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Effects of Adrenoxyl on Blood Coagulation Mechanism and Vasomotor Response.

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(Introduced by J. A. Shannon.)

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Cannon and Mendenhall[1] have shown that adrenalin shortens the coagulation time of blood. Roskam, Derouaux and associates[2] studied the effect of small doses of adrenalin on the bleeding time and reported a hemostatic action following the administration of the drug.

Adrenoxyl is a relatively stable derivative of adrenalin* developed by Braconier and associates.[3] Adrenochrome, adrenoxime,[4,5] and adrenoxyl,[6] oxidative derivatives of adrenalin, are reported as being capable of reducing the bleeding time approximately 25%. The adrenoxyl effect is demonstrable 15 minutes after intravenous injection in rabbits and 30 minutes after injection in man. The optimal doses are 0.001 mg in the rabbit and 0.1 to 0.5 mg in man. Derouaux[6] and Bacq[7] report that the circulation in rats and rabbits remains unaltered by the above doses of Adrenoxyl. These investigators emphasize lack of any sympathomimetic effect as reflected by the absence of change in the blood pressure and pulse rate in animals following injection of the drug. Roskam and associates believe that Adrenoxyl has a vasomotor effect which is responsible for the rapid occlusion of injured vessels. On the other hand, Desfontaines[8] has found a vasodilator effect of adrenochrome in man and recommends it for the treatment of hypertension.

As a preliminary study of the pharmacological properties of Adrenoxyl in relation to the bleeding time, we followed the administration of the drug with these screening procedures: Clotting time; prothrombin time; pulse wave velocity; skin temperature; and bleeding time determination. Such procedures would yield information concerning alterations in coagulability of blood and the vasomotor response of the injured vessels.

Methods and Results. Adrenoxyl prepared by Labaz Laboratories in ampules containing 0.1 and 0.5 mg in solution, was used in this investigation.† The prothrombin time and bleeding time determinations were performed on rabbits of both sexes. All other studies were made on healthy men between 17 and 39 years of age.

1. *Clotting time* (Table I). Blood samples were taken from the arm veins of 6 seated individuals and drawn directly into capillary tubes by a method devised by Pichotka and Reichel.[9] The method consists of drawing blood from a vein by a 22 gauge needle connected to a capillary tube by a paraffin joint. The method is designed to limit the possible errors caused by surface and tissue factors. The capillaries were broken every 30 seconds. Three to 5 determinations were considered to be sufficient to give mean values under conditions of

* The monosemicarbazone of adrenochrome.

1 Cannon, W. B., and Mendenhall, W. L., *Am. J. Physiol.*, 1914, **34**, 225.

2 Derouaux, G., and Roskam, J. Physiol. Soc., June 5, 1937, through *J. Physiol.*, 90: Proc., 65, 1937.

3 Braconier, F., LeBihan, H., and Beaudet, C., *Arch. Internat. de pharmacodyn. et therap.*, 1943, **69**, 181.

4 Green, D. E., and Richter, D., *Biochem. J.*, 1937, **31**, 597.

5 Derouaux, G., *Arch. Internat. de pharmacodyn. et therap.*, 1941, **66**, 202.

6 Derouaux, G., *Arch. Internat. de pharmacodyn. et therap.*, 1943, **69**, 142.

7 Bacq, Z. M., *Presse med.*, 1947, **16**, 175.

8 Desfontaines, G., *Ann. biol. clin. Par.*, 1948, **6**, 138.

9 Pichotka, J., and Reichel, H., unpublished data.

† Supplied by Squibb Institute for Medical Research, New Brunswick, N. J.

TABLE I.
Effect of Adrenoxyl on Clotting Time.

	Control							
Case	No. of determinations	Avg value (sec.)	Possible deviation (sec.)	Dose (mg)	Time after injection (min.)	No .of determinations	Avg value (sec.)	Possible deviation (sec.)
1	3	330	±30	.10	10-50	5	362	±52
2	3	470	±60	.45	10-45	4	420	±30
3	4	405	±45	.50	15-20	4	435	±75
4	3	370	±30	.45	15-20	4	367	±37
5	5	450	±60	.25	10-40	4	510	±45
Avg		405	±45				419	±45

TABLE II.
Effect of Adrenoxyl on Prothrombin Time.

