

## STUDIES IN EXPERIMENTAL EXTRACORPOREAL THROMBOSIS.

### I. A METHOD FOR THE DIRECT OBSERVATION OF EXTRACORPOREAL THROMBUS FORMATION.

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Pulmonary embolism is one of the problems still a challenge to medicine and surgery. In the Mayo Clinic, the necropsy records show that it has been responsible for 7.3 per cent of the postoperative deaths during the last 10 years. Its annual toll throughout the world must be very large, and is probably much greater than is generally accepted, or than most statistics indicate, since pulmonary embolism is readily overlooked at necropsy unless sought for as a routine and specifically. Because of its important bearing on surgical mortality (2-11) we are making a study of thrombosis with the hope that eventually some means may be found whereby postoperative pulmonary embolism may be avoided or prevented.

In connection with this problem, one of us recalls some earlier studies carried on with John J. Abel, in which a dialyzing apparatus or an artificial kidney was used for the removal of diffusible substances from the circulating blood of living animals. In those experiments it was necessary to combat thrombosis constantly despite the fact that hirudin was employed in generous quantities as an anticoagulant. From this experience, it was thought that a similar, but simpler, apparatus might be employed advantageously in the study of the various stages of formation of thrombi, particularly that of white thrombi.

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*Principle of the Method.*

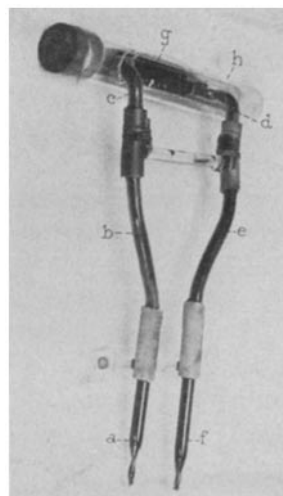
The method is simple: it consists in the introduction into the circulation of an extracorporeal vascular loop. By means of a cannula, the artery of the animal is attached to a collodion dialyzing tube, immersed in physiologic solution of sodium chloride, or other form of solution, and the blood is returned to the animal's body by another cannula attached to the vein. Before the instrument is attached to the vessels, the tubes and cannulas are completely filled with a solution of sodium chloride which approximates in its salt content the plasma of the animal, and this fluid is displaced into the body when the circulation through the apparatus is established.

During the experiment, the blood flows in a perfectly enclosed system and is returned to the body within a few seconds without having been exposed to contact with the air. On the way, it passes through the collodion tube which permits of an interchange of diffusible substances between the blood and the fluids in the outside container.

Since the inner surface of the apparatus is a foreign surface, coagulation occurs rapidly in the normal animal, within 4 to 10 minutes, but coagulation of the blood may be delayed or prevented by paraffining the tubes or by the previous injection of anticoagulants, such as heparin and hirudin, into the vein. Despite the presence of these anticoagulants, white thrombi are formed and thrombosis eventually occurs. The rate of thrombus formation can be influenced by various substances introduced into the circulation directly, or indirectly, and especially locally by dialysis through the collodion membrane, and the factors affecting each stage of clot formation may thereby be analyzed and assayed. The apparatus lends itself readily to the study of the influence of the mechanical, physical and chemical factors affecting the process of thrombosis. The experiments are carried out under complete trichloro-tertiary-butylalcohol anesthesia and are usually interrupted within 3 or 4 hours. The proportion of blood in the extracorporeal loop (about 2 cc.) is practically negligible so far as the physiology of the circulation of the animal is concerned.

*The Apparatus.*

The apparatus (Text-fig. 1) is simply an artificial circulatory loop permitting of extracorporeal blood flow. It consists of two parts connected by a collodion tube, which is immersed in a tube used as a container for dialyzing solutions. A glass frame holds the apparatus in place. The centrifugal and centripetal parts of the apparatus are attached respectively to the carotid artery and jugular vein. For purposes of handling and cleansing, each part is made in three sections, which are connected by means of rubber tubing. An ordinary arterial glass cannula, *a*, (about 4.5 cm. in length) is attached to the vessel and connected with the side tube *b*, which is about 6 cm. long and curved slightly to facilitate its connection with the corner tube *c* (5 cm.), which is curved in two directions, horizontally to approach the collodion tube *g*, which connects it with the other half of the apparatus, and downward to provide for immersion of the collodion membrane in the solution of the outside container *h*. The side tube and corner tube are paraffined. The collodion tube is attached to the two corner tubes by ligatures. The containing jacket is a short, stoppered test-tube with an opening in the side, which permits the immersion of the collodion tube in the solution of the dialyzing system. When the experiment is in progress, the blood flows from the artery through the cannula, side tube and corner tube of the arterial part into the collodion tube, and then into the corner tube *d*, side tube *e*, and cannula *f*, of the centripetal part back to the veins of the animal.



