mechanism of action by which the histamine content of the worm is increased by piperazine in the presence of histamine remains uncertain but increased absorption across the body wall and relaxation of the muscle around the mouth (thereby increasing ingestion) are possibilities.

We would like to thank the Dunmow Flitch Bacon Factory for providing facilities for collecting *Ascaris suum* samples. This work was carried out during the tenure of an A.R.C. grant (J.L.P.).

References

BALDWIN, E. & MOYLE, V. (1947). An isolated nerve-muscle preparation from *Ascaris lumbricoides*. *J. exp. Biol.*, 23, 277-291.

MIYAGAWI, Y. (1961). Histamine in *Ascaris lumbricoides* and the effect of some anthelmintics on it. *Jap. J. Parasitol.*, 10, 419-428.

PHILLIPS, J.L., STURMAN, G. & WEST, G.B. (1975). The presence of histamine in the tissues of *Ascaris suum*. *Comp. Gen. Pharmac.* In press.

Site of action of an anti-inflammatory fraction from normal human plasma

JEMIMA K. BADCOCK, A.W. FORD-HUTCHINSON*, M.J.H. SMITH & J.R. WALKER

Department of Biochemical Pharmacology, King's College Hospital Medical School, London SE5 8RX

A fraction isolated from normal human plasma contains a substance of low molecular weight, below 500, which shows anti-inflammatory activity in animal tests in which the emigration of circulating leucocytes is a major factor (Ford-Hutchinson, Smith, Elliott, Bolam, Walker, Lobo, Badcock, Colledge & Billimoria, 1975). We have now studied its effects on the release of complement-derived chemotactic factors and anaphylatoxin from rat serum.

Preparations of the plasma fraction which caused a significant reduction in the accumulation of leucocytes in sponges implanted subdermally in the intact rat were tested at the same time for their effects on the directed migration of isolated rat leucocytes using the Boyden chamber technique (Goetzl & Austen, 1972) and on the production of anaphylatoxin by the method of Kleine, Poppe & Vogt (1970). Antigen-antibody complex was used as the activator of the classical pathway of complement and zymosan and *E. coli* endotoxin as activators of the alternate pathway.

The results (Table 1) show that the plasma fraction inhibited the release of chemotactic factors and anaphylatoxin when complement was activated by zymosan or endotoxin but not by antigen-antibody. However, there was no interference with the actions of the released chemotactic factors and anaphylatoxin on either the rat peripheral leucocytes or the isolated guinea-pig ileum. It is suggested that the active substance in the plasma fraction inhibits the C3 activator system in rat serum (Götze & Müller-Eberhard, 1971). This effect may be of some significance because of the recent observation (Goldstein & Weissmann, 1974) that leucocyte lysosomes contain a material causing a non-immune activation of the complement system thus amplifying the inflammatory response through a positive feed-back loop mechanism.

This work was supported by grants from the National Research Development Corporation, the King's College Hospital and Medical School Research Committee, the Wates Foundation, and the Nuffield Foundation.

Table 1 Effects of plasma fraction on the release of chemotactic and anaphylatoxic activities from rat and guinea-pig serum.

Addition to serum	*Chemotaxis (no. of cells per high power field)*	*Anaphylatoxin (% change: activator = 100)*
Zymosan	14.7 ± 1.7 (9)	
Zymosan + PF	8.6 ± 0.6 (9)*	−40 ± 8 (5)*
Endotoxin	22.4 ± 0.7 (9)	
Endotoxin + PF	13.2 ± 0.7 (9)*	
Antigen-antibody	14.9 ± 1.1 (9)	
Antigen-antibody + PF	15.1 ± 0.4 (8)	+2 ± 3 (3)

Results given as mean ± s.d., number of separate experiments in brackets. PF, plasma fraction (0.1 ml); * $P < 0.001$, significant difference from the corresponding value with activator alone.

References

FORD-HUTCHINSON, A.W., SMITH, M.J.H., ELLIOTT, P.N.C., BOLAM, J.P., WALKER, J.R., LOBO, A.A., BADCOCK, J.K., COLLEDGE, A.J. & BILLIMORIA, F.J. (1975). Effects of a human plasma fraction on leucocyte migration into inflammatory exudates. *J. Pharm. Pharmac.*, 27, 106-112.

GOLDSTEIN, I.M. & WEISSMANN, G. (1974). Generation of C5-derived lysosomal enzyme-releasing activity (C5a) by lysates of leucocyte lysosomes. *J. Immunol.*, 113, 1583-1588.

GOETZL, E.J. & AUSTEN, K.F. (1972). A neutrophil-immobilizing factor derived from human leucocytes. *J. exp. Med.*, 136, 1564-1580.

GÖTZE, D. & MÜLLER-EBERHARD, E.J. (1971). The C3-activator system: an alternate pathway of complement activation. *J. exp. Med.*, 134, 905-1085.

KLEINE, I., POPPE, B. & VOGT, W. (1970). Functional identity of anaphylatoxin preparations obtained from different sources and by different activation procedures. 1. Pharmacological experiments. *Eur. J. Pharmac.*, 10, 398-403.

Time-dependent potentiation and inhibition by 5-hydroxytryptamine of platelet aggregation induced by ADP

F. MICHAL & MINA MOTAMED*

Department of Pharmacology, University Medical School, Hills Road, Cambridge CB2 2QD

Platelet aggregation by adenosine diphosphate (ADP) can be either potentiated or, under certain conditions, inhibited by 5-hydroxytryptamine (5-HT) (Baumgartner & Born, 1968). We have investigated these opposing effects. Platelet aggregation was measured in human citrated platelet-rich plasma by the photometric method (Born, 1962). The uptake of 5-HT into platelets was measured with 5-HT labelled with ^{14}C.

In the presence of 5-HT, platelet aggregation by ADP was first accelerated and then decelerated (Figure 1). The acceleration was greatest when 5-HT and ADP were added simultaneously. With increasing intervals between the addition of 5-HT and the subsequent addition of ADP, the potentiation disappeared and was followed by inhibition. The inhibition increased for 10 min and then decreased; after 30 min the aggregation velocity was again similar to that of controls.

Earlier work (Born, Juengjaroen & Michal, 1972) showed that platelet aggregation by 5-HT is inhibited strongly by methysergide ($K_i \approx 0.03\ \mu M$) and weakly by imipramine ($K_i \approx 10\ \mu M$) whereas the uptake of 5-HT by platelets is inhibited strongly by imipramine ($K_i \approx 0.3\ \mu M$) and weakly by methysergide ($K_i \approx 125\ \mu M$). Methysergide at 0.25 μM completely inhibited both potentiation and inhibition by 5-HT of aggregation by ADP. Imipramine at 0.5 μM, and also at 5 μM which inhibited 5-HT uptake completely, only partially prevented the potentiation but did not prevent the inhibition nor the subsequent recovery. Ouabain at up to 100 μM influenced neither potentiation nor inhibition.

