A TEST FOR ANTITHROMBIN IN THE BLOOD.*

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The part that antithrombin plays in the human blood in inhibiting coagulation is a question concerning which there is a decided difference of opinion. Although it is admitted generally by physiologists that antithrombin is present in the circulating blood, there is a marked divergence as to the significance and importance which should be accorded it. On the one hand, we note that Morawitz, whose theory of coagulation has gained wide acceptance, assigns to antithrombin no function in the theory of coagulation which he has elaborated. This omission seems to be a weak link in the chain which he has constructed, as any theory is necessarily incomplete which leaves out of consideration a substance which is regularly present in the circulating blood. On the other hand, Howell considers antithrombin to be a very important constituent, ascribing to it the part of maintaining the fluidity of the blood, in that coagulation ensues only when the antithrombin is rendered inert by the neutralizing effect of the zymoplastic substance in the shed blood or in the tissues. As stated, however, there is no diversity of opinion regarding the normal occurrence of antithrombin, so that it would seem worth while to study this substance clinically from a quantitative point of view, in order to obtain fuller data from which to judge its importance. The term antithrombin is employed in a functional sense to designate any substance or substances in the plasma which tend to inhibit coagulation. It is realized that a terminology of this kind cannot be absolutely satisfactory, especially from a chemical point of view. Nevertheless, it appears to be justifiable; it is, for example, in accordance with the physiological studies in immunity, where all the substances,—complement, amboceptor, etc.,—exist only from a functional viewpoint.

There have been very few quantitative examinations of antithrom-

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bin in the human blood. This, probably, may be accounted for by the fact that it has been overshadowed by the striking importance of other substances in the blood,—the thrombin, fibrinogen, and other active principles, which may be grouped as the positive coagulative factors,—and in part by the fact that there has been no simple method for making such tests. The few estimations of this nature which have been carried out have been reported by Howell, who used a method which he has devised. Although this method seems to be satisfactory, it is by no means simple, and is hardly suited to wide clinical use. In the first place, it requires the preparation of a pure thrombin. This is extracted from pig fibrin by treating it with an 8 per cent. solution of sodium chloride, followed by repeated extractions of the coagulable proteins by means of chloroform. The test also requires the preparation of a solution of fibrinogen which must contain no prothrombin; that is, must not clot merely upon the addition of calcium. It requires considerable experience to prepare both of these substances satisfactorily; the fibrinogen solution, which is not the salted fibrinogen of Hammarsten, but a dialyzed plasma, is especially difficult to obtain and to maintain free from contamination of prothrombin.

In the course of testing the coagulability of oxalated plasma from various sources, an examination which included a test for antithrombin, it was found that for clinical use a simple method could be employed, which requires neither a preparation of thrombin nor of fibrinogen. The method is carried out as follows:

About nine cubic centimeters of blood are aspirated and put into one cubic centimeter of 1 per cent. sodium oxalate. The blood is centrifugalized and the plasma removed in the usual way. The plasma is then recalcified by adding 2, 3, 4, and 5 drops, respectively, of a 0.5 per cent. calcium chloride solution. In this way we ascertain the general coagulability of the plasma, which is the composite of a number of interacting factors,—prothrombin, fibrinogen, antithrombin, etc.—and we determine at the same time the optimal amount of calcium for this particular plasma.

If we heat some of this plasma to 60° C., the prothrombin, as is well known, is destroyed and the fibrinogen is coagulated. After filtering off the coagulum we have a plasma which contains anti-

thrombin and practically no prothrombin. The strength of this antithrombin may be ascertained, for clinical purposes, as follows:

First, we prepare human plasma from a normal case just as we prepared the oxalated plasma which is to be tested. Five drops of this plasma are put into five thoroughly cleansed vials. The first of these serves as a control; to the second three drops of normal antithrombin are added; to the third five drops of normal antithrombin; to the fourth three drops of the antithrombin that is to be tested; and to the fifth five drops of this antithrombin. All tubes are equalized in amount by the addition of normal salt solution, and the mixtures are allowed to remain in contact for fifteen minutes. The plasma is then recalcified by the addition of 0.5 per cent. calcium chloride, the number of drops which are added having been determined by the general coagulability test, which should always precede the antithrombin test. As a rule, four drops have been found to be the optimal amount.

TABLE I.

Antithrombin Test.

A.

Normal.			Hemophilia.		Interval.
Control.	3 drops antithrombin.	5 drops antithrombin.	3 drops antithrombin.	5 drops antithrombin.	
−	−	−	−	−	2 min.
+	+	+	+	+	4 min.
+++	+++	++	+++	++	6 min.
		+++		+++	8 min.

B.

Normal.			Purpura.		Interval.
Control.	3 drops antithrombin.	5 drops antithrombin.	3 drops antithrombin.	5 drops antithrombin.	
−	−	−	−	−	4 min.
+	+	+	+	+	6 min.
+	+	+	+	+	8 min.
+++	++	++	+	+	10 min.
	+++	++	++	++	12 min.
		++	+++	++	14 min.
		+++		+++	16 min.

Table I *A* illustrates a test of this kind in an atypical case of hemophilia where there was a deficiency of calcium in the blood. We note that antithrombin was present in the plasma of the patient to no

greater degree than in the normal plasma; in both instances coagulation was rapid and but slightly delayed by the addition of three and of five drops of antithrombin. Table I *B* illustrates a similar test in a case of purpura. Here we likewise find no increase in antithrombin.

Antithrombin is judged to be in excess where a marked delay in coagulation is brought about in the tubes to which it has been added, as compared with the coagulation in the control tube. As in the case of the coagulation time, no arbitrary norm can be set up; the results should be well defined to warrant the conclusion that there is an excess of antithrombin.

