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Heparin and Plasma Albumin in Relation to Thromboplastic Action of Trypsin, Cephalin and Brain Extracts.

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Evidence has recently been presented for the existence of a *thromboplastic enzyme* factor in blood clotting.[1] Its rôle is to mobilize cephalin and calcium for thrombin formation.[2] With recently standardized technical procedures,[3] observations have been extended to include the known normal antithrombic factors.

The degree to which different amounts of heparin can inhibit clotting of citrated dog plasma by crystalline trypsin, under varying conditions of calcium and cephalin mobilization, is evident in the controlled experiments of Table I.

Sufficient heparin can greatly delay coagulation even under conditions most favorable to the action of trypsin, namely, abundance of added cephalin and calcium. Nevertheless, clotting eventually occurs in all tubes. The heparin inhibition varies inversely with the clot-aiding efficacy of the thromboplastic mixture.

Table II shows the varying extent of "antiprothrombic" action of heparin during the formation of thrombin from recalcified prothrombin in the presence of (1) aqueous brain extract,* (2) its equivalent of isolated phospholipids, (3) a comparable solution of cephalin, (4) crystalline trypsin.

The heparin is able completely to prevent thrombin formation in the presence of cephalin and (practically) in the case of the slightly more potent solution of isolated P-lipids. With whole brain extract there is delayed activation (confirmed) only evident in the first minute of incubation. Thereafter, the thrombin formed in the presence of heparin is as potent as in the control. Trypsin behaves very similarly to the crude thromboplastin, namely, evincing some delay in the early minutes of prothrombin activation. The occurrence of a minor degree of the same phenomenon in the control (*sans* heparin)

1 Ferguson, J. H., and Erickson, B. N., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 625.

2 Ferguson, J. H., and Erickson, B. N., *Am. J. Physiol.*, 1939, **126**, 661.

3 Ferguson, J. H., *J. Lab. and Clin. Med.*, 1938, **24**, 273.

* Thromboplastin prepared from a dry brain preparation kindly supplied by Dr. A. J. Quick. Solution analysed: Total P-lipid = 11 mg. incl. Cephalin = 4.6 mg; Protein = 104 mg %.

TABLE I.

Effects of Varying Concentrations of Heparin upon Clotting of Citrated Dog Plasma by Crystalline Trypsin, Cephalin, and Calcium.

	Trypsin (mg in 0.25 cc)	Dist. water (cc)	Cephalin (cc of 1:1000)	N/10 $CaCl_2$ (cc)	Heparin* (mg in 0.25 cc)	Cit. Plasma (cc)	Clotting-time (38°C)
1.	—	.75	.25	—	—	1	No clot
2.	—	.75	—	.25	—	1	325 sec
3.	—	.5	.25	.25	—	1	100 "
4.	—	.5	—	.25	0.05	1	1340 "
5.	—	.5	—	.25	0.1	1	No clot
6.	—	.5	—	.25	1.0	1	" "
7.	—	.25	.25	.25	0.05	1	240 sec
8.	—	.25	.25	.25	0.1	1	375 "
9.	—	.25	.25	.25	1.0	1	No clot
10.	.25	.75	—	—	—	1	>2<7 hr
11.	.25	.5	.25	—	—	1	90 sec
12.	.25	.5	—	.25	—	1	58 "
13.	.25	.25	.25	.25	—	1	22 "
14.	.25	.5	—	—	0.05	1	>2<7 hr
15.	.25	.5	—	—	0.1	1	>2<7 "
16.	.25	.5	—	—	1.0	1	>7<15 "
17.	.25	.25	.25	—	0.05	1	>2<7 "
18.	.25	.25	.25	—	0.1	1	>2<7 "
19.	.25	.25	.25	—	1.0	1	>7<15 "
20.	.25	.25	—	.25	0.05	1	135 sec
21.	.25	.25	—	.25	0.1	1	2 hr
22.	.25	.25	—	.25	1.0	1	>7<15 "
23.	.25	—	.25	.25	0.05	1	29 sec
24.	.25	—	.25	.25	0.1	1	45 "
25.	.25	—	.25	.25	1.0	1	>2<7 hr

*Ordinary commercial heparin (H., W. and D.'s.)

TABLE II.

Effects of Heparin on the Formation of Thrombin from Recalcified Prothrombin in the Presence of Various Thromboplastic Agents.

Thrombic mixture (T) = 4 cc prothrombin soln. + 1 cc activator(s) *plus* inhibitor (suitably diluted), incubated at 15°C. All mixtures include $CaCl_2$ (0.05 m.eq. Ca per 5 cc T). Cephalin, trypsin, and heparin : mg per 5 cc T. Clotting-times (sec) at 38°C : 1 cc fibrinogen (prothrombin-free) + 0.5 cc T.

