Factors affecting the coagulation time of blood. VI, The effect of rapid progressive hemorrhage upon the factors of coagulation / by Katherine R. Drinker and Cecil K. Drinker from the Laboratory of physiology in the Harvard Medical School and the Medical Clinic of the Peter Bent Brigham Hospital, Boston.

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It is an old observation that progressive bleeding decreases the coagulation time of the blood. Gray and Lunt[1] have summarized the literature on this subject. We will, however, review briefly the chief explanations which have been offered for this decrease in coagulation time.

Hewson[2] in 1780 attributed the increased coagulability to a probable change in "that state of the blood vessels on which the thinness and lessened tendency of the lymph to coagulate depends; which surely is a very curious circumstance." Nasse[3] in 1842 and Brücke[4] in 1857 confirmed the observation as to the increased coagulability but showed at the same time a decrease in the fibrin content of the blood. This fact led them to conclude that the fibrin content and the rate of coagulation of the blood do not necessarily run parallel. Milian[5] in 1901 making his observations on hemorrhage following capillary puncture, thought that the increased coagulability was due to a local accumulation of a coagulating substance, stored in the skin and

[1] Gray, H. and Lunt, L. K.: American Journal of Physiology, 1914, xxxiv, 332.

[2] Hewson, W.: Experimental Inquiries into the Properties of the Blood, 1780, Part I, Experiment XX, 55.

[3] Nasse, H.: Handwôrterbuch der Physiologie (Wagner), 1842, I, 75.

[4] Brücke, E.: Archiv für Pathologische Anatomie und Physiologie und für Klinische Medizin (Virchow), 1857, xii, 81, 172.

[5] Milian, G.: Mémoires de la Société de Biologie, 1901, liii, 556, 576.

in the tissues and released when they were wounded. Arloing[6] in the same year, experimenting with venous hemorrhage and finding the same shortening of coagulation time, opposed Milian's view of a local secretion of coagulating substance by the cells of the skin and tissues but suggested the possibility of an increase in fibrin ferment, resulting from an alteration in the blood when it came in contact with the lumina of the tubes which carried it from the veins to the receiving flasks. Arthus[7] in 1902 suggested that the acceleration of the coagulation time might be due, not to a quantitative increase in the amount of fibrin ferment in the blood after hemorrhage, but to an acceleration of its production. Hartman[8] in 1909 was unable to choose between diminished oxygen, augmented carbon dioxide, augmented fibrin ferment, and augmented flow of tissue thrombokinase. Von den Velden[9] in the same year explained the decreased coagulation time as the result of an augmented thrombokinase which reached the blood stream through an influx of tissue juice, and confirmed the observation of Nasse and Brücke that an immediate decrease in the fibrin content of the blood accompanies the decrease in coagulation time.

In order to throw further light on this interesting problem and to determine whether or not a quantitative alteration in the different factors of coagulation corresponding to a change in coagulation time might be observed, we undertook the series of experiments which are reported in this paper. In each experiment determinations of the coagulation times and analyses of the factors of coagulation were made before and after successive hemorrhages.

I. METHODS OF WORK

The technique of obtaining and examining the specimens of blood and of determining the coagulation times was as follows. The test animal was first anaesthetized with urethane (2 grams

[6] Arloing, F.: Mémoires de la Société de Biologie, 1901, liii, 675.

[7] Arthus, M.: Journal de Physiologie et de Pathologie Générale, 1902, iv, 273.

[8] Hartman, J.: Münchener Medizinische Wochenschrift, 1909, lvi, 796.

[9] Von den Velden, R.: Archiv für Experimentelle Pathologie und Pharmakologie, 1909, lxi, 37.

per kilo). The femoral or in some cases the carotid artery was next exposed, ligated distally and clamped proximally. A glass cannula washed with oxalate solution was then slipped into an oblique cut in the artery but not tied, the clamp was released, and the blood allowed to flow from the cannula. Blood obtained in this way supplied the specimens to be analyzed for the factors of coagulation and the specimens upon which coagulation time was determined. The specimens to be analyzed for the factors of coagulation were collected in glass graduates containing 1 per cent sodium oxalate in a 0.9 per cent sodium chloride solution in the proportion of 1 cc. of oxalate solution to 8 cc. of blood. A few drops of blood were first allowed to drop into a graduate containing the oxalate; the specimens upon which the coagulation time was determined were next collected in two test tubes (diameter 10 mm.), each graduated to hold 1 cc.; after which procedure the collection of the specimen to be analyzed was completed. The purpose of this order of work was to obtain for the determination of coagulation time a specimen of blood which had not stood in contact with the injured vessel wall and yet one which represented the condition of affairs in the body before the bulk of the hemorrhage occurred. The amount of the hemorrhage varied in different animals depending on their size. At the end of the bleeding the vessel was clamped proximally and the cannula removed.