Rabbit No.	Normal Prothrombin time (sec)	Dose	Post Adrenoxyl time (min.)	Prothrombin time (sec.)	Change
2	18 18 18 19 19	1	20	18 19	0
4	19 17 19 18 19	1.5	40	20 24 23 20	Increase, slight
8	17 17 17 17 16	3	45	clotted	- - - -
4	18 16 18 17	3	30	19 17 17 18 16	0
8	19 18 19 18 19	1	30	19 17 19 19 19	0
5	19 18	0.5	30	19 19	0

study. After the control measurements Adrenoxyl in a dosage of 0.10 to 0.50 mg was injected intravenously, and the clotting time of four to five separate blood samples was measured at intervals varying between 10 to 50 minutes later.

The clotting time had an average standard deviation of 4.4% in each subject in the control, and a mean standard deviation from the corresponding normal values of 4.8%. The individual differences between the pre- and post-Adrenoxyl findings fall within the range of physiological deviation.

2. *Prothrombin time* (Table II). Determinations were made on 5 rabbits before and after Adrenoxyl administration on blood samples obtained by intracardiac tap at stated intervals. The samples were collected in dry syringes and transferred immediately into test tubes containing 0.1 molar sodium oxalate. Prothrombin time was determined by the Quick method.[10] The applied dosage of Adrenoxyl varied between 0.0005 and 0.003 mg. No essential change was noted in the prothrombin time following Adrenoxyl injection.

3. *Pulse wave velocity* (Table III). Determinations were carried out by the photographic method of Frank[11] and Wexler.[12] Rubber membrane capsules and rubber tubes were used for air transmission of the impulses. The mirrors on the membranes were illuminated by a light beam from a projector, provided with a 3-slit lantern slide. The pulse curves of the carotid, femoral, and radial arteries were recorded by means of a photo-electric highspeed kymograph. The pulse wave velocity of the aorta (CA) and of the brachial (CB) artery was computed from the difference between the rise of the carotid, the femoral, and the radial curves. From these values the quotient, CB/CA, was calculated. Pulse rate and the blood pressure were also measured. Control determinations were followed by intravenous injections of 0.15 to 0.50 mg Adrenoxyl. The pulse rec-

[10] Quick, A. J., *Amer. Journal Clin. Path.*, 1940, **10**, 222.

[11] Frank, O., *Z. f. Biol.*, 1903, **44**, 445; 1905, **46**, 441; 1929, **89**, 289.

[12] Wezler, K., *Z. f. Biol.*, 1935, **96**, 261; *Z. f. Breislaufforsch.*, 1935, **27**, 721.

TABLE III.
Effect of Adrenoxyl on Circulation.

Case	Dose	Time of determinations (min after inj.)	PR—Beats per minute	BP (mm Hg)	CA (m/sec.)*	CB (m/sec.)*	CB/CA
6	.15	Resting	74	120/70	6.37	7.45	1.17
		20	80	125/70	6.75	8.10	1.20
7	.20	Resting	60	100/65	4.05	4.95	1.22
		10	60	115/70	4.12	5.03	1.22
8	.20	Resting	81	120/75	5.55	6.42	1.16
		10	70	120/70	5.18	5.77	1.11
9	.10	Resting	69	110/70	5.90	7.97	1.35
		60	67	110/70	5.74	7.80	1.36
10	.10	Resting	51	115/70	6.27	6.41	1.02
		50	55	115/70	6.25	6.49	1.04
11	.50	Resting	52	110/70	6.63	9.54	1.44
		15	54	110/70	6.26	9.14	1.46
12	.50	Resting	45	110/70	5.50	7.62	1.38
		15	43	115/70	5.78	7.68	1.33
13	.50	Resting	66	120/75	6.80	9.04	1.31
		15	67	120/75	7.04	9.12	1.30

* Meters per second.
PR—Pulse rate.
BP—Blood pressure.
CA—Pulse wave velocity in aorta.
CB—Pulse wave velocity in brachial artery.

TABLE IV.
Effect of Adrenoxyl on Skin Temperature.

Case	Dose (mg)	Time after injection (min.)	Mean skin temp. (°C)		Room temp. (°C)
			Normal	Adrenoxyl	
12	.5	40	31.85	31.44	28.7
14	.5	25	31.11	31.01	26.9
15	.5	30	31.60	31.73	27.7
16	.5	40	30.80	30.70	27.9

ords were repeated immediately and 10-50 minutes later. During the whole procedure the subjects were maintained in the supine position. Following the injection of Adrenoxyl, deviations of the pulse wave velocity were negligible. Maximal change of the quotient, CB/CA, did not exceed 35%, which falls within physiological variations.

