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Influence of Adrenalin on Fibrinogen.

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Vosburg and Richards[1] first noted that blood coagulated more rapidly in animals after administering adrenalin, and Cannon and his coworkers reasoned from their experiments with adrenalin and from other evidence that the liver furnished some factor to increase the clotting process of the blood. In a study to determine just what this factor was, Grabfield[2] concluded that adrenalin decreases the coagulation time by increasing the amount of prothrombin in the circulating blood.

Grabfield could find no change in the anti-thrombin or in the fibrin content of the blood following the administration of adrenalin, but he says: "The fibrin determinations were made by the heat coagulation method of Whipple and Hurwitz, an unsatisfactory and unreliable method for this work because, chiefly, the amounts of blood required for accurate results are too large to obtain from cats without introducing the factor of hemorrhage."

In reviewing the problem it seemed that fibrin instead of prothrombin may have been an active factor in initiating the decreased coagulation time and that Grabfield's work should be repeated, using larger animals. Cannon was able to show that the increase in blood sugar concentration after adrenalin could not alone decrease the coagulation time, and since it has been shown by Whipple and Foster[3] that fibrin is probably stored in the liver, it was decided to study particularly this substance.

Dogs were injected subcutaneously with 1:1,000 adrenalin chloride. Of the 6 animals used, 4 were healthy and 2 had chronic secondary anemia from hemorrhage. Coagulation time was determined by a modified method of Howell[4] using test tubes 12 mm. in diameter. Blood sugar determinations were made by the method of Folin[5] and fibrin by the method of Schultz, Nicholes, Schaefer.[6]

We chose arbitrarily to inject, subcutaneously, 1 and 2 cc.

[1] Vosburg and Richards, *Am. J. Physiol.*, 1903, **9**, 35.

[2] Grabfield, G. P., *Am. J. Physiol.*, 1916, **42**, 46.

[3] Foster and Whipple, *Am. J. Physiol.*, 1922, **58**, 365.

[4] Howell, *Arch. Int. Med.*,, 1914, **8**, 76.

[5] Folin, *J. Biol. Chem.*, 1929, **82**, 83.

[6] Schultz, Nicholes, Schaefer, *Am. J. Path.*, 1925, **1**, 101.

amounts of the adrenalin solution in dogs weighing approximately 12 kilos. This equals about 0.01 and 0.02 mg. per kilo respectively. The dogs were fasted 18 hours before the experiments were made. The blood was drawn directly into dry syringes from the iliac vessels by skin puncture in quantities not exceeding 25 cc.

The protocol shows in detail the time relations in each experiment together with the changes in fibrin and sugar content in the blood following the injection of adrenalin.

PROTOCOL.

Exp.	Time	Mg.	Fibrin per 100 cc.	% Increase of fibrin	Blood sugar mg. per 100 cc.	Increase	Coagulation time, min.
I	11:11		316.0		85		4
	11:17	adrenalin 1 cc.					
	11:32		407.6	91.6	121	29.0	2
II	10:09		363.9		76		6
	10:44		324.0		76		2
	10:55	adrenalin 1 cc.					
	11:10		482.5		85		1
	11:25		396.0	119.6	90	35.6	3
III	11:07		455.7		77		6
	11:09	adrenalin 1 cc.					
	11:19		541.7		121		5
	11:29		540.8	85.1	113	18.7	4
IV	9:00	(arterial)	354.0		62		6
	10:10		320.8		62		2½
	10:15	adrenalin 1 cc.					
	10:30		403.1		85		1½
	2:50	(highly excited)	421.6	100.8	108	31.4	2½
V	10:52		343.0		77		7
	10:55	adrenalin 1 cc.					
	11:10		398.0		133		3
	2:06		311.2	55.0	109	16.0	6
VI	Anemic dog, Hb 55%						
	1:46		242.4		80		3½
	1:47	adrenalin 2 cc.					
	1:57		290.5		87		3
	2:03.5		365.0		91		1
	2:11		346.5	122.0	91	50.5	1
VII	Anemic dog, Hb. 58%						
	1:56		368.6		83		2½
	1:57	adrenalin 2 cc.					
	2:09		439.2		86		2
	2:14		512.7		93		1½
	2:24		553.5	194.9	95	52.9	1½
VIII	Anemic dog, Hb. 60%						
	11:22	A. M.	306.7		101		7
	1:23.5	P. M.	303.9		97.6		7
	1:25	adrenalin 2 cc.					
	1:39		470.4		116		4
	1:56.5		478.4		150		2
	2:12.5	(arterial)	438.1	171.7	161	55.9	4

Unless indicated otherwise, venous blood was used.