			Thrombin formation period, *minutes*, at 15°C)					
T	Thromboplastic agent	Inhibitor	1′	5′	10′	20′	30′	60′
1.	†Cephalin (0.1)	—	—	50″	20″	11″	9″	9″
2.	″ ″	Heparin (0.5)	—	∞	∞	∞	∞	∞
3.	‡Brain P-lipids (0.025)	—	50″	20″	10″	8″	6″	6″
4.	″ ″ ″	Heparin (0.5)	∞	∞	∞	∞	*1800″	*360″
5.	Thromboplastin (0.25 cc) incl. 0.025 mg P-lipid	—	35″	16″	9″	7″	7″	7″
6.	″ ″ ″ ″ ″ ″ ″	Heparin (0.5)	*720″	16″	10″	7″	7″	7″
7.	Trypsin (0.125)	—	∞	30″	15″	12″	11″	8″
8.	″ ″	Heparin (0.5)	∞	97″	32″	13″	11″	9″
9.	″ (0.05)	—	∞	95″	30″	15″	14″	11″
10.	″ ″	Heparin (0.5)	∞	∞	660″	70″	32″	18″

*Indicates incomplete coagulation.

†The cephalin was an old preparation which appears to have lost somewhat more than three-fourths of its original potency.

‡Quick's ″thromboplastin″ extracted with alcohol-ether (3:1) ; acetone-insoluble P-lipids recovered in ether-petroleum ether, dried, and prepared in aqueous solution (1:10,000).

may be evidence for an enzyme inhibitor in the prothrombin preparation.

Fresh tissue extracts have a much greater thromboplastic potency than can be accounted for on the basis of their phospholipid content. There is some evidence for this even in the present experiment in which the aqueous extract has been kept for 3 days in the ice-box, pending analyses.

These data afford additional evidence that the close analogy of thromboplastin and trypsin extends to their behavior in the presence of heparin. The antiprothrombic action of heparin is so brief in these cases, as compared with tests using isolated P-lipids, that it is clear how Quick[4] and Brinkhous, Smith, *et al.*,[5] missed the classical effect.[6]

Antithrombic actions of heparin and albumin. Table III confirms Quick's[7] finding of the synergism of the otherwise-negligible "antithrombic" actions of heparin and plasma *albumin* (the dialysed residue after $(NH_4)_2SO_4$ pptn. of plasma globulins), with one important exception. There is no evidence of any *progressive* inactivation of thrombin, although tests were made on thrombic mixtures of the 4 types shown in Table II and with the addition of (1) albumin, (2) heparin, and (3) albumin plus heparin. The phenomena of Table III, significantly, were reproducible with *each type* of thrombic mixture.

Conclusions. There is no valid reason to suppose that the fundamental process of thrombin formation differs as between the phospholipid and the enzymic modes of activation.[2] The fact of the

TABLE III.

Synergism Between Antithrombic Actions of Heparin and Plasma Albumin. Thrombin = 4 cc prothrombin soln. + 0.5 cc cephalin (1:500) + 0.5 cc $CaCl_2$ (N/10), aged for over 2 hours but stabilized by low temperature (10°C).

	Thrombin (cc)	Dist. Water (cc)	Heparin (mg in 0.25 cc)	Albumin (cc)	Fibrinogen* (cc)	Clotting-time (38°C)
1.	.5	.75	—	—	1	15 sec
2.	.5	.5	.05	—	1	20 "
3.	.5	.25	—	.5	1	37 "
4.	.5	—	.05	.5	1	25 *min.*
5.*	.5	—	.05	.5	1	24 "

*Fibrinogen added exactly 1 minute after last anticoagulant, except in 5 where the heparin, albumin and thrombin were allowed to interact for 10 minutes.

[4] Quick, A. J., *Am. J. Physiol.*, 1936, **115**, 317.

[5] Brinkhous, K. M., Smith, H. P., Warner, E. D., and Seegers, W. H., *Am. J. Physiol.*, 1939, **125**, 683.

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

[7] Quick, A. J., *Am. J. Physiol.*, 1938, **123**, 712.

significant quantitative differences in the cited tests shows that the so-called "antiprothrombic" effect of heparin is not to be interpreted as an alteration of the prothrombin itself, but rather as a manifestation of antagonisms between the heparin and the thromboplastic factors. There is an experimental difference between the simple addition of cephalin and its enzymic mobilization from protein-phospholipid combinations.

The results of all clotting experiments must be evaluated with reference to (1) quantitative relationships between the various factors, (2) the question of cephalin mobilization, and (3) time factors involved in thrombin formation, the thrombin-fibrinogen interaction, and in the overcoming of the inhibitory mechanisms. It is significant that thromboplastic enzyme has demonstrable powers of overcoming the "antiprothrombic" inhibitory mechanisms. The "antithrombic" action of heparin-albumin mixtures is immediately evident and is not dependent upon the type of thromboplastic agent used in the preparation of the thrombin.

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The Sugar Tolerance of Alcoholic Patients.

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In previous publications from these laboratories[1, 2] we have reported studies on the effect of glucose on the oxidation of alcohol. We found that the oxidation of alcohol *in vitro* was accelerated when glucose was simultaneously oxidized.[1] Studies on the effect of glucose on the oxidation of alcohol in man revealed that there was a moderate increase after glucose administration, and a more marked increase after glucose and insulin.[2] It is well known that alcoholic patients eat very little while on a drinking debauch, and it was suggested that the difference between the effect of the glucose and the glucose plus insulin might be due to an impaired ability of the patients to oxidize the added glucose without insulin because of their previous low carbohydrate intake.

1 Goldfarb, W., and Bowman, K. M., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 471.

2 Goldfarb, W., Bowman, K. M., and Parker, S., *Am. J. Physiol.* (Proc.), 1939.