As soon as the blood for determining coagulation time was collected, the tubes were placed in a water bath and kept at 37°–38°C. until they could be inverted without dislodging the clots. Observations were made by gently tilting the tubes at the end of five minutes and then at the end of every minute. Each coagulation time was taken with a stop-watch which was started as soon as the blood began flowing into the tube.

At an interval—usually of twenty minutes—the procedure described above was repeated, and a second determination of coagulation time (represented by the average of the two tubes) was made, and a corresponding oxalated specimen obtained to be analysed for the factors of coagulation. A fresh cannula or the old cannula freshly washed was used. This process of

bleeding at intervals of about twenty minutes was continued until the animal died. The later hemorrhages were of course smaller than the earlier ones, but varied in amount in different experiments.

After the death of the animal the specimens of oxalated blood were centrifugalized for 10 minutes at high-speed, and the plasma pipetted off. A portion of the plasma from each specimen was used to make *prothrombin* determinations; a portion was used to make *antithrombin* determinations; and a third portion (10 cc. if possible) was used to make *fibrinogen* determinations. In five experiments platelet counts[10] were made before each hemorrhage.

The methods of making prothrombin and antithrombin determinations were those described by Howell[11] and reviewed by Drinker and Hurwitz in an article not yet published. The fibrinogen determinations were made by the heat coagulation method.

II. EXPERIMENTAL DATA

Nine experiments were done on cats and eight on rabbits. In every experiment in which the coagulation time as a whole decreased there was a decrease in the amount of antithrombin. In all but two cases there was a steady fall in the fibrinogen content. The behavior of the prothrombin was irregular, on the whole tending first to increase slightly in amount and then to decrease. This irregularity in the prothrombin reaction depends to a certain extent upon the variations in antithrombin. With a fall in antithrombin one would expect a relative increase in prothrombin, though the absolute amount of the substance remained constant. In view of this fact the slight relative fall in prothrombin occurring in a number of our experiments in association with a fall in antithrombin, indicates a still greater absolute fall in the amount of prothrombin in the blood.

The following protocol illustrates the method by which our results were obtained. In this case the amount of prothrombin increased slightly while the amounts of antithrombin and fibrinogen decreased.

[10] Wright, J. H. and Kinnicutt, R.: Journal of the American Medical Association, 1911, lvi, 1457.

[11] Howell, W. H.: Archives of Internal Medicine, 1914, xiii, 76.

PROTOCOL—EXPERIMENT IV

Date, August 22, 1914.
Weight cat, 2 kilos. Urethane anaesthesia.
Anaesthesia begun, 11.40 a.m.
Operation begun, 12.03 p.m.

First Bleeding

Time, 12.11 p.m.
Amount, 23.5 cc.
Coagulation time (1) 14 minutes, 11 seconds.
(2) 13 minutes, 49 seconds.
Average, 14 minutes.

Second Bleeding

Time, 12.30 p.m.
Amount, 18 cc.
Coagulation time (1) 12 minutes, 12½ seconds.
(2) 14 minutes.
Average 13 minutes, 6¼ seconds.

Third Bleeding

Time, 12.50 p.m.
Amount, 18 cc.
Coagulation time (1) 12 minutes, 7 seconds.
(2) 12 minutes, 23 seconds.
Average 12 minutes, 15 seconds.
Death at 12.52 p.m.

ANTITHROMBIN DETERMINATION

Specimen 1

Thrombin drops	*Antithrombin drops*	*Time Interval minutes*	*Fibrinogen drops*	*Coagulation minutes*
3	1	15	10	15
4	1	15	10	10½
5	1	15	10	7¾
6	1	15	10	6
			Average,	9.81

Specimen 2

3	1	15	10	13½
4	1	15	10	9¼
5	1	15	10	6½
6	1	15	10	5¼
			Average,	8.62

Specimen 3

Thrombin drops	*Antithrombin drops*	*Time Internal minutes*	*Fibrinogen drops*	*Coagulation minutes*
3	1	15	10	6
4	1	15	10	5¼
5	1	15	10	4
6	1	15	10	3¼
			Average,	4.62

Control

Thrombin drops	*Fibrinogen drops*	*Coagulation minutes*
3	10	5
4	10	4

In making antithrombin determinations the selection of a definite end-point is somewhat difficult. The antithrombin delays or even prevents the formation of a solid clot. In our opinion the first definite appearance of coagulum in the clear solutions used is the safest end-point to employ, and we have used it throughout our determinations. In all of our experiments the average of the four antithrombin observations on each specimen has been used to represent the antithrombin factor in that specimen.

PROTHROMBIN DETERMINATION

Specimen 1

Oxalated Plasma drops	*0.5% $CaCl_2$ drops*	*Coagulation minutes*
5	2	5
5	3	5
5	4	4½
5	5	4½
5	6	4½

Tube containing optimum amount of $CaCl_2$ = 4½ minutes.

Specimen 2

5	2	3½
5	3	3½
5	4	3½
5	5	3
5	6	3

Tube containing optimum amount of $CaCl_2$ = 3 minutes.

