NOTES ON THE STANDARDIZATION OF BLOOD COAGULANTS.*

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In spite of the fact that there are numerous preparations on the market which are recommended for the purpose of quickening the coagulation of blood, there has not as yet been published a satisfactory method of testing their activity. The standardization of blood coagulants differs from the standardization of drugs in that the preparations cannot be made too active, since the stronger they are the better are the results obtained and, consequently, it is only necessary to determine that they possess a certain minimum activity. The preparations on the market consist of three general types—one derived from blood itself containing thrombin, a fibrin ferment (an English product), another type derived from tissues containing cephalin and the third type (also derived from tissues but by a different procedure) containing tissue fibrinogen.

The various preparations as found on the market differ not only in the manner in which they act, which depends on their content of thrombin, cephalin or tissue fibrinogen, but also in their potency, purity and stability.

It should be noted at this point that while the coagulants containing the various active substances will render the blood more coagulable *in vivo* or *in vitro*, each under a certain set of conditions, they all act in a different manner. The thrombin preparations may be injected intravenously (1) under ordinary conditions without causing intravascular clotting and death, but they will almost instantly clot shed blood. Subcutaneous injections are without effect on the clotting time of blood.

The cephalin preparations, while they have a strong coagulating effect on shed blood and recalcified citrated or oxalated plasma mixed with blood serum, will not cause intravascular clotting (2) even though injected in large amounts. The fibrogen (tissue fibrinogen) preparations will cause almost instant death by intravascular clotting (3) when injected intravenously even in small amounts and will greatly reduce the clotting time of recalcified citrated blood or shed blood taken from the animal's heart, uncontaminated with tissue juices. Subcutaneous injections or oral administrations will greatly reduce the clotting time of blood(4 and 5).

In view of the different effects produced by the blood coagulants on the market, it would be impossible to devise a single test which would even indicate the relative merits of all the coagulants, but all of the published tests have much in common. The standard tests are briefly (6) a comparison of the clotting time of blood *in vitro* between samples mixed with the coagulant and those mixed with a similar amount of physiological salt solution. Generally the test is conducted upon citrated or oxalated blood plasma to which some blood serum (for products of the second class) or a calcium salt, has been added at the time of testing to enable coagulation to take place. A qualitative standard is adopted which indicates in a general way the potency of the preparation. While these tests are more or less accurate and comparable results can be obtained when the same plasma is used, they are complicated by two steps—the addition of a decalcifying agent or clot preventive and the readdition of blood serum or a calcium salt. The technique of the tests would

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be considerably simplified as well as several possible sources of error eliminated if these two steps could be avoided.

EXPERIMENTAL.

The proposed method is the testing of the effect of a coagulant upon blood drawn directly from an animal. It was early found that the time required for the clotting of blood drawn over a tissue surface, as from the marginal ear vein of a rabbit, decreased with every successive drawing over the same surface. This is manifestly due to the effect on the blood of contact with the exposed tissue. Table I shows the irregularities from tests by this procedure. Table II shows that when the blood is drawn directly from the heart with a well-oiled syringe uniform results can be obtained on each animal. Table III shows the effect of the coagulant when blood so drawn is divided into two parts, one part being mixed with a coagulant and the other with a physiological salt solution. Unstarved rabbits were used. Table IV shows the results obtained when starved animals were used. That the coagulation time is affected by the state of digestion has been shown by Mills (7). These results show that it is important to use animals that have been starved. A period of 24 hours was found to give good results.

TABLE I.

THE CLOTTING TIME IN MINUTES OF SUCCESSIVE BLEEDINGS FROM THE SAME VENOUS PUNCTURE.

Rabbit.	From fresh puncture.	After 5 minutes.	After 10 minutes.	After 15 minutes.
No. 10	4.15	3.50	2.80	1.70
No. 13	3.62	1.75	2.30	1.40
No. 9	2.80	1.15	1.80	1.50
No. 12	3.20	2.30	1.60	1.00

TABLE II.

NORMAL CLOTTING TIME OF RABBIT BLOOD OBTAINED BY HEART PUNCTURE.

		Clotting time, minutes.				
Date of test.	Rabbit.	1st.	2nd.	3rd.	4th.	Average.
5–14–24	No. 10	3.09	3.45	3.50		3.35
5–14–24	No. 7	4.10	4.25	3.50		4.27
5–14–24	No. 9	4.30	4.40	4.15		4.27
5–15–24	No. 9	3.20	3.24	3.30	3.15	3.30
5–15–24	No. 7	2.70	2.65	2.40		2.58
5–15–24	No. 32	2.60	2.90	2.70		2.73

TABLE III.

EFFECT PRODUCED BY THE SAME LOT OF TISSUE FIBRINOGEN ON THE CLOTTING TIME (IN VITRO) OF BLOOD DRAWN FROM SEVERAL UNSTARVED RABBITS.

		Clotting time in minutes.		
Date of test.	Rabbit.	Blood + salt average 2 tests.	Blood + coagulant average 2 tests.	% Reduction.
5–26–24	No. 10	4.00	4.60	15*
5–26–24	No. 35	3.35	1.60	.52
5–26–24	No. 9	3.80	3.10	18
5–26–24	No. 36	3.85	2.15	44
5–26–24	No. 37	2.90	2.05	29
6–3–24	No. 9	2.95	1.25	58
6–3–24	No. 35	2.85	0.95	67
6–3–24	No. 13	2.70	0.70	74
6–3–24	No. 36	3.05	0.50	84
6–3–24	No. 59	2.50	0.80	68

* Increased.

TABLE IV.

EFFECT PRODUCED BY THE SAME LOT OF TISSUE FIBRINOGEN ON CLOTTING TIME (IN VITRO) OF BLOOD DRAWN FROM SEVERAL STARVED RABBITS.

Rabbit.	Clotting time of blood. Normal, average 2 tests.	With fibrogen, average 2 tests.	% Reduction of clotting time.
No. 9	3.55	1.15	68
No. 12	2.30	0.95	59
No. 13	2.85	0.97	66
No. 35	3.25	1.10	66
No. 36	3.20	1.30	60
No. 59	2.90	1.10	63
No. 10	3.40	1.15	67
		Average reduction	64%

TECHNIQUE.

Into a depression of a clean porcelain spot plate which has been kept at room temperature (25° C.) one standard platinum loop (1 mm., or about 1/100 cc.) of a physiological salt solution is introduced and a similar amount of the coagulant to be tested, is placed in an adjacent spot. One cubic centimeter of arterial heart blood is now withdrawn from the test animal, a starved rabbit, by means of a well-oiled syringe fitted with an 18 gauge $1^1/_2$-inch needle. The puncture is made through the 5th inter-costal space into the left ventricle. If by accident venous blood is obtained, it is discarded. Half of the blood so obtained is mixed with the salt solution and the other half with the coagulant. The clotting time is determined as the elasped time between mixing the blood with the coagulant and the first appearance of a fibrin thread. The fibrin threads are demonstrated by drawing fine L-shaped glass rods through the blood at the rate of 5 or 6 times per minute until a visible fibrin thread is picked up.

Since the normal clotting time of the blood obtained from various rabbits varies, percentage reductions in the clotting time have been calculated.

SUMMARY.

The accepted methods of testing the activity of blood coagulants are complicated by two steps which can be avoided and are subject to variation owing to the source and age of the citrated plasma. The proposed method eliminates these factors and is accurate when employed under controlled conditions of temperatures, etc.

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