THE USE OF HERUDIN IN THE TRANSFUSION OF BLOOD*

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The present communication is the last of three reports on experimental work undertaken to develop a more widely useful method of blood transfusion than those already existent. This work was inspired by the recent advances in the study of blood coagulation by W. H. Howell and other investigators of the Baltimore school, which suggested that the practical difficulties of developing a safe and easy transfusion method might be met on a more rational basis in the light of better knowledge; and that the newer laboratory methods of estimating the various factors of coagulation would be a help in forming a more precise judgment as to the efficacy or non-efficacy of measures calculated to prevent harmful changes in transfused blood.

The first part of our experimental work was concerned with influencing the initiative changes of coagulation through preventing or hindering thromboplastin formation by the use of paraffin and a suitable instrumental technic.[1]

The second part of this work resulted in the further development of a practical form of apparatus adapted to paraffin and herudin methods, and especially designed to enable a single operator to perform a transfusion, if necessary, without trained assistance.[2]

The third part of our investigations, concerning herudin, has been in progress along with the work already reported, but we have thought best to reserve the publication of this work until satisfied that the results warranted practical application.

Oxalated, citrated and fluorided plasmas are well known in the physiologic laboratories, and sodium citrate is reported to have been used as an anticoagulant for small quantities of transfused blood. Oxalate and citrate solutions act by fixing the calcium of the blood, which is a necessary factor in spontaneous coagulation. This decalcification is, of course, a change produced by a chemical reaction in the blood, and is theoretically, at least, undesirable. The use of herudin as an anticoagulant is not open to this objection.

Herudin is the active principle of a secretion derived from the buccal glands of the pond-leech, *Sanguesuga medicinalis,* and has been classed by Franz as a secondary albumose. Its physiologic properties are variously regarded by different investigators. Morawitz believes that it acts by neutralizing thrombin and prothrombin (thrombogen). Shittenhelm and Bodong believe that it neutralizes neither of these factors but reacts with some, as yet unknown substance, which is derived from the plasma. Mellanby concludes, from what appears to be substantial experimental evidence, that herudin contains an antibody for prothrombin and also a very energetic antibody for thromboplastin (kinase).

It may be concluded fairly from the available evidence that herudin has a decided effect on the prothrombin-antithrombin balance and that it has a neutralizing action on thromboplastin. This action is best illustrated diagrammatically.

The scheme of representation is based on Howell's theory of the mechanism of coagulation, and provides, we believe, an adequate explanation of the phenomena which have been observed. According to this theory the potential factors of coagulation may be expressed as shown in the diagram of the circulating blood (Fig. 1 *A*). The fibrinogen and calcium occupy a neutral position but have a potential affinity for prothrombin. The antithrombin and prothrombin have an affinity for each other and are loosely combined. This combination constitutes a balance, the so-called antithrombin-prothrombin balance, which, in normal circulating blood, is not quite equal, because there is a slight

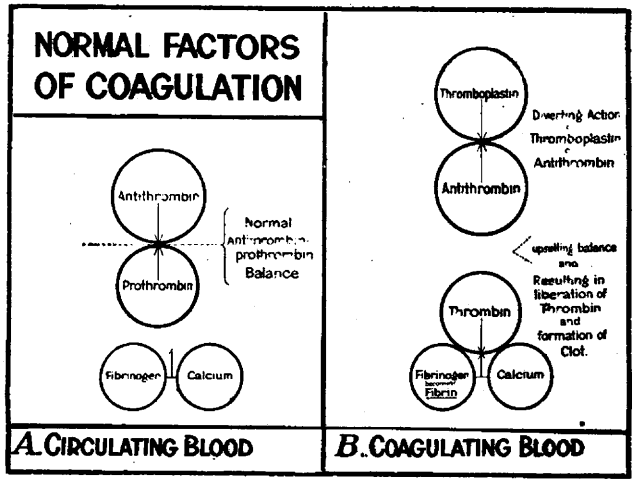

Figure 1.

preponderance of the antithrombin. If anything happens to upset this balance, so that the antithrombin side is weakened, then some of the prothrombin is released, and, in the presence of free calcium, becomes thrombin; and this thrombin reacts with fibrinogen to form a clot.