Specimen 3

Ozalathd Plasma drops	*0.5% $CaCl_2$ drops*	*Coagulation minutes*
5	2	3½
5	3	3¼
5	4	3
5	5	2½
5	6	2½

Tube containing optimum amount of $CaCl_2$ = 2½ minutes.

The end-point in each prothrombin reaction is reached at the moment when the tube can be inverted without dislodging the clot. Great care must be taken not to jar the tubes while the clots are forming. In all of our experiments the prothrombin factor in each specimen is represented by the lowest figure in the series—i.e., the time required for clotting in the tube containing the optimum amount of calcium.

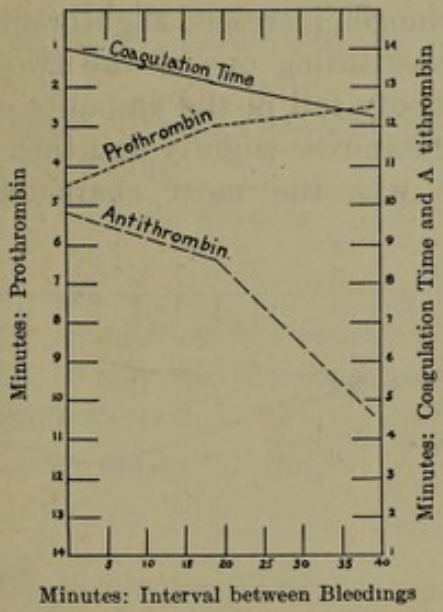

Fig. 1. Experiment IV.

FIBRINOGEN DETERMINATION

Specimen 1: 0.2137 gram in 100 cc. plasma.
Specimen 2: 0.1822 gram in 100 cc. plasma.
Specimen 3: 0.1350 gram in 100 cc. plasma.

Figure 1 (Exp. IV) represents the results of this experiment plotted graphically.

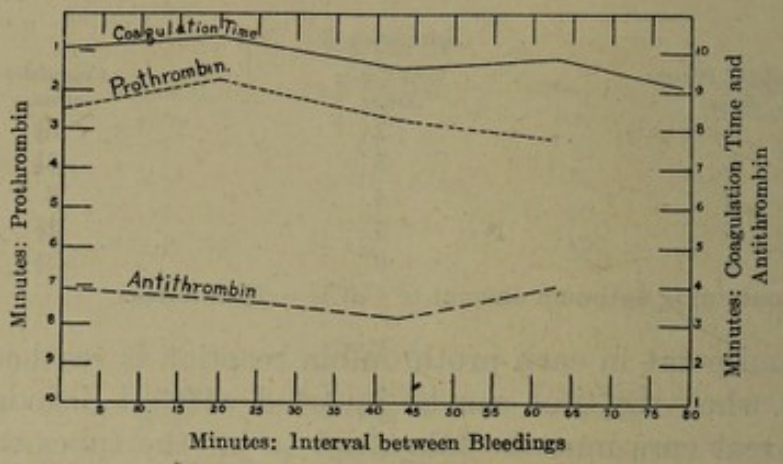

Fig. 2. Experiment IX.

Figure 2 (Exp. IX) represents the results of an experiment in which there was very little variation in the coagulation time, and a correspondingly small variation in the amount of antithrombin. The amount of fibrinogen increased slightly and then fell slightly, this experiment constituting one of the two exceptions to our observation of a steady fall in the amounts of fibrinogen. The amount of prothrombin rose slightly and then fell slightly, which in our experience was the most characteristic prothrombin behavior.

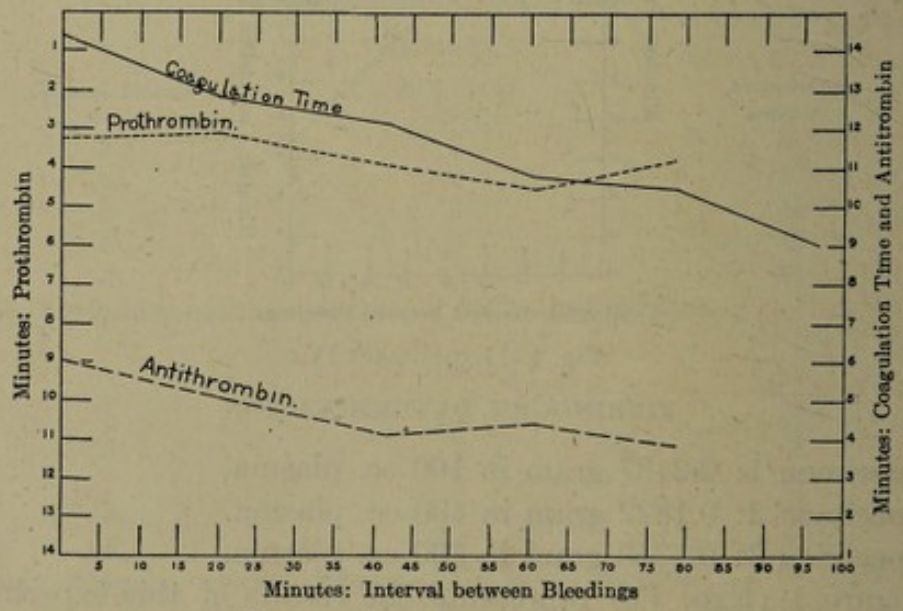

Fig. 3. Composite Curve, Cat.

