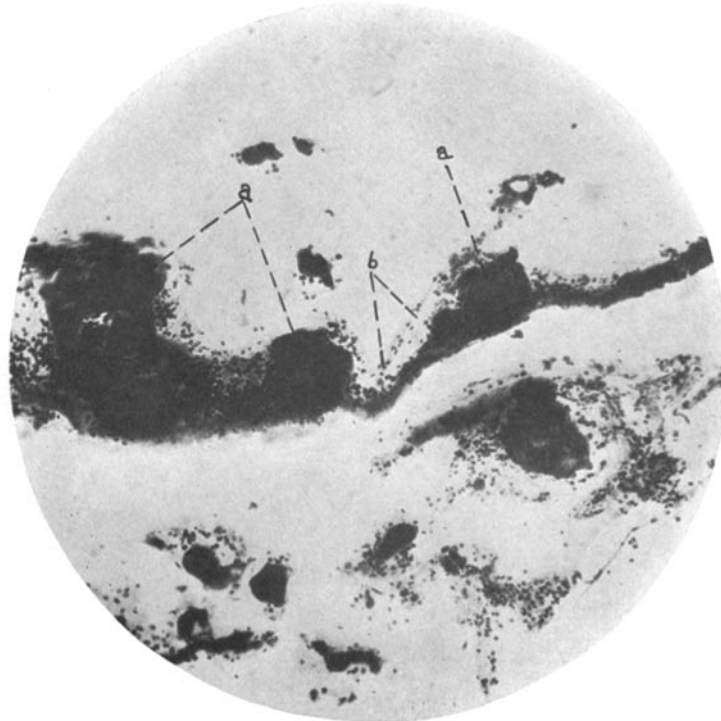


FIG. 4

(Shionoya: Extracorporeal thrombosis. III.)