This action is seen in Figure 1 *B*. Here the coagulative process is initiated by the advent of another element, thromboplastin, which, having a very marked affinity for antithrombin, diverts it from its prothrombin attachment, and clotting ensues in the manner indicated.

In Figure 2 it will be seen that the action of herudin is to intervene and bind the thromboplastin, thus preventing the deviation of antithrombin from its prothrombin attachment.

There is considerable literature on the experimental use of herudin and there are some reports on its therapeutic use by intravenous injection for eclampsia, but no mention of its use as an anticoagulant for transfusing human blood.

From our experimental work it has become evident that herudin affords a convenient alternative for the paraffin method of transfusion under most circum-

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* The spelling used in this article for the name of the active principle of the secretion derived from the buccal glands of the pond-leech is that given to it by the discoverer, Friedrich Franz.—ED.

* Because of lack of space, this article is here abbreviated by omission of the bibliography. This, however, will appear in the author's reprints, a copy of which will be sent by the authors on receipt of a stamped, addressed envelope.

1. Satterlee, H. S., and Hooker, R. S.: Experiments to Develop a More Widely Useful Method of Blood-Transfusion, Arch. Int. Med., January, 1914, p. 51.

2. Satterlee and Hooker: The Further Development of an Apparatus for the Transfusion of Blood, Surg., Gynec. and Obst., 1914 [not yet published].

stances. The amount of herudin necessary, with our apparatus, is so small that its use may not be contraindicated even in those pathologic conditions in which there is already an excess of antithrombin or a deficiency of prothrombin in the circulating blood of the recipient.

Sievert has published a series of experiments which indicate that intravenous injections of herudin in rabbits are toxic in very large doses, that is from 25 to 50 mg. per kilogram of body-weight, the toxicity showing itself by changes in respiration and temperature, apathy, somnolence and albuminuria. Sievert states that the higher dosage, if quickly repeated, may result fatally, and that necropsy reveals hyperemia and petechial hemorrhages of the kidney and spleen but no other lesions. Other observers have found comparatively little or no ill effect from the intravenous use of large doses of herudin. Kaposi has given 40 mg. of herudin in normal salt solution by intravenous injection to a 2-kg. rabbit without harming the animal, and designates this proportion, 20 mg. per kilogram of body-weight, as the normal dose for

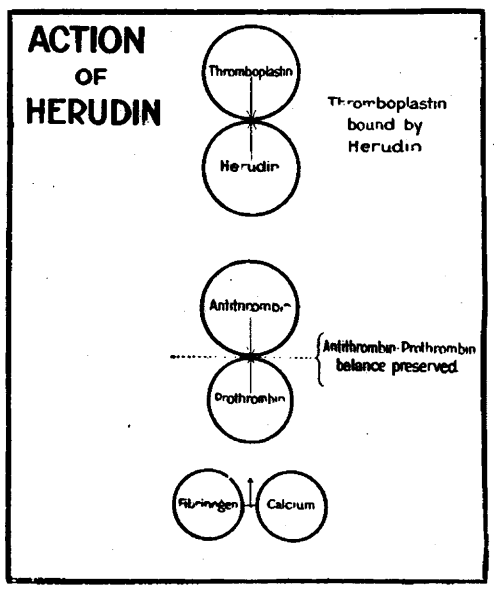

Figure 2.

experimental work. Rimann and Wolf, using this dosage, particularly state that no disturbance of health was noted as a result. Cowie has given thirty-five intravenous injections of herudin to a rabbit in doses increasing from 10 mg. to 22 mg. in twenty-six days, and maintained the last-mentioned dosage until the final injection, the total period of treatment covering fifty-four days. At the end of this time the animal had gained in weight and was in every respect perfectly well. Bodong has given from 23 to 73.25 mg. per kilogram of body-weight to rabbits, and states that it has no influence on the circulation or the respiration and is in no other way harmful to the animal. Von Hertzen and Ohman have confirmed Bodong's observation in a series of twelve experiments and concluded that herudin has no disturbing effect on the heart and blood-vessels. Abel, Rowntree and Turner have used very large quantities of herudin in "vividiffusion" experiments on dogs without apparently impairing the normal physiologic condition of the surviving animals. Dienst reports that he has given 200 mg. in 50 c.c. of salt solution by intravenous injection to a patient with very severe eclampsia, with most excellent results; and Engelmann has reported fourteen cases of eclampsia treated in this way with doses of from 200 to 300 mg.