Figure 3 is a composite curve embodying the coagulation times and the antithrombin and prothrombin determinations in our nine experiments on cats. The points to be noted are: (a) the fall in the amount of antithrombin corresponding to the decrease in coagulation time; and (b) a slight increase and then a slight fall in prothrombin. The final increase shown in the curve represents a single determination and is therefore unimportant. The fibrinogen determinations on cats are shown in Table 2, which contains a summary of the results of our entire series of experiments.

Discovering that at room temperature during a short period of contact with thrombin solution, the antithrombin in rabbits acts much more potently than does the antithrombin in cats, we determined to use the rabbit as a test animal.

The following protocol represents a typical rabbit experiment.

PROTOCOL—EXPERIMENT XV

Date, October 9, 1914.
Weight rabbit, 2.6 kilos. Urethane anaesthesia.
Anaesthesia begun, 10.35 a.m.

First Bleeding

Time, 11.22 a.m.
Amount, 13 cc.
Coagulation time (1) 18 minutes, 54 seconds.
(2) 18 minutes, 10 seconds.
Average 18 minutes, 32 seconds.

Second Bleeding

Time, 11.22½ a.m.
Amount, 13 cc.
Coagulation time (1) 18 minutes, 20 seconds.
(2) 18 minutes, 5 seconds.
Average 18 minutes, 12½ seconds.

Third Bleeding

Time, 11.47 a.m.
Amount, 12 cc.
Coagulation time (1) 15 minutes, 58 seconds.
(2) 15 minutes, 52 seconds.
Average 15 minutes, 55 seconds.

Fourth Bleeding

Time, 11.47½ a.m.
Amount, 12.5 cc.
Coagulation time (1) 14 minutes, 45 seconds.
(2) 16 minutes, 22 seconds.
Average 15 minutes, 33½ seconds.
Death at 11.55 a.m.

ANTITHROMBIN DETERMINATION

Specimen 1

Thrombin drops	*Antithrombin drops*	*Time Interval minutes*	*Fibrinogen drops*	*Coagulation minutes*
3	1	15	7	71¾
4	1	15	7	56
5	1	15	7	47¾
6	1	15	7	41¼
			Average,	54.18

Specimen 2

3	1	15	7	80½
4	1	15	7	62½
5	1	15	7	52
6	1	15	7	23½
			Average,	54.62

Specimen 3

3	1	15	7	57
4	1	15	7	46
5	1	15	7	33¾
6	1	15	7	15¼
			Average,	38

Specimen 4

3	1	15	7	64
4	1	15	7	47
5	1	15	7	33¼
6	1	15	7	23
			Average,	41.81

Control

Thrombin drops	*Fibrinogen drops*	*Coagulation minutes*
3	7	3½
4	7	2¼

PROTHROMBIN DETERMINATION

Specimen 1

Oxalated Plasma drops	*0.5% $CaCl_2$ drops*	*Coagulation minutes*
5	2	$8\frac{3}{4}$
5	3	$6\frac{1}{2}$
5	4	$8\frac{3}{4}$
5	5	$7\frac{1}{2}$
5	6	$7\frac{3}{4}$

Tube containing optimum amount of $CaCl_2$ = $6\frac{1}{2}$ minutes.

Specimen 2

5	2	8
5	3	6
5	4	$7\frac{3}{4}$
5	5	$7\frac{1}{4}$
5	6	$9\frac{1}{4}$

Tube containing optimum amount of $CaCl_2$ = 6 minutes.

Specimen 3

5	2	$11\frac{1}{2}$
5	3	$8\frac{3}{4}$
5	4	$9\frac{3}{4}$
5	5	$9\frac{1}{4}$
5	6	$11\frac{1}{4}$

Tube containing optimum amount of $CaCl_2$ = $8\frac{3}{4}$ minutes.

Specimen 4

5	2	$9\frac{1}{4}$
5	3	$10\frac{1}{2}$
5	4	$10\frac{1}{2}$
5	5	10
5	6	$10\frac{1}{2}$

Tube containing optimum amount of $CaCl_2$ = $9\frac{1}{4}$ minutes.

FIBRINOGEN DETERMINATIONS

Specimens 1 and 2 (mixed): 0.3240 gram in 100 cc. plasma.

Specimens 3 and 4 (mixed): 0.2475 gram in 100 cc. plasma.

Figure 4 (Exp. XVI) shows a marked decrease in coagulation time; a marked fall in antithrombin; a fall in fibrinogen; first a decrease and then a final increase in prothrombin.