The essential difference between this antithrombin test and that of Howell is that plasma is used as a basis instead of a fibrinogen solution. The validity of employing plasma in this way may be determined by preparing solutions of hirudin of varying strengths and titrating them upon plasma; in other words, by substituting hirudin for human antithrombin. Tests of this nature were carried out, first upon horse plasma, and later upon normal human plasma. In both cases the plasma was clear and had a coagulation time of not over ten minutes. Table II *A* illustrates an experiment of this kind. We may note that dilutions of 1 to 20,000, 30,000, and 40,000 of hirudin were employed, and that in each instance 1, 3, and 5 drops of one of these antithrombin solutions were added to the human plasma. It will be seen that in each of the three tests the sequence of coagulation is in direct ratio to the number of drops of antithrombin added, and that in general the more dilute the hirudin, the less its inhibiting effect. A test (table II *B*) of normal human antithrombin upon its own plasma has been added to this table to enable a comparison between the strengths of the hirudin used and of human antithrombin. It will be seen that, according to the test, human antithrombin is about equal to a 1 to 40,000 solution of hirudin.

If we turn again to table I, we notice that in addition to the tests of plasma in the case of purpura and of hemophilia, it includes titrations of antithrombin upon autogenous plasma. In these instances the plasma was normal. The same autogenous test may be carried out in pathological cases; *e. g.*, in hemophilia, as shown in table III, which reproduces an equilibrium test of this kind with the

same plasma which is reported in table I. This test evidently can not be considered an antithrombin test, as we employ plasma from a pathological case in order to ascertain the degree of antithrombin. It is termed an equilibrium test because it gives us information as to the balance which obtains in the plasma between all positive and negative factors concerned in coagulation. If the balance is in a state of delicate adjustment, the addition of a small amount of antithrombin will suffice greatly to delay coagulation, whereas if there is an excess of prothrombin and allied substances, this addition will bring about but a slight increase in the coagulation time. We see this when we compare the two tables. In table I, where two equilibrium tests of normal plasma are shown, the addition of three drops of antithrombin resulted in either slight or no delay, and five drops brought about at most a retardation of from ten to sixteen minutes. These tests must not be considered exceptions to the rule, although the delay when three drops of antithrombin are added is generally more marked, and when five drops are added, the period of complete coagulation is postponed to fifteen or twenty minutes. On the other hand, with the plasma of the hemophiliac, referred to in table I, the addition of the same amounts of antithrombin was sufficient to retard coagulation markedly (table III). Comparative tests such as these demonstrate that the mere coagulation time does not furnish complete information as regards the power for clotting which exists in plasma. There are latent potential coagulative factors which come to light only when inhibiting substances are added to the plasma. For example, a normal plasma (table II *A*) coagulated in ten minutes; the plasma of the hemophiliac (table III) in twelve minutes, that is, almost in the same length of time; nevertheless, upon the addition of five drops of its own antithrombin, the former coagulated in sixteen minutes, whereas the latter took forty-three minutes to clot. This test is not recommended for clinical use, because the testing of antithrombin upon abnormal plasma is erroneous. However, it is highly significant from one point of view: it shows that the circulating blood is not delicately balanced in regard to its coagulability. When we reflect that in adding three and five drops of antithrombin to five drops of plasma we are more than doubling its normal content of antithrombin, and that never-

theless coagulation generally ensues with but slight retardation, we must conclude that there exists a considerable factor of safety in the mechanism of the coagulation of the circulating blood. If such were not the case, serious hemorrhage, as the result of a slight temporary excess of antithrombin, would be a constant danger.

TABLE II.

A. Hirudin.

Dilution 1 :20,000.

1 drop hirudin.	3 drops hirudin.	5 drops hirudin.	Interval.
−	−	−	5 min.
+	+	−	8 min.
++	+	+	10 min.
++	++	+	11 min.
++	++	+	12 min.
+++	++	++	14 min.
	++	++	16 min.
	+++	++	18 min.
		++	20 min.
		+++	22 min.

Dilution 1 : 30,000.

1 drop hirudin.	3 drops hirudin.	5 drops hirudin.	Interval.
−	−	−	5 min.
+	+	+	8 min.
++	++	+	10 min.
+++	++	++	11 min.
	++	++	12 min.
	+++	++	14 min.
		+++	16 min.

Dilution 1 : 40,000.

1 drop hirudin.	3 drops hirudin.	5 drops hirudin.	Interval.
−	−	−	5 min.
+	+	+	8 min.
+++	++	++	10 min.
	++	++	11 min.
	++	++	12 min.
	+++	++	14 min.
		+++	16 min.

B. Antithrombin Test.

Control.	1 drop antithrombin.	3 drops antithrombin.	5 drops antithrombin.	nterval.
+	+	+	−	5 min.
++	++	++	+	8 min.
+++	+++	+++	++	10 min.
			++	11 min.
			++	12 min.
			+++	14 min.

TABLE III.

Equilibrium Test.[1]

Control.	3 drops antithrombin.	5 drops antithrombin.	Interval.
−	−	−	6 min.
+	+	+	10 min.
+++	+	+	12 min.
	+++	+	15 min.
		++	35 min.
		+++	43 min.

SUMMARY.

A test is described for the estimation of antithrombin in the blood. The chief advantage of the test is that it is simple, and does not require the preparation of fibrinogen and of thrombin, which are difficult to prepare and to maintain in a pure state. The principle consists in titrating the antithrombin against normal human plasma; in this way we obtain an estimation of its power to delay coagulation. As the result of examinations carried out by this method, it would seem that there is a wide factor of safety as regards the amount of antithrombin in the human blood, and that this inhibiting substance may be increased to a considerable degree without markedly delaying or endangering clotting.

[1] The same case as in table I *A*.