Rimann and Wolf, in a series of eight experiments on the circulating blood of rabbits, have found that 20 mg. of herudin per hundred gm. of blood (normal dosis of Kaposi) renders the blood uncoagulable for four and a half hours, and that 10 mg. per hundred gm. of blood results in a shortening of the period of incoagulability to from one and a half to one and three-fourths hours. In these experiments the herudin was injected intravenously and the amount of circulating blood was calculated as one-tenth of the body-weight. The evidence of a coagulative tendency in the circulating blood following the injection was obtained by introducing a thread of catgut within the blood-current, according to the method of Trendelenburg. This catgut was withdrawn at varying intervals and the earliest appearance of fibrin formation was noted.

EXPERIMENTAL BASIS OF THE METHOD

Our first aim in experimenting with herudin was to determine the minimal amount of this substance which, when used in conjunction with the best available technic, would serve to prevent coagulative changes for a sufficient length of time to insure a safe transfer of blood from donor to recipient. The first experiment of this series was designed to show the amount of herudin necessary to defer coagulation from one-half to three-quarters of an hour when mixed with blood which was withdrawn directly from the vein so that there was the least possible chance of contamination with traumatized tissue. In this experiment no paraffin was employed, the blood being taken from a vein in 1-c.c. glass pipets and transferred immediately to six glass tubes containing varying amounts of herudin in 0.9 per cent. sodium chlorid solution.

From this experiment it was estimated that about 3.5 mg. of herudin to 100 c.c. of blood would be requisite for purposes of transfusion without the aid of paraffin, provided good technic was employed in obtaining the blood free from admixture of tissue juices.

This, in actual trial with our regular apparatus, was found to be the case; but the time of onset of coagulation varied within fairly wide limits — from seven to fifteen minutes.

When a paraffin coating was applied to the tip and the neck of our transfusion pipet it was found that the amount of herudin could be reduced by half, or to 1.5 to 1.8 mg. per hundred c.c. of blood, and that under these conditions there was also a twofold lengthening of the time of onset of coagulation. This meant a fourfold increase of efficiency of the herudin in the presence of a paraffin lining of the tip of the transfusion pipet.

As compared with a 30-c.c. piston-syringe with cannula 1 mm. in diameter and 30 mm. in length, 4.0 mg. per hundred c.c. of blood gave a clotting-time of ten minutes and thirty seconds for the syringe method, whereas 1.5 mg. per hundred c.c. of blood gave a clotting-time of thirty minutes for the paraffin-sealed pipet; and this, by a simple computation, shows a relative efficiency of approximately 1:8 in favor of the pipet.

To demonstrate the effect of herudin as applied to our method of transfusion, we have appended a table including all of our experimental and clinical transfusions with herudin in which no special factors were introduced (as varying pressures in delivery of blood, etc.), which would influence the onset of coagulation in the blood which was reserved in the pipet for observation. Experimental Transfusions 5 and 6, in which no paraffin was used as a lining for the tip of the pipet, are included in this table for the special purpose of comparison with our preferred method, in which the tip of the pipet is paraffin-sealed and lined.

EXPERIMENTAL AND CLINICAL TRANSFUSION WITH HERUDIN

Case and Experiment No.	Amt. of Blood Transfused c.c.	Amount of Herudin Used mg.	Dilution of Herudin with NaCl Sol.	Time of Onset of Coagulation, Minutes	Time of Complete Coagulation, Minutes
*Exp. Transfusion 5.....	160	3	1/1,000	†	12
*Exp. Transfusion 6.....	150	3	1/1,000	†	13
Exp. Transfusion 2.....	100	5	1/1,000	40	†
Exp. Transfusion 7.....	150	3	1/1,000	22	†
Exp. Transfusion 11.....	200	3	1/500	29½	33
Clin. Case 1:					
Cylinder A	230	3	1/500	20	†
Cylinder B	200	3	1/500	16	†
Clin. Case 2:					
Cylinder A	220	3.4	1/450	35	†
Cylinder B	220	3.4	1/450	30	†
Exp. Transfusion 15	160	3	1/500	21⅚	†
Exp. Transfusion 17	150	3	1/500	28½	35
Exp. Transfusion 18.....	220	3	1/500	35	38‡
Exp. Transfusion 19	220	3	1/500	30	38

* In these experiments the tip of the pipet was not lined with paraffin.
† Not observed.
‡ No clot at 38.