Figure 5 (Exp. X) shows a decrease in prothrombin after two bleedings and then a slight increase. The fibrinogen has in-

creased slightly (0.0168 gram) instead of falling. The coagulation time and the antithrombin have both decreased steadily.

Figure 6 is a composite figure embodying the coagulation times and the antithrombin and prothrombin determinations in

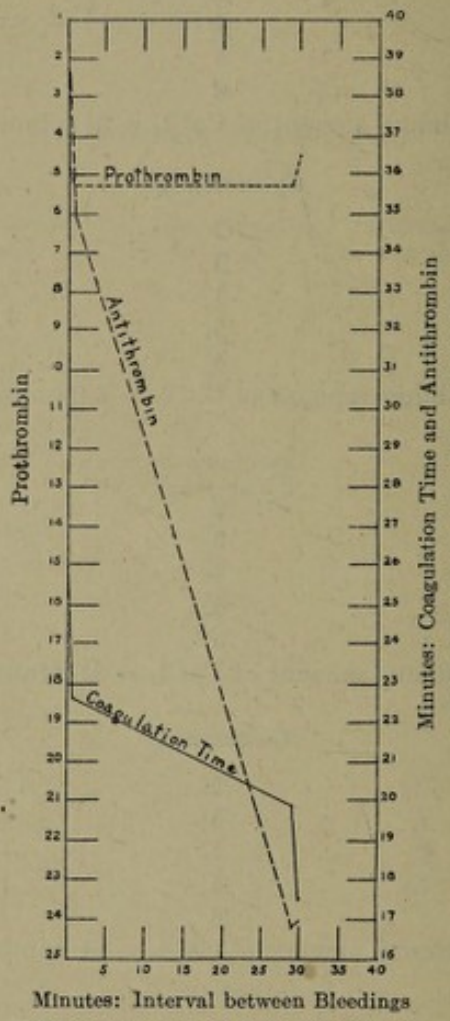

Fig. 4. Experiment XVI. Minutes 1 to 16 in the right hand column, being unnecessary in the figure, are omitted in order to save space.

our eight experiments on rabbits. It shows the same features which we have demonstrated in the composite curve of our experiments on cats: (a) a steady fall in antithrombin corresponding to a decrease in coagulation time; and (b) a slight increase, then a slight fall in the amount of prothrombin, and a final return to practically the original amount (two observations).

Our fibrinogen determinations on rabbits are shown in Table 2. The individual determinations vary less than those made upon cats but, with one exception, they show the same downward trend.

Thinking that possibly a marked change in the number of platelets after severe hemorrhage might be a factor to be con-

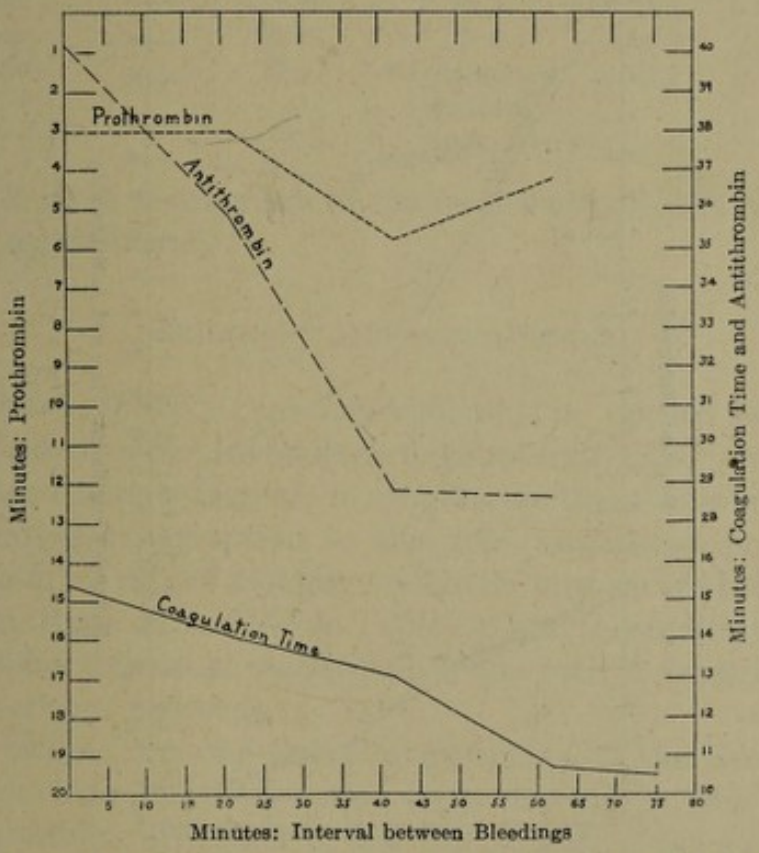

Fig. 5. Experiment X. Minutes 1 to 10 and 17 to 28 in the right hand column, being unnecessary in the figure, are omitted in order to save space.

sidered, we made platelet counts in five experiments before the first hemorrhage and then after each bleeding, but found practically no change in their number.