The fasting fibrin levels averaged 334.3 mg. from 8 determinations, the average maximum increase after adrenalin was 36.3% when the blood was analyzed between 15 and 30 minutes following the injection.

The blood sugar determinations were made as a check upon the action of adrenalin, and are included in the protocol for this reason. There was no definite relationship between the rise in blood sugar and the decrease in coagulation time following the use of adrenalin.

The coagulation time decreased, averaging about 60%, and in each instance the decrease was definite. There was a consistent relation between the height of the fibrin values and the decrease in coagulation time, as can be seen from the protocols. We could detect no difference in results by the use of 1 or 2 cc. of adrenalin solution.

The blood fibrin increased at 10 minutes after the injection but the maximum values appeared somewhat later, and followed fairly consistently the rise in blood sugar.

In several experiments the animal was anesthetized with amytal, but this precaution to avoid the element of excitement did not affect the results. Except in one instance the dogs were accustomed to the procedure in the experiments reported.

The experiment was performed upon 2 normal human subjects using 0.6 cc. adrenalin subcutaneously. There was a marked increase in the blood fibrin. (Table I.)

TABLE I.
Showing response to adrenalin in 2 human subjects at end of 10 and 15 minutes.

	Time	Fibrin	Blood Sugar	Blood Pressure
Case A	8:25 A. M.	333.0	93.4	110/80
	8:26 Adrenalin 0.6 cc.			
	8:37	437.7	123.8	140/100
Case B	8:30 A. M.	227.0	93.5	130/80
	8:31 Adrenalin 0.6 cc.			
	8:38	261.0		180/90
	8:45	468.6	168.8	150/85

It was established in Cannon's experiments that when the abdominal viscera were excluded from the circulation, the clotting process was not hastened by the injection of adrenalin. Foster and Whipple[3] apparently have demonstrated that fibrin comes from the liver. However, it cannot be stated positively as they suspected that fibrin is made in the liver. Our experiments seem to demonstrate that fibrinogen at least is stored in the liver and that the action of adrenalin is to cause its quick mobilization into the blood stream.

Its action would be analogous in this respect to the increase of sugar in the blood stream following the administration of adrenalin.

After adrenalin injections the fibrin values remain high for several hours, and probably would have to be considered in interpreting nitrogen metabolism studies such as have been reported recently by Watkins and Smith.[7]

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Oxidation of Cobaltous Cysteine.

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Michaelis and Barron[1] and Michaelis and Yamaguchi[2] have shown that cobalt reacts with cysteine to form a product which has a high reduction intensity. They also showed that this reaction product can be oxidized with air, ferri-cyanide, and phenolindophenol to a brown cobaltic cysteine complex. We have found that cobaltous cysteine reacts in a different manner toward each one of these oxidants. Indigo disulfonate produces a quantitative conversion of cobaltous cysteine to the cobaltic cysteine complex. All other oxidants except those which contain a quinone group result in the formation of the cobaltic cysteine complex and cystine in different proportions.

Oxidation with ferricyanide produces a unique type of electrometric titration curve because all of the cobaltous cysteine is removed from solution by addition of a half of the total amount of oxidant. The last half of the curve represents oxidation of cysteine to cystine.

Cysteine reacts with quinone and with the quinone group of dibromophenol-indophenol with the formation of an addition product between the dye and the thiol group similar to the addition of an aromatic thiol group to quinone. One molecule of cysteine reacts with 1 molecule, that is, 2 equivalents of the dye. Ninety per cent of cobaltous cysteine is oxidized to the cobaltic cysteine complex with dibromophenolindophenol, and 10% of the cysteine combines with the dye. The amounts of cobaltic complex and cystine

[7] Watkins, O., and Smith, George Van S., *Am. J. Physiol.*, 1931, **96**, 28.

[1] Michaelis, L., and Barron, E. S. C., *J. Biol. Chem.*, 1929, **83**, 191.

[2] Michaelis, L., and Yamaguchi, S., *J. Biol. Chem.*, 1929, **83**, 367.