THE STANDARDIZATION OF BLOOD COAGULANTS.*

BY HERBERT C. HAMILTON.

Hemorrhage, whether due to an abnormal condition of the blood or to lacerated tissues intentional or otherwise, has long been the subject of careful consideration.

Wiggers[1] found that in the different stages of pulmonary hemorrhage pituitary extracts, by their action in raising systemic arterial pressure, while at the same time lowering that in the pulmonary circuit, are peculiarly adapted to its control. This agent, however, has no direct effect on the rate of blood coagulation, its peculiar effectiveness being due to its action on the heart and vessels.

Adrenalin is frequently used and with very good results since it acts to constrict the vessels locally, retarding the flow of blood and permitting the formation of the clot. But so far as known, it has no action on the character of the blood to hasten coagulation.

In hemorrhage, while any step which will favorably influence coagulation is to be recommended, no procedure is equal to one which will shorten the coagulation time of the blood itself, promoting the formation of the clot at the exposed surface of the ruptured vessels and thus more quickly sealing them.

The accepted theory of blood coagulation and its remaining fluid in the vascular system is that the substance essential to the formation of fibrin from fibrinogen is thrombin, which is held in the form of prothrombin by a hypothetical anti-thrombin or anti-prothrombin. Possibly both substances are present, the one to hold the prothrombin, the other to destroy or combine with any thrombin which may be formed. It is very difficult seriously to disturb the equilibrium which maintains intravascular fluidity. When the tissues are lacerated, however, the shed blood, if of normal character, will soon form a clot and tend to seal the wound.

From the edges of the wound the tissue fluids exude, and from the blood platelets, which quickly disintegrate when exposed to the air, similar substances are set free. These two sets of fluids mix with the blood and start the process of coagulation which consists essentially in liberating the prothrombin from its antithrombin binding and in promoting the reaction first between prothrombin, thrombokinase and calcium ions by which thrombin is produced, then between the thrombin and fibrinogen, by which fibrin is split off and the clot formed. Morawitz[2] designated this substance which initiates coagulation as thrombokinase.

The change which has taken place appears to be two-fold. If we consider coagulation as an abnormal condition of the blood, the first departure from the normal is the cessation of metabolism by which the globulins are returned to the blood. When cytoglobulin,[3] the anti-coagulative substance—anti-thrombin—is no longer poured into the blood, prothrombin is set free and predominates.

With the calcium of the blood and in the presence of the tissue fluid containing thrombokinase, which now appears and constitutes the second abnormal condition, thrombin is produced and the clot-formation at once proceeds if the normal blood has been in proper equilibrium. Some have assumed that the office of the

* Read before Scientific Section, A. Ph. A., New York meeting, 1919.

[1] Wiggers, *Archives of Int. Med.*, 1914, p. 14.

[2] Morawitz, *Deutsch Archiv. f. Klin. Med.*, 179, p. 1.

[3] Schmidt, "Zur. Blutlehre.," Leipzig, 1893.

thrombokinase is two-fold, but proof has been obtained that the latter has no action on the anti-thrombin.[4]

Anti-thrombin seems to be a product of the liver, and its activity or the quantity present in the blood may be greatly increased by the injection of peptone, which can render the blood incoagulable.

Thrombokinase, the name originally given to the tissue and cell juices which initiate coagulation, was believed by Wooldridge[5] to owe its effectiveness to the lipoid lecithin, although he also observed that lecithin, derived from the yolk of eggs, is inactive in this respect.

Howell[6] and his co-workers established the fact that the active agent is lipoid cephalin, which exists in largest proportion in the brain cells, but also occurs in most tissues. Howell further states that cephalin, however carefully prepared, has only a limited period of activity and is dangerous for hypodermatic or intravenous administration. Dr. Hess stated that it should never be used intravenously although intravascular clotting may not be the invariable consequence.

The value of thrombin, prepared from fresh blood serum, is also limited since, unless treated to prevent change, it soon reverts to the inactive metathrombin. In any case the action of thrombin is limited to the amount present since the reaction between thrombin and fibrinogen is strictly quantitative. Rettger.[7]

Davis[8] found that intravenous injection of purified thrombin in relatively large quantities, instead of causing intravascular clotting, had the effect of prolonging the clotting time. There appeared to be an increased formation of antithrombin to compensate for the excess of thrombin.

Blood serum, containing fresh active thrombin, or serum treated to retain the thrombin in an active form, when used in sufficient quantity is an effective agent in shortening the coagulation time of hemophilic blood.