A comparison of the average results of our experiments on cats and those on rabbits is of interest. The behavior of the prothrombin in the two series of experiments was in general the same—a slight initial increase and then a slight decrease in

amount. In both series there was a steady fall in fibrinogen: in the cats an average decrease of 0.0762 gram per 100 cc. of plasma after three bleedings: in the rabbits an average decrease of 0.0770 gram after three bleedings and 0.0811 gram after four bleedings. The following table gives a comparison of the per-

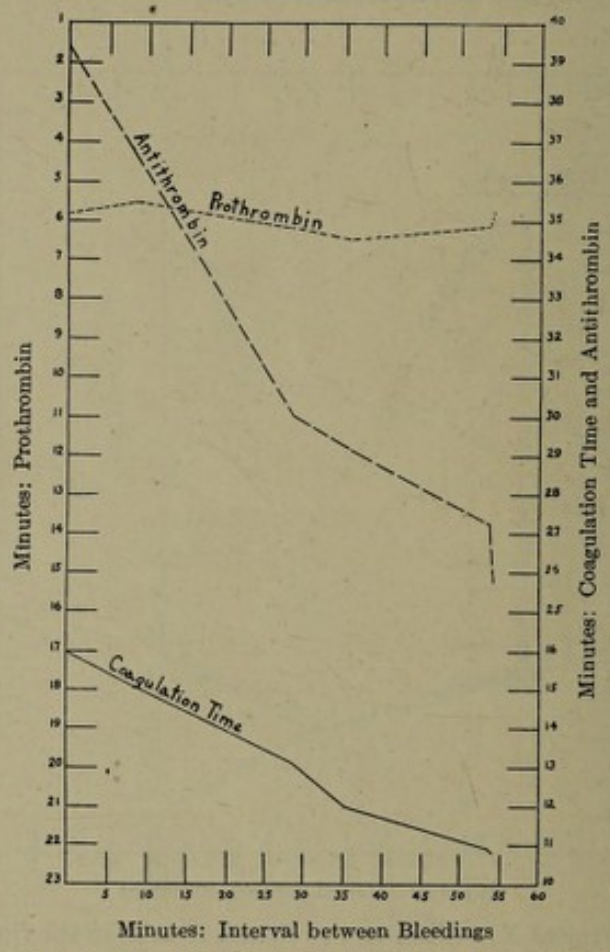

Fig. 6. Composite Curve, Rabbit. Minutes 1 to 10 and 17 to 25 in the right hand column, being unnecessary in the figure, are omitted in order to save space.

centages of the average decrease in coagulation times and in the amounts of antithrombin following each hemorrhage in the two series of experiments.

TABLE 1

	CAT		RABBIT	
	Percentage decrease in coagulation time	Percentage decrease in antithrombin	Percentage decrease in coagulation time	Percentage decrease in antithrombin
	per cent	*per cent*	*per cent*	*per cent*
2d specimen	11.1	15.1	5.4	6.7
3d specimen	15.4	30.9	17.8	23.6
4th specimen	25.0	26.4	24.8	25.9
5th specimen	27.0	34.6	31.6	30.8
6th specimen	37.4	No specimen obtained	32.2	34.7

Table 2 gives a summary of the results obtained in our entire series of experiments.

DISCUSSION AND CONCLUSIONS

Coagulation Time. Our experiments, in accord with the observations of other investigators, show that rapid progressive hemorrhage causes a decrease in coagulation time. An occasional animal proves an exception to this rule, and shows practically no change in its rate of clotting no matter how severe the hemorrhage (e.g., Exps. II and XI in Table 2), but the majority of the animals show a gradual steady fall in the rate of coagulation as the hemorrhage proceeds.

Antithrombin. In our series of experiments, the decrease in coagulation time is accompanied by a decrease in the amount of antithrombin. Whether or not this change is the result of a simple dilution of the blood by an influx of fluid from the tissues, or whether it is due to a decrease in the amount of antithrombin formed, we are unable to say. In either case the fact of diminution remains. Gray and Lunt[12] have shown that there is no decrease in coagulation time after hemorrhage in an anterior animal (i.e. an animal in which the aorta and vena cava are ligated just above the diaphragm). This observation may be easily reconciled with our experiments. If the decrease in antithrombin

[12] Gray and Lunt: Loc. Cit. (1).

