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[1] Baker, F. N., VanDemark, N. L., Salisbury, G. W., and VanTienhoven, A., *J. Animal Sci.*, **11**, 783 (1952). Salisbury, G. W., and Kinney, jun., W. C. (unpublished results, 1953). Kinney, jun., W. C., M.S. thesis, University of Illinois (1954).

[2] VanDemark, N. L., and Sharma, U. D., *J. Dairy Sci.*, **40**, 438 (1957).

Phenols as Inhibitors of the Heparin Co-factor of Plasma

PHENOLS counteract the antithrombic activity of plasma and serum[1]. The antithrombic activity is present in a protein fraction which also contains the co-factor required for the anticoagulant effect of heparin[2]. The antithrombic and the heparin co-factor activity are present in the α-globulin fraction of human plasma[3]. It was therefore of interest to test the influence of phenols on heparin activity in plasma.

The results indicate that the phenols tested partly counteracted the heparin effect in human oxalated plasma. On a molar basis *p*-cresol is more active than the other compounds ; a similar behaviour was noted with phenols in the counteraction of antithrombic activity.

Phenols accelerate the thrombic fibrinogen reaction[4] by a factor of approximately 2. The observed shortening of the thrombin clotting time by phenols in the presence of heparin is far greater, therefore it cannot be due solely to this effect. The phenomenon could be due either to destruction of heparin or to a partial inactivation of the heparin co-factor. Heparin is not destroyed by phenol since phenol can be used for the quantitative isolation of heparin from plasma[5]. Further evidence that pyrocatechol does not inactivate heparin itself was obtained from experiments in which twice as much heparin was added to oxalated human plasma under conditions similar to those given in Table 1, both in the presence and absence of 0·36 *M* pyrocatechol. The recovery of heparin was approximately 80 per cent in both cases.

It is therefore most likely that the phenols have the property of inhibiting the co-factor of heparin activity. The physiologically occurring phenols—adrenaline, noradrenaline, tyrosine, natural œstrogens and synthetic œstrogens—in saturated solutions in the buffer system used had no effect on heparin activity.

Table 1. INFLUENCE OF PHENOLS ON HEPARIN ACTIVITY
Reaction mixture consisted of 0·2 ml. plasma, 0·05 ml. veronal buffer, *p*H 7·1, containing the phenols and 0·05 ml. veronal buffer, *p*H 7·1, containing heparin. Incubation for 1 min. at 37° C. Addition of 0·1 ml. human thrombin dissolved in veronal buffer, *p*H 7·1

Molarity of compound		Heparin (int. units)	Thrombin clotting time (sec.)
Phenol	0·36	0·5	12
Phenol	0·22	0·5	19
p-Cresol	0·15	0·5	11–12
p-Cresol	0·09	0·5	18·5
Pyrocatechol	0·36	0·5	11–12
Pyrocatechol	0·22	0·5	20
0		0	7
Phenol	0·36	0	5
p-Cresol	0·15	0	5
Pyrocatechol	0·36	0	5
0		0·5	>300

Heparin antagonists have been described to occur in crude preparations of adrenocorticotrophic hormone[6]. However, the mechanism was not established. It should be pointed out that for the preparation of corticotrophin solution, phenol is used often and this, of course, would affect heparin activity in plasma.

There are two classes of heparin antagonists: basic substances which bind heparin, and thus annul the heparin effect, and the phenols which counteract the heparin co-factor.

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[1] Fantl, P., *Biochem. J.*, **57**, 416 (1954).

[2] Howell, W. H., and Holt, E., *Amer. J. Physiol.*, **47**, 328 (1918).

[3] Lyttleton, J. W., *Biochem. J.*, **58**, 15 (1954).

[4] Gerber, C. F., and Blanchard, E. W., *Amer. J. Physiol.*, **44**, 447 (1945).

[5] Homan, T. D. H., and Lens, F., *Biochim. Biophys. Acta*, **2**, 333, (1948). Monkhouse, F. C., and Jaques, L. B., *J. Lab. Clin. Med.*, **36**, 782 (1950).

[6] Weissbecker, L., and Hitzelbieger, A., *Klin. Wochenschr.*, **31**, 288 (1953). Köhler, V., *Naturwiss.*, **42**, 99 (1955).

A Hitherto Unknown Blood Clotting Defect in Hæmophilia and Christmas Disease

MANY consider that the blood clotting defect in hæmophilia and Christmas disease is due to a deficiency of one of the blood clotting factors, namely, antihæmophilic globulin[1] or Christmas factor (plasma thromboplastin component)[2]. Others believe that the anomaly is produced by a derangement in the balance between a lipid inhibitor and a platelet plasma co-factor[3].

While investigating the specificity of different forms of plasma thromboplastin[4], we noticed that incomplete plasma thromboplastin—that is, thromboplastin formed in the absence of antihæmophilic globulin or Christmas factor—clotted normal substrate (plasma) in a shorter time than hæmophilic or Christmas disease (Table 1). This difference in the times of clotting was not materially affected on the addition of antihæmophilic globulin or normal serum (Christmas factor) to the substrates, respectively. This indicated that it was due to a

Table 1. EFFECT OF INCOMPLETE PLASMA THROMBOPLASTIN ON NORMAL AND PATIENTS' SUBSTRATES

Reagents incubated with 0·025 *M* calcium chloride (equal volumes)	Clotting time of substrate (sec.)*	
	Normal plasma	Patients' plasma
Hæmophilic plasma adsorbed by aluminium hydroxide (1 : 5) Normal serum (1 : 10) Platelet suspension	40	59
Normal plasma adsorbed by aluminium hydroxide (1 : 5) Christmas disease serum (1 : 10) Platelet suspension	32	47

* The activity of the incomplete plasma thromboplastin formed in the incubation mixture was tested after 6 min., by taking 0·1 ml. thereof together with 0·1 ml. 0·025 *M* calcium chloride, adding them simultaneously to 0·1 ml. substrate at 37° C., and recording the clotting time.

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