In the case of hemophiliacs there is an abnormal condition due either to an excessive amount of anti-thrombin or a deficiency of prothrombin, or in any particular case both conditions may prevail. It is logical, therefore, to keep in mind the conditions possibly present and to use a product capable of meeting all the requirements.

From the time of the earliest attempts to influence the coagulation time, fresh blood and blood serum have been used. But the difficulty and danger in the former, and the unreliability of the latter, stimulated research to develop a more dependable coagulant.

Among those resulting from various researches are products from blood platelets[9], from brain substance,[10] from blood serum,[11] from tissue extracts and from a combination of these.[12]

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[4] Lapenta, V. A., Paper read before Delaware-Blackford Co. Medical Societies at Muncie, Ind., Apr. 4, 1919.

[5] Wooldridge, "Beitrage zur Physiologie," 1887.

[6] Howell, "The Coagulation of the Blood," 1916–17.

[7] Rettger, *Amer. Jour. Physiol.*, 24, 406, 1909.

[8] Davis, *Ibid.*, 38, 233, 1911.

[9] Fonio, *Corresp.-Blatt f. Schweiz Aertzl.*, No. 13–15, 1913.

[10] Hess, *Jour. A. M. A.*, 1915, p. 64.

[11] Clowes and Busch, *N. Y. Med. Jour.*, 97, 16, 1913.

[12] Lapenta and Walters, *Therapeutic Gazette*, 34, 24, 1918.

Coagulen is a powder prepared from blood platelets by fractional centrifuging followed by careful desiccation and dilution with milk sugar. It is claimed to be a preparation of lipoid material, one gramme of which represents 20 grammes of dried blood. The active agent of coagulen is a thromboplastin which, according to Howell and others, is nothing else than cephalin and identical with the extract of brain substance. The required dose is not less than 20 Cc.

Thromboplastin, kephalin and hemagulen are names applied to the extract of brain tissue. This is obtained either by extracting with Ringer's solution an artificial blood plasma, and obtaining more or less of a suspension, or by extracting with an ether-acetone-alcohol reagent, by which a yellow lipoid residue is obtained.

Most of these are for local application only or principally, since there is danger of intravascular clotting if intravenously administered.

Blood serum contains no fibrinogen and probably no prothrombin or thrombokinase. It does contain thrombin although in a more or less inactivated form, due to the gradual recombination with antithrombin and the production of a so-called metathrombin.

Coagulase is a purified product obtained by precipitation of fresh horse serum by which an easily soluble powder is obtained, representing practically the active thrombin. The dose is 10 mils of the solution.

Hemostatic serum is a coagulant in liquid form, of which the dose is only 1 to 2 Cc. for any form of administration. It contains three distinct substances all of which are concerned in the process of blood coagulation: 1st, prothrombin, the form in which thrombin exists in the blood; 2nd, thrombokinase or tissue extract, but not derived from brain tissue; and 3rd, a substance which for lack of a more specific name is called anti-antithrombin, a substance similar to antitoxin in that it neutralizes the excess of anti-thrombin and lowers the anticoagulative power of the blood.

With hemostatic serum, therefore, the surgeon is in a position to counteract abnormal conditions of the blood whichever one is responsible for the slow coagulative power, and at the same time to initiate the process of coagulation when the prothrombin content has been augmented and the antithrombin rendered inactive.

The statement has been made that some of the coagulants mentioned have only a limited period of usefulness, unless proper precautions have been taken to remove or inactivate certain accompanying substances. How can it be shown that these precautions have been taken? Clinical reports have shown the effectiveness of freshly prepared extracts of brain tissue or blood platelets. But in the life-or-death cases when a blood coagulant is required are we to depend on the fact that the efficiency of a similar preparation had once been demonstrated?

I have tested three extracts of brain tissue and in only one case has any material shortening of the coagulation time been observed. Only one extract of blood platelets was available for test and in no case, by any means of testing, was it possible to demonstrate coagulative properties.

It is not possible by any known method of examination other than one closely allied to the physiological to determine whether a coagulant of this character is active.

As in other cases where a valuable medicinal agent is not amenable to chemical

standardization, it became necessary to evolve a physiological test in order to have some dependable means of measuring the hemostatic value of these agents. While clinical evidence is, in the final analysis, the sole criterion, clinical evidence is usually slow and often unsatisfactory. As a means of laboratory control over the activity of a product of such vital importance some more flexible method is imperatively demanded.

For testing blood derivatives, such as normal horse serum and coagulose, a method has been applied by which the coagulation time of horse plasma is measured. The test blood is drawn into a solution of sodium citrate or oxalate and the mixture centrifugalized to throw out the corpuscles to facilitate observation. Definite proportions of the coagulant and plasma are mixed and the time recorded when a firm clot is formed. A standard is selected, the maximum period during which the plasma may remain uncoagulated. This test, as shown by the chart, is very satisfactory as a laboratory measurement of value where applicable.