TABLE 2

Cat

Specimens	COAGULATION TIME 1	2	3	4	5	6	INTERVAL BETWEEN BLEEDINGS 1–2	2–3	3–4	4–5	5–6	ANTITHROMBIN 1	2	3	4	5	6	PROTHROMBIN 1	2	3	4	5	6	FIBRINOGEN 1	2	3	4
	min. sec.	min. sec.	min. sec.	min. sec.	min. sec.	min. sec.	min.	min.	min.	min.	min.	min.	min.	min.	min.	min.	min.	min.	min.	min.	min.	min.	min.	gm.	gm.	gm.	gm.
Exp. I	11 38	10 36½	9 45	7 44			14	30	19			7.56	6.00	5.50	5.00			2	2	4	4½			Determination			Lost
Exp. II	10 35½	10 11	10 0	9 ¾			16	15	15			Determination Lost						2½	2½	2	4½			0.4758	0.4016	0.3408	
Exp. III	19 0	12 27	14 54½	15 7			28	16	19			6.75	6.50	4.87	4.87			3½	3	3¼	5			0.0675	0.0652	0.0644	
Exp. IV.	14 0	13 6½	12 15				19	20				9.81	8.62	4.62				4½	3	2½				0.2137	0.1822	0.1350	
Exp. V	15 27½	11 16	11 31½	11 35			20	20	20			Determination Lost						2½	4½	3½	5			0.3611	0.3116	0.2430	
Exp. VI	15 16½	12 59½	10 47				20	29				4.50	3.68	3.62				5	3½	3½				0.2216	0.1049	0.0974	
Exp. VII	19 34½	20 44½	18 12½				23	23				4.12	3.31	3.18				3	5	8½				0.5342	0.5062	0.4134	
Exp. VIII	14 0	13 33	12 32	11 35	11 54½	9 1	20	21	20	20	18	5.43	4.00	4.12	3.81	3.93		4	3½	4½	5	3¾		0.0843	0.0843	0.0562	0.0481
Exp. IX	10 4	10 20	9 35½	9 49½	9 6		20	23	20	16		3.93	3.62	3.18	4.00			2½	1¾	2¾	3¼			0.2373	0.2666	0.2361	
Average in minutes	14.4	12.8	12.17	10.8	10.5	9.01	20	21.88	18.83	18	18	6.01	5.10	4.15	4.42	3.93		3.27	3.13	3.86	4.54	3.75		0.2744	0.2403	0.1982	0.0481

Rabbit

Exp. X	15 26	14 4½	13 2½	10 45½	10 31		21	21	20	13		40.18	35.81	28.80	28.68			3	3	5½	4¼			0.2925	0.2947	0.3093	
Exp. XI	15 5½	14 35	15 17				20	21				23.06	24.50	20.93				2¾	2½	3¾				0.3240	0.2812	0.2328	
Exp. XII	15 56	13 34	9 3	12 41	12 28	12 25	½	21½	½	21	½	41.50	39.68	32.43	33.56	30.12	25.56	5½	6½	8	6	6½	6	Determination not made			
Exp. XIII	12 16	11 54	8 8½	8 55	9 48	9 15	½	20½	½	20½	½	36.06	36.37	28.68	23.81	24.43	25.87	8½	6	5½	6½	5½	5½	Determination not made			
Exp. XIV	9 37	11 17	9 39	9 21			21	½	24			38.12	31.43	39.06	23.12			9½	8¾	4½	4¼			Determination not made			
Exp. XV	18 32	18 12½	15 55	15 33½			½	24½	½			54.18	54.62	38.00	41.81			6½	6	8¾	9½			0.3240	0.3240	0.2475	0.2475
Exp. XVI	25 55	22 38	19 53	17 30½			½	28½	1			38.62	35.06	16.75	16.87			4¾	5½	5½	4½			0.2621	0.2621	0.1968	0.1968
Exp. XVII	15 4	14 40	14 4	9 15			2	24½	1½			43.75	36.56	36.18	36.68			6½	6½	7¾	9½			0.5062	0.5062	0.3375	0.3375
Average in minutes	15.98	15.11	13.12	12.00	10.92	10.83	8.25	20.25	6.85	18.16	0.5	39.43	36.75	30.10	29.21	27.27	25.71	5.81	5.56	6.18	6.46	6.12	5.75	0.3417	0.3336	0.2647	0.2606

which we have observed is due to a decrease in the amount formed, we may assume that antithrombin is formed in one of the abdominal organs. In support of such an assumption there is considerable evidence to show that the liver is very active in antithrombin production.

If the decrease in antithrombin is a matter of dilution, we know that the major portion of the fluid entering the blood after hemorrhage comes from the abdominal region. This fluid which enters the blood after hemorrhage is, in our opinion, lymph, not tissue juice. If it could be shown that tissue juice—the fluid obtained from wounded tissues—entered the blood after hemorrhage, we could readily explain our diminished amount of antithrombin by assuming that a large portion of it was neutralized or fixed by the thromboplastin in the tissue juice. In this way the amount of free antithrombin in the blood would be diminished, and our tests for it would show this reduction. But lymph, on the contrary, is not rich in thromboplastin. Howell[13] has shown very recently that lymph contains prothrombin and antithrombin in the same concentration as blood plasma, but much less thromboplastin. There is, therefore, a relative excess of antithrombin in lymph which explains its long coagulation time. Addition of thromboplastic material (tissue extract or kephalin solutions) causes lymph to clot promptly and firmly. In view of these facts it would seem that an influx of lymph into the blood after hemorrhage would bring with it a relative excess of antithrombin, which would increase the coagulation time of the blood instead of decreasing it. It is perhaps more reasonable to suppose that the decrease in antithrombin which we have demonstrated is the result of diminished antithrombin production.