PRACTICAL APPLICATION OF THE METHOD

In making use of herudin for transfusion we have employed our regular pipets and cannulas, but we have dispensed with the paraffin coating of the cylinders. We have prepared the pipets by introducing from 3 to 5 c.c. of a 1:500 solution of herudin in salt solution, flowing this liquid very thoroughly over the interior of the cylinder and draining away the excess through the tip of the pipet just previous to use. From 1.5 to 2 c.c. of this solution, or approximately 3 mg. of herudin, are retained by adherence to the walls of the pipet, and for more than twenty minutes this is sufficient to prevent 220 c.c. of blood, drawn into the pipet, from clotting.

With the exceptions already noted, the operation with herudin is conducted in precisely the same way as with the paraffin-coated apparatus. The preparation of the pipets is the same, except that the first coating of the cylinders with paraffin is omitted. A partial coating with paraffin, however, is advisable. This coating should be done by aspirating the sterile, melted paraffin mixture just within the neck of the cylinder and expelling it again. This use of paraffin, from the tip to the neck, is primarily to insure an air-tight junction of the pipet tip with the metal bushing, and of the latter with the neck of the cylinder, but it also has a demonstrable effect in lessening thromboplastin formation during the aspiration of blood, and permits the employment of a minimal quantity of herudin. The herudin method may be employed without any paraffin whatever by using a 1:300 solution of herudin for coating and with care that the interior of the pipet tip is perfectly smooth and brightly polished, and that all connections are tight against air-leakage; but these contingencies are so surely and easily provided for by aspirating a little sterile paraffin just above the neck of the pipet, that the latter method is to be preferred. Any small receptacle with a cover and alcohol heating device will answer the purpose for sterilizing and holding the paraffin. The hardened paraffin may thus be carried with the apparatus, and the sealing process may be done at the time of operation, allowing ten or fifteen minutes for the pipet to cool, before coating with herudin solution.

It would, no doubt, be possible to obtain a more accurate estimate of the coagulation factors in transfused blood, and also of the inhibiting effect of herudin, by quantitative determinations of thrombin and of thromboplastin in specimens of such blood under varying conditions. We believe, however, that the simple observation of onset of coagulation together with the amounts of blood and of herudin, as recorded in the table, justify our conclusions. We hope at some future time to follow our present work with a more complete investigation of the subject with the aid of more precise laboratory methods.

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ARTHROPLASTY FOR INTRA-ARTICULAR BONY AND FIBROUS ANKYLOSIS OF TEMPOROMANDIBULAR ARTICULATION

REPORT OF NINE CASES *

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Arthroplasty, or the technic for the formation of new joints, may be divided into seven different stages in its evolution, and each stage has been initiated or created by the work of a single individual and then his succeeding school. These stages are:

1. The formation of fibrous or flail joints, as of the shoulder and elbow (Langenbeck, Ollier, Julius Wolff and others). These were desired sequences following resections for diseased joints, as tuberculosis, syphilis, pus infections, etc.

2. The restoration of mobility in a bony ankylosed joint by the interposition of muscle and fibrous tissue between the separated ends at the ankylosed joint, as in the mandible (Helferich, 1893, who was the father of this method).

3. Pseudo-arthrosis developing after osteotomies in the neighborhood of joints (Lorenz), as in hip ankylosis.

4. The transplantation of pedicled flaps of fascia and fat and capsule, with the production of a movable, sliding joint and a hygroma (Murphy, 1902), in the mandible, hip, knee, elbow, shoulder and wrist.

5. The homotransplantation of the articular ends and surfaces of bone (Lexer, 1906), particularly in the knee.

6. The transplantation of free fat and fascia (Lexer).

7. The interposition of foreign material to make the joint, from Péan's metallic joint down to Kraske, Baumgarten, Roser and Baer's heterovisceral implantations.

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