A similar test is theoretically applicable to all the agents mentioned by eliminating the decalcifying agent and keeping the blood plasma fluid by careful drawing and at low temperature.

There is no immediately apparent reason why it is not applicable to hemostatic serum, a product which combines the essential elements of all the other blood coagulants, but repeated experiments have failed to shorten the coagulation time of horse plasma under the conditions described. It was necessary, therefore, to work out a different method since the clinical test—the final deciding criterion—is ordinarily not immediately available.

The logical method for measuring coagulation time is that for which the Bifi-Brooks coagulometer was devised. A large drop of blood, a series of loops in a platinum wire and means for keeping the film of blood in the loops moist and warm, are all that is required for carrying out the test. Very accurate measurements can be made on blood from the human subject and on any one with a coagulation time sufficiently long to permit measurement when the time is greatly shortened.

My experience with the dogs' blood, however, seemed to prove that the apparatus is not readily adapted to the test of coagulation time after hemostatic serum had been administered. The fibrin film in the loop forms so promptly that the shortened time cannot be accurately measured. It is obscured by variations in the behavior of the films of fibrin which do not uniformly retain the web across the loop. This limitation was sufficient to prevent that accuracy which is essential to a proper reading of results.

The method that was finally adopted, as a tentative measure at least, seems not to differ materially from that used for coagulose, except that the action of the hemostatic serum is *in vivo* rather than *in vitro*. The method in detail makes use of an anesthetized dog to facilitate making intravenous injections and the frequent withdrawals of blood for observation.

The dog is anesthetized with chloretone (trichlor tertiary butyl alcohol) by intraperitoneal injection.[13] A femoral vein and a carotid artery are opened and glass cannulae inserted, for injecting the solution and drawing out samples of

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[13] Rowe, L. W., *Journ. Pharm. and Exp. Therap.*, 9, 107, 1916.

blood. A convenient amount of blood, about 3 Cc., is drawn into a clean test-tube and immediately placed in water at 40° C. At one-minute intervals it is observed to note the beginning and progress of coagulation, the amount of coagulation being roughly recorded as, — (minus) when no evidence of fibrin formation has appeared, 1 (meaning 1 plus) when this is first observed, 2 (++) and 3 (+++) as it proceeds and 4 (++++) when coagulation is complete and the tube can be inverted without disturbing the clot. In some cases a tough film forms on the surface while the volume of blood below remains fluid. This should be broken so that correct observations can be made.

Before the second and subsequent samples of blood are drawn, the cannula must be carefully cleaned to remove the clot and all adhering particles, and an amount of blood about equal to the sample to be used is discarded.

Several samples are drawn and tested to determine the normal coagulation time before making the injection. After injection of hemostatic serum the first evidence of shortened coagulation time usually does not appear in less than 15 minutes. It may be delayed as long as 1 hour before any consistent shortening of the time is observed. Usually, however, it is advisable to test a sample of blood every 15 minutes. If no effect from an active coagulant is observed in 1$^1/_2$ hours, the dog is probably highly resistant to the effect of a hemostatic and must be discarded. While such a condition is not easily explainable, failure is not an unusual occurrence in physiological testing.

Another difficulty which occasionally appears in attempting to carry out tests by this method is, that shortening in the coagulation time occurs with no apparent reason. This has also been observed by Drinker and Drinker[14] but our results differ from their observations because the hemorrhage is not so severe as is shown in their technic.

The standard for hemostatic serum is a shortening of the coagulation time to one-third or one-fourth the normal time for that test animal. Clinical tests have repeatedly verified the correctness of the results by this test and seem to establish definitely the fact that this is a dependable means of assay. The method was suggested by some work described by Howell[15] in which cephalin was injected intravenously. The production of thrombin was demonstrated by this method just as definitely as when added to the blood outside the body.

It is possible and even probable that by using mixtures of pure fibrinogen and thrombin, or other combinations of blood constituents, the test will be further simplified to eliminate the test animal; experiments so far have not proved satisfactory.

Until such work has been successfully prosecuted, we are content to depend on the physiological test which seems in every way to run parallel to clinical results.

PROTOCOLS OF TESTS.

In this and all subsequent tables the first column of figures refers to the time of observation of the sample of blood.

At the head of the columns that follow is the actual time when the sample of blood is drawn or sample of coagulant is injected.

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[14] Drinker and Drinker, *Amer. Jour. of Physiol.*, 38, 233, 1915.