4. *Skin temperatures* (Table IV). Readings were taken from undressed recumbent subjects by means of a Dermalor instrument having a standard deviation of ± 0.2° C. The control readings were performed 30, 40, and 50 minutes after the undressing, in a room temperature of 27° to 29° C. The skin temperature measurements were repeated 20, 30, and 40 minutes after an intravenous injection of 0.5 mg Adrenoxyl. The mean value was determined for each selected body site (forehead, ears, nose, chin, shoulders, forearm, hand, fingers, umbilicus, knee, mid-leg, feet, and toes) on the basis of 3 "control" and 3 "test" measurements. Skin temperature determinations remained essentially unchanged following administration of Adrenoxyl.

5. *Bleeding Time.* Determinations were made on 12 rabbits anesthetized by sodium amytal, intravenously administered. Small, uniform, through and through incisions were made along the caudal margin and tip of the ears. The incised wounds were placed under a uniform saline drip. The time was recorded between the moment of incision and the moment of cessation of bleeding. The error of cutting a large vessel was avoided by transillumination. After a satisfactory control time was established, Adrenoxyl (0.001 to 0.0025 mg) was injected intravenously and periodic bleeding time determinations were

made over the ensuing 60 minute period. No significant reduction in bleeding time was noted following the injection of Adrenoxyl.

Discussion. Insofar as venous clotting time and prothrombin time reflect the speed of fibrin production, Adrenoxyl does not foster an accelerated process under the stated experimental conditions. It is, however, conceivable that tissue thromboplastic activity can be altered by Adrenoxyl and that the alteration is not evident where venous or heart sampling reduces the amount of tissue thromboplastin to the minimum.

After administration of Adrenoxyl, observations of the pulse rate, the blood pressure, the arterial wall tonus (as reflected by the pulse wave velocity), and the vaso-motor response of the superficial vessels (as evaluated by skin temperature determinations), do not reveal data which would suggest alteration in the vasomotor response that would not fall within normal physiological limits.

Bleeding time experiments are inconclusive since the experimental error of the technique used is of such great magnitude. We were unable to isolate real evidence that Adrenoxyl reduces the bleeding time under the stated experimental conditions. It is interesting to us to note that when an animal under light anesthesia responds to the stimulus of an incision on the ear, the bleeding time is reduced. This finding raises the question of advisability of collecting bleeding time data on unanesthetized animals.

Conclusions. 1. Under the stated experimental conditions Adrenoxyl does not influence (a) the speed of the coagulation process, and, (b) the vasomotor response.

2. The method of bleeding time determination in the experimental animal in our hands does not give conclusive results, therefore we are unable to confirm or deny the findings of Roskam and associates.

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Evaluation of Certain Dangers in the Use of Jet Injection Technic.

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As a substitute for the hypodermic syringe, a jet injector which utilizes the principles of the Diesel fuel injector has been described by Hingson and Hughes.[1] In the administration of substances by the hypodermic syringe, the possibility of an accidental intravenous injection is recognized. The present study was designed to investigate the possibility of a similar accidental intravenous injection with the "Hypospray."

Experimental Work. Dogs anesthetized with 30 mg per kg of sodium pentobarbital intravenously were given atropine sulfate by vein until electrical stimulation of the cut right vagus nerve failed to slow the heart. Both jugular veins were exposed by a medial approach so they could be seen by eversion of the edges of the incision. Blood pressure was continuously recorded by a mercury manometer connected to one carotid artery. Known doses of epinephrine were injected by syringe into the right jugular vein.

A "Hypospray"* with a 125 pound spring was loaded with a cartridge† containing 0.25 cc of a 0.4% solution of epinephrine hydrochloride. By eversion of the edge of the incision the left jugular vein was exposed to view. The tip of the cartridge was then pressed firmly against the skin on outside of the shaved neck and so aligned that the vein lay directly between the cartridge tip and the

[1] Hingson, R. A., and Hughes, J. G., *Anesth. and Analg.*, 1947, **26**, 221.

* Courtesy of the R. P. Scherer Corp., Detroit.

† Courtesy of the Gelatin Products Corp., Detroit.