Prothrombin. The behavior of the prothrombin in our experiments varied in different animals: occasionally it increased steadily in amount, occasionally it decreased steadily, but in the majority of cases it first increased slightly and then decreased slightly. The only conclusion in regard to the prothrom-

[13] Howell, W. H.: American Journal of Physiology, 1914, xxxv, 483.

bin which we feel warranted in making is that the prothrombin changes in our series of experiments do not offer any explanation for the decrease in coagulation time which occurred.

Fibrinogen. The fibrinogen in our experiments, as estimated by the heat coagulation method, gave marked variations as to the amount present in different animals, a fact in accord with Whipple's observations,[14] but remarkably uniform results as to the effect of hemorrhage on the fibrinogen content of the blood of individual animals.

As time has passed we have felt more and more certain that this method of estimating fibrinogen is open to question. In Table 2, it may be noted that while one cat shows a normal content of 0.0675 gram of fibrinogen per 100 cc. of plasma, another cat shows a normal content of 0.5342 gram. These figures give the maximal variation, 0.4667 gram, which we found between two different animals. Whipple's maximal variation in dogs was 0.6686 gram per 100 cc. of plasma, but he never obtained in normal animals the very low figures of 0.0675 gram and 0.0843 gram which we encountered twice in this series of experiments, and which we have found a number of times in some experiments on rabbits not yet reported. None of these animals possessing an apparently very low fibrinogen content showed any bleeding tendencies but seemed normal and healthy in every way. We have taken every precaution to assure ourselves that there has been no technical error in the application of the method, and we are convinced that there is enough variation in the reactivity of fibrinogen to heat to render the heat coagulation method of determining this substance of somewhat questionable accuracy.

Our data in regard to fibrinogen is as follows. With the exception of two cases (Exps. IX and X) all of our determinations showed a steady decrease in the amount of fibrinogen following each hemorrhage. The average fall from the normal in all cases of specimens obtained at the third bleeding was 0.0766 gram per 100 cc. plasma.

[14] Whipple, G. H.: American Journal of Physiology, 1914, xxxiii, 50.

Dreyer[15] has shown that the fibrinogen content of the blood increases after hemorrhage provided the interval between bleedings is a period of twenty-four hours. It is possible that in our experiments the apparent reducing effect of rapid progressive hemorrhage upon the fibrinogen content of the blood, is in reality a false one. It may be that instead of an actual fall in the amount of the fibrinogen, there occurs some alteration in the fibrinogen itself which diminishes its responsiveness to the heat coagulation test. On the other hand we must remember that Nasse, Brücke, and von den Velden have all reported an immediate diminution in the fibrin content of the blood following severe hemorrhage, and at the same time an increase in the speed of coagulation.

There remains to be considered the question as to whether or not the results which we have obtained in our analyses of the factors of coagulation after hemorrhage are sufficient to explain the decrease in coagulation time which occurred, but this question unfortunately must remain unanswered. A decrease in antithrombin will of course favor more rapid clotting, but whether or not the decrease which we have shown is sufficient to compensate for a certain amount of diminution in prothrombin and for a possible diminution in fibrinogen also, we are unable to say. The fact that there was very little change in antithrombin in two of our experiments in which the coagulation time was practically constant seems suggestive in this connection.

We believe that the chief interest in these experiments lies in our demonstrations of a variation in antithrombin. Except for the experiments of Davis[16] showing the production of an antithrombin wave as the result of thrombin injections and the work of various investigators on the increase in antithrombin as the result of peptone injections, all previous work on the antithrombin in the blood has indicated that this substance is remarkably constant. Thrombin and peptone injections produce

[15] Dreyer, G. P.: Studies from the Biological Laboratory of the Johns Hopkins University, 1893, v, 77.

[16] Davis, D.: American Journal of Physiology, 1911, xxix, 160.

an increase in the amount of antithrombin. Our experiments are the first instance in which there has been reported a positive decrease in the antithrombin content of the blood.

SUMMARY

1. Rapid progressive hemorrhage causes a progressive decrease in coagulation time. An occasional animal proves an exception to this rule and shows no change in its rate of clotting, no matter how severe the hemorrhage.

2. Antithrombin decreases in amount when the coagulation time decreases and remains practically constant when the coagulation time is unchanged.

3. Prothrombin tends first to increase slightly in amount and then to decrease slightly.

4. Fibrinogen, estimated by the heat coagulation method, decreases as hemorrhage progresses.

5. Platelets counts do not vary with rapid progressive hemorrhage.

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