[15] Howell, "The Harvey Lectures," 1916–1917, p. 296.

The figures below the time are symbols:

1, signifies beginning of fibrin formation; 2 and 3, progressive clotting; 4, solid clot when tube is inverted.

Coagulation Time Tested by Effect in Vivo.
Hemostatic Serum—℞ 050879.
Tested Aug. 22, 1919.

Minutes	10:00	10:10	10:20	10:22	10:51	11:01	11:08	11:11	11:18	11:36
1	—	—	—	Inj.	—	1	3	2	3	4
2	—	—	—	2	—	2	4	3	4	
3	1	1	—	Cc.	1	2		4		
4	1	2	1	sample	1	3				
5	2	2.	2		2	4				
6	2	3	2		3					
7	3	3	3		4					
8	3	3	3							
9	4	4	4							

Coagulation time shortened from 9 minutes to 1 minute.

Sample A.—From Blood Serum.
Tested July 22, 1919.

Minutes	1:13	1:57	1:58	2:16	2:35	3:40
1	—	Inj.	—	—	—	—
2	—	solution	—	1	1	—
3	—		—	2	2	—
4	—		1	3	4	—
5	1		2	3		—
6	2		3	4		1
7	2		4			2
8	3					3
9	4					4

Coagulation time shortened to one-half normal.

Sample A.—From Blood Serum—Coagulation Test.
Using Decalcified Sheep Plasma.

Minutes	Normal	0.025	0.05	0.1	0.2	0.4	0.6
15		1	1	1	2	4	4
25		1	1	1	2	4	4

Coagulation complete in 15 minutes with 0.4 Gm. of Sample A.

In the serum tests the columns are headed by the amount of sample added to plasma.

Sample B.—Tests of Action in Vivo.
Tested July 23, 1919.—From Blood Platelets.

Minutes	10:27	10:33	10:40	10:53	10:55	11:01	11:11	11:18	11:21	11:38
1	1	1	1	Inj.	1	1	1	Inj.	1	1
2	2	1	2	2 Cc.	3	2	2	2 Cc.	2	2
3	3	2	3	sample	3	3	3	more	3	3
4	3	3	3		4	3	3	of	4	4
5	4	3	4			4	3	same		
6		4					4			

Same Test Continued.

Minutes	1:12	1:20	1:31	1:40	1:53	2:17	3:27
1	1	Inj.	1	2	2	1	1
2	1	2 Cc.	2	3	3	2	1
3	2	more	3	4	3	3	2
4	3	of	4		4	4	3
5	4	same					3
6							4

Sample B—(Second Test).

Minutes	9:58	10:05	10:15	10:21	10:29	10:40	10:53	11:02	11:15	11:39	11:46	1:14
1	—	—	1	—	Inj.	—	—	—	—	1	1	1
2	1	1	2	1	4 Cc.	—	—	—	—	2	2	1
3	1	1	2	1		1	1	1	1	3	2	2
4	2	2	3	2		3	1	1	2	3	3	2
5	3	2	3	3		4	2	2	3	3	4	3
6	3	3	4	4			3	3	4	4		3
7	4	4					4	4				4

Coagulation time not materially shortened.

Sample B.—From Blood Platelets.

Coagulation tests using horse plasma *in vitro.*

Minutes	Normal	0.05	0.1	0.12	0.15
1	—	—	—	—	—
2	—	—	—	—	—
3	—	—	—	—	—
4	—	—	—	—	—
5	—	—	—	—	—
6	—	—	1	—	1
7	—	—	1	1	1
8	1	1	1	1	1
9	1	1	1	1	1
10	1	1	2	1	2
11	1	1	3	1	2
12	2	2	4	2	2
13	3	3		3	3
14	4	3		4	4
15		4			

Practically no shortening of the coagulation time resulted from use of this agent.

Sample C.—From Brain Tissue.

Tested July 23, 1919, by action *in vivo.*

Minutes	10:13	10:22	10:31	10:34	10:47	11:00	11:15	11:29	11:53	1:17
1	—	—	—	Inj.	—	—	—	—	—	—
2	—	—	—	2 Cc.	—	—	—	—	1	1
3	1	1	1	sample	1	1	1	1	2	1
4	1	1	2		1	1	1	2	3	2
5	2	2	3		2	2	2	4	3	2
6	2	3	3		2	3	3		3	3
7	3	4	4		3	4	4		4	3
8	4				4					4

Test continued.

