Table 1

Final venom concentration in plasma (μg/ml.)	Residual Factor VIII activity after incubation for: 1 h	2 h	4 h
0·5	150	120	—
1	135	100	75
2	115	78	70
4	62	56	33
5	56	—	—
10	<10	—	—
Buffer	100	100	100

Incubation system:
3·6 ml. plasma + 0·4 ml. glyoxaline buffer or solution of venom in buffer. After 1, 2 and 4 h incubation at 37° C, 1 ml. incubation mixture was removed from each tube, adsorbed with $Al(OH)_3$ and assayed for Factor VIII activity. The residual Factor VIII activity was expressed as a percentage of the buffered plasma sample incubated for the same length of time.

$CaCl_2$. After incubation at 37° C for 1 h the fibrin clots formed were washed in saline, digested and assayed for total nitrogen content by the micro-Kjeldahl method. It was found that the amount of fibrin formed by the action of the venom was the same as the amount formed when the plasma was clotted by recalcification.

With alumina-adsorbed fibrinogen-free plasma as starting material, attempts were made to precipitate Factor VIII activity by the alcohol fractionation procedure of Cohn[10]. Whereas in normal plasma Factor VIII is precipitated along with fibrinogen in Fraction I at a final ethanol concentration of 8 per cent, we found that in the defibrinated plasma maximal precipitation of Factor VIII activity took place at a final ethanol concentration of 15 per cent. It is well recognized that the precipitation characteristics of plasma proteins are altered in the absence of fibrinogen, and Gobbi[11] has shown that higher concentrations of alcohol are required to precipitate Factor VIII activity from the plasma of patients with congenital afibrinogenaemia than from normal plasma.

The action of the venom in clotting plasma fibrinogen and yet sparing Factor VIII is consistent and reproducible and it should be possible by its means to prepare fibrinogen-free Factor VIII for experimental purposes and possibly also for therapeutic use.

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Inhibition of Adenosine Diphosphate-induced Platelet Aggregation by Histamine

It is now well known that adenosine and adenosine monophosphate (AMP) strongly inhibit the aggregation of human and rabbit platelets which occurs in the presence of adenosine diphosphate (ADP). Adenosine and AMP are effective at a concentration of approximately 10^{-7} M, and are the most potent naturally occurring inhibitors known. O'Brien[1] has shown that antihistaminics, antimalarials, and local anaesthetics, all at approximately

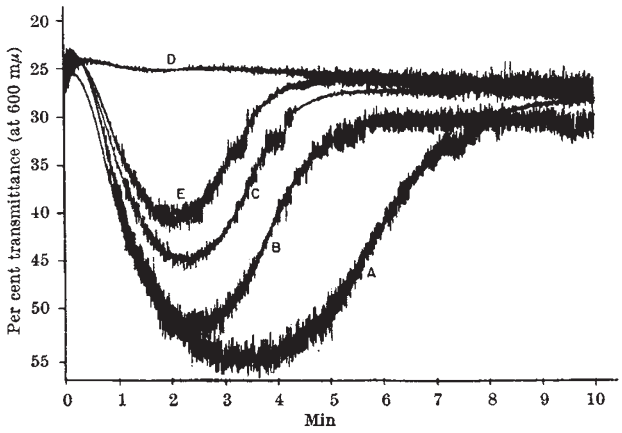

Fig. 1. Inhibition of ADP-induced platelet aggregation. At zero time 10^{-6} M ADP was added to rabbit platelet-rich plasma which had been incubated 1 min with: *A*, 0·9 per cent sodium chloride solution; *B*, histamine, 5×10^{-5} M; *C*, histamine, 10^{-4} M; *D*, histamine, 10^{-3} M; *E*, adenosine, 10^{-6} M

10^{-3} M, inhibit ADP-induced platelet aggregation. It has recently been reported by Salzman and Chambers[2] that substituted amino-acids, at a concentration of $1{\cdot}5 \times 10^{-2}$ M, inhibit ADP-induced platelet aggregation.

It has now been found that histamine inhibits ADP-induced aggregation of rabbit platelets. Changes in light transmittance of platelet-rich plasma on addition of ADP were recorded at 23° C, as described by Born[3] and O'Brien[4]. Fig. 1 shows the inhibitory effect of various concentrations of histamine when this was added to the plasma 1 min before the addition of ADP ; all concentrations are final concentrations in the test system. The results were similar when histamine remained in contact with the plasma for 10 min prior to the addition of ADP; but when histamine was incubated with plasma for 30 min at 37° C, then cooled rapidly to 23° C, there was no inhibition of ADP. This may be explained by the action of plasma histaminase during the 37° C incubation period; in addition, it has been shown that there is increased platelet responsiveness to ADP in platelet-rich rabbit plasma which has been incubated at 37° C, and then cooled rapidly to 23° C[5]. Imidazole (10^{-3} M) did not inhibit ADP-induced platelet aggregation. A series of substituted imidazoles, each tested at 10^{-3} M, were ineffective as inhibitors of ADP:

2-aminoimidazole	β-(*N*,*N*-dimethylaminoethyl)imidazole
2-methylimidazole	4-(β-*n*-propylaminoethyl)imidazole
2-mercaptoimidazole	4-(β-isopropylaminoethyl)imidazole
4,5-imidazoledicarboxmorpholide	4-(β-*n*-butylaminoethyl)imidazole
2-*n*-butylmercaptoimidazole	4-(β-di-*n*-butylaminoethyl)imidazole

O'Brien[6] has reported that histamine does not inhibit ADP-induced aggregation of human platelets, and this may be yet another example of species-determined differences in the behaviour of platelets toward both aggregating agents and their inhibitors. There is no obvious explanation of the mechanism by which histamine inhibits ADP-induced platelet aggregation. Butcher and Sutherland[7] have shown that imidazole stimulates cyclic phosphodiesterase, the enzyme which converts cyclic 3′,5′-AMP to AMP. It is possible, therefore, that histamine might stimulate cyclic phosphodiesterase and so result in the production of an inhibitory level of AMP. Evidence against this concept is the lack of inhibition to ADP-induced platelet aggregation by imidazole.

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