TEXT-FIG. 1. Extracorporeal loop.

*Technic of the Experiment.*

Rabbits, dogs or cats are suitable for such experiments, but throughout all our experiments rabbits have been employed; compared with dogs, they are more economical in the use of the anticoagulants.

Anesthesia is induced by the intraperitoneal injection of a saturated solution of trichloro-tertiary-butylalcohol in olive oil (from 1.5 to 2 cc. for each kilo of body weight). The apparatus is prepared, the glass side tube and corner tube paraffined, the collodion tube tied in place and the whole apparatus filled with physiologic sodium chloride solution.

The cannulas are introduced in the usual way, particular care being taken to produce as little trauma as possible. The vessels are clamped gently and carefully, to preserve, so far as possible, the integrity of the intima. With the cannulas tied in place, the cut wounds of the vessels are outside of the circuit, and hence, little, if any tissue juice or thromboplastic substance finds its way into the circulation in the extracorporeal loop. All the experiments are conducted under practically identical conditions. If anticoagulants are administered, unusual care must be exercised in the isolation and ligation of all vessels. This is necessary in the absence of clotting resulting from the anticoagulants, otherwise large quantities of blood may be lost, even from the smallest vessels.

The carotid artery and jugular vein are clamped until the apparatus is attached and then the artificial circulation is established, the blood flowing from the carotid artery into the tubes and back into the animal through the jugular vein. The blood flow can be seen clearly, particularly at points of constriction and in the neighborhood of curves. Currents, eddies and swirling motion may be obvious, particularly in the cannula where the arterial blood enters, and in the collodion tube. When doubt exists, proof of continued circulation can be readily obtained by clamping off the jugular vein and observing the presence or absence of ballooning of the vessel proximal to the clamp.

Thrombus formation can be directly observed through the collodion wall of the sac. This is more likely to occur in the neighborhood of wrinkles and irregularities in the surface of the collodion tube. Thrombi are visible first as pin-point areas, growing and radiating gradually in a direction counter to the blood stream and may attain considerable size (0.5 by 0.05 cm.). Clotting of the blood or the actual formation of the red thrombus may or may not occur, depending on the conditions of the experiment.

At will, any stage of the thrombus formation may be studied through

direct observation with the tube *in situ* or after the removal of the collodion tube. In the early stages of clotting, after the removal of the tube the surface may be gently washed with sodium chloride solution and observed directly, or studied under the microscope with or without the aid of special stains; or the specimen may be prepared and embedded in paraffin for section staining and ordinary pathologic study. The clots themselves may also be subjected to study.

#### DISCUSSION.

This method lends itself to the study of thrombus formation and the part played by various factors under many varying conditions. Thus the influence of mechanical, physical, physiologic, pharmacologic and pathologic factors may be determined, measured and to a certain extent assayed. Mechanically, several factors influence the rate of coagulation. The absolute and relative size of the tubes employed, the nature of the surfaces exposed, constriction, dilatation or angulation of the channel or changes in hydrostatic pressure resulting from differences in the levels of the blood stream, all influence the rate of blood flow and of coagulation. Physically, the temperature of the fluid surrounding the collodion tube is of importance. Physiologically, the vigor of the circulation and the size of the animal play some rôle. Pharmacologically,<sup>1</sup> drugs may be introduced which affect vigor and rate of circulation, or which directly accelerate or inhibit the process of coagulation. These may be injected intravenously, or diffusible drugs may be introduced into the extracorporeal loop locally by means of dialysis. Pathologic processes may be induced experimentally in animals, such as obstructive jaundice, chronic arsenic or phosphorus poisoning, and the influence of these diseases on the process of thrombosis determined. Finally, many combinations of these various factors may be studied simultaneously such, for instance, as the influence of transfusion or of various thromboplastic substances on the

<sup>1</sup> Since the phenomena of agglutination of platelets and of coagulation of blood are so imperfectly understood it has seemed better to undertake a wide range of experiments even including such as might *a priori* be expected to be fruitless. Results at variance with present hypotheses might help clarify these vexing problems.

process of coagulation in the presence of induced obstructive jaundice, or the influence of calcium on coagulation in a jaundiced or in a heparinized animal. The influence of these various factors is already under investigation and will form the basis of subsequent reports.

## SUMMARY.

A new method has been described for the study *in vivo* of thrombus formation in blood circulating extracorporeally in an artificial loop. This method permits of control of many factors, and hence of intensive study of thrombus formation under many and varied conditions. It admits of separate study of the formation of white thrombi and of the deposition of fibrin, which may apparently be independent processes.

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