Minutes	1:28	1:33	1:38	1:50	1:55	2:34	2:58	3:12	4:11	4:21
1	Inj.	1	1	1	1	3	1	3	3	2
2	4 Cc.	3	2	2	2	3	2	4	4	3
3	sam-	4	2	3	3	4	3			4
4	ple		4	4	4		4			
5										

The action was both prompt and lasting when a second injection of 4 cc. was made. Not only did it appear in 5 minutes but the shortened coagulation time extended over a period of 3 hours.

The dog was alive the next morning although useless for further examination. The effect

of the thromboplastin was so strong as to clot the blood in the artery making it difficult to obtain samples.

Shortened coagulation time from 7 minutes (original) to 2 minutes.

Sample C.—From Brain Tissue.

Coagulation test using horse serum.

Minutes	Normal	0.05	0.1	0.15	0.2
1	—	—	—	—	—
2	—	—	—	—	—
3	—	—	—	—	—
4	—	1	1	—	1
5	—	2	2	—	2
6	—	4	4	1	4
7	—			4	
8	—				
9	—				
10	1				
11	3				
12	4				

Coagulation time shortened to 6 minutes.

Sample D.—From Brain Tissue.

Material emulsified with normal salt solution.—Tested July 23, 1919.

Minutes	2:30	3:30	3:40	3:41	3:54	4:05	4:22
1	—	—	Inj.	—	—	—	—
2	—	—	2 Cc.	1	1	1	1
3	1	1	emul-	1	1	1	1
4	2	1	sion	2	1	2	2
5	2	1		2	2	3	3
6	3	2		3	2	4	4
7	3	3		3	3		
8	4	4		4	4		

Sample D (Second Test).

Tested Aug. 11, 1919.

Minutes	9:58	10:06	10:50	10:53	10:57	11:11	11:21	11:31	11:33
1	—	—	—	Inj.	—	—	—	—	—
2	1	1	1	2 Cc.	1	1	1	1	1
3	2	2	2		2	2	2	2	2
4	2	3	2		2	3	3	3	3
5	3	3	3		3	4	3	3	4
6	3	4	3		4		4	4	
7	4		4						

Coagulation time scarcely affected.

TEST OF HEMOSTATIC SERUM—℞ 049606.

Same day. Same dog.

Minutes	1:15	1:26	1:28	2:17	2:30	2:45	2:55
1	—	Inj.	1	—	—	1	2
2	1	2 Cc.	2	1	1	2	4
3	2	hem.	3	2	2	4	
4	2	Serum	3	3	3		
5	3		3	4	4		
6	4		4				

Coagulation time shortened from 6 to 2 minutes.

Sample E.—From Brain Tissue.

The material was injected in form of an emulsion with normal salt solution.—Tested Aug. 9, 1919.

Minutes	10:49	10:56	11:13	11:25	11:40	1:45	2:25
1	—	—	Inj.	1	1	1	—
2	1	1	5 Cc.	1	2	2	1
3	2	2	emulsion	2	3	3	2
4	2	2		3	4	3	2
5	3	3		4		4	3
6	3	3					4
7	4	4					

Coagulation time only slightly affected.

TEST OF HEMOSTATIC SERUM.

Same day. Same dog.

Minutes	2:25	2:35	2:37	3:10	4:09	4:15
1	—	Inj.	1	1	2	2
2	1	2 Cc.	2	2	2	4
3	2	of	3	3	4	
4	2	sample	3	4		
5	3		4			
6	4					

Coagulation time shortened from 6 minutes to 2 minutes in $1^{2}/_{3}$ hours.

An illustration of one form of failure which occasionally occurs in attempting to test a coagulating agent.

May 21, 1919.

Minutes	10:33	10:43	10:49	10:52	11:48	1:31	2:11	3:00
1	—	1	1	4	2	1	1	2
2	—	2	4		4	3	2	3
3	1	3					3	
4	2	4						
5	2							
6	2							
7	3							
8	3							
9	4							

No injection was made into this dog as the coagulation time would not remain uniform at any time.

RESEARCH LABORATORY
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DETROIT, MICH.

ABSTRACT OF DISCUSSION.

DR. PITTENGER: I just wish to state that during the past several months I have had considerable experience in testing blood coagulants. Although we have not done as much work towards perfecting the method as Dr. Hamilton brings out in his paper, we have obtained practically the same results, *viz.*, that there are quite a few preparations on the market which appear to be valueless as to their coagulant properties, and some others which apparently do the work very well.

I employed a method somewhat similar to his in which I used very small paraffined glass test-tubes. The only difference between Dr. Hamilton's technique and that which I employed was that our experiments were carried out at room temperature. We anesthetized a dog, tied a paraffin-coated cannula into the carotid artery, and then withdrew four or five tubes of normal blood. The coagulant was then injected into the saphenous vein and samples of blood drawn