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Modus Operandi of Commercial Heparin.

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The full explanation of the clot-inhibiting action of heparin (Howell and Holt's[1] anticoagulant liver extract) has awaited the reconciliation of the conflicting results of various investigators. The present experiments were designed to assist in the search for the experimental variables involved. Three technical points received special attention: (1) adequate controls[2]; (2) the time factor in certain reactions; (3) the effective concentration zones of the chief reagents as measured by a dilution method.

0.5 cc. of a moderately weak thrombin mixture [prothrombin soln. (10) + 0.1% cephalin (1) + N/10 $CaCl_2$ (1)] gave solid clots in 1.0 cc. of prothrombin-free dog fibrinogen in 15-60 sec.[2] Successive dilutions of a 1% stock solution of commercial (H. W. & D.'s) heparin were used in the following tests (all performed at 38°C.).

A. A true *antithrombic action* could be demonstrated by adding 1-3 mg. heparin (1:1000 soln.) per one cc. of 'ripe' (*i. e.*, maximally activated) thrombin and subsequently testing its clotting power. As long as the reagents were added simultaneously it was immaterial whether the heparin was added to the thrombin or to the fibrinogen first. In other words the inhibition was 'immediate'. Nevertheless, if the heparin was incubated with the thrombin before the clotting test, the neutralization was definitely enhanced. The rapid and solid clots with control thrombins substantiate Howell's (Cekada[3]) claim that "antithrombin is absent from the solutions of prothrombin prepared by the acetone method." There is nothing to suggest that this antithrombic action of heparin is other than direct. A flocculation of fibrinogen by the heparin proved troublesome in some of the tests but it was found possible to prevent this by controlled alkalinization with N/10 KOH.

B. 0.2-0.1 mg. heparin per 1 cc. of thrombic mixture (proved by control tests *(A.)* to lack enough direct antithrombic action to prevent the formation of good clots) were able completely to inhibit

[1] Howell, W. H., and Holt, E., *Am. J. Physiol.*, 1918, **47**, 328.

[2] Ferguson, J. H., *Am. J. Physiol.*, 1936, **117**, 587.

[3] Cekada, E. B., *Am. J. Physiol.*, 1926, **78**, 512.

the elaboration of thrombin from the prothrombin, cephalin, calcium mixture. Whereas the control 'activation curve' (*sine* heparin) reached a steady level (maximal activation) in some 5-10 min., the series containing the weak heparin solutions were not active even after 60-90 min. This is the classical *antiprothrombic* action of Howell.[1]

C. Thrombic mixtures (v. supra) were incubated with varying dilutions of heparin until the minimal effective zone for the antiprothrombic effect was reached. The test was then repeated with stronger cephalin (1%) added to the mixture. The diminution of the antiprothrombic action of heparin as the result of the extra *cephalin* added is clearly seen in Table I.

TABLE I.

A. Composition of Thrombic Mixtures (incubated at 38°C.).

	Prothrombin cc.	Dist. Water cc.	Cephalin cc. %	Heparin cc.	N/10 $CaCl_2$ cc.
I	5	1.0	.5 (0.1)	—	.5
II	5	—	.5 (0.1)	1.0 (1:1000)	.5
III	5	0.5	.5 (0.1)	0.5 (1:3000)	.5
IV	5	0.5	.5 (0.1)	0.5 (1:1000)	.5
V	5	—	.5 (0.1) .5 (1.0)	0.5 (1:1000)	.5

B. Clotting-times for Prothrombin-free Fibrinogen (38°C.).

Age of mixture		15 sec.	1 min.	5 min.	10 min.	20 min.	30 min.	60 min.
Mixture	I	15¾ min.	6¾ min.	45 sec.	25 sec.	20 sec.	25 sec.	45 sec.
"	II	∞	∞	∞	∞	∞	∞	∞
"	III	480 sec.	490 sec.	145 sec.	95 sec.	40 sec.	45 sec.	45 sec.
"	IV	Overnight	Overnight	—	55½ min.	35 min.	25 min.	5 min.
"	V	4 hr.	4 hr.	—	75 sec.	40 sec.	40 sec.	40 sec.

D. Regarding the ability of cephalin to neutralize the direct antithrombic action of our heparin solutions, the results were less conclusive. If clotting was completely prevented, a moderate excess of cephalin over heparin (dry weights) did not appear to affect the result. However, in all cases in which the heparin merely delayed coagulation, the tubes containing extra cephalin showed quicker and better clots. Controls proved that no increase in the potency of the thrombin itself resulted from the additional cephalin.

These preliminary data are offered with no intention of relying on them for any theory as to the rôle of heparin in blood coagulation. They merely serve to indicate the type of tests to which any prepared anticoagulant should be subjected. It is highly probable that inconsistencies in the experimental literature are chiefly the result of working with quantities of the respective reagents without regard to the zone of minimal effective amounts. A large excess of

potent thrombin might readily mask any antithrombic action. The phospholipid antagonisms, in our experience, are difficult to demonstrate except with limiting dilutions of heparin. The dilution method permits experimentation in the zone of minimal quantities of reagents which we are still unable to isolate and work with in pure chemical form.

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Reflex Inhibition of the Human Heart: Complete A-V Block and Parasystole.

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Certain phenomena observed in the electrocardiogram have forced the question: May reflex augmentation of inhibitory tone during the systolic rise in arterial pressure produce effects which wax and wane within the time of a single cycle, or are the reflex effects smoothed out so that little change in the degree of inhibition occurs within a single cycle?

The experimental results of Brown and Eccles[1] suggest that the first alternative may be the true one. In their experiments, however, one (or 2) stimuli were applied to the vagus nerve and time was then allowed for full recovery from inhibition before another stimulus was given. If a stimulus be applied every 2 cycles, or less, conditions as they sometimes obtain in the human heart are more closely approximated. A curve illustrating the time course of vagus slowing is shown in Fig. 1. Make-break shocks were applied about every 650σ to the peripheral end of the cut right vagus of a dog under urethane anesthesia. The other vagus was also cut. Electrocardiograms were taken. The uninhibited cycle was 350σ. Several experiments were carried out with similar results.

The curve shown rests upon a background of inhibition resulting from the frequency of nerve stimulation. The zero point on the ordinates is an arbitrary point of reference. The latency of inhibitory effect is 170σ; the ascending limb of the curve is about 300σ.

In several experiments the rate of vagus stimulation was only slightly slower than the inhibited sinus rate. Under these condi-

[1] Brown, G. L., and Eccles, J. C., *J. Physiol.*, 1934, **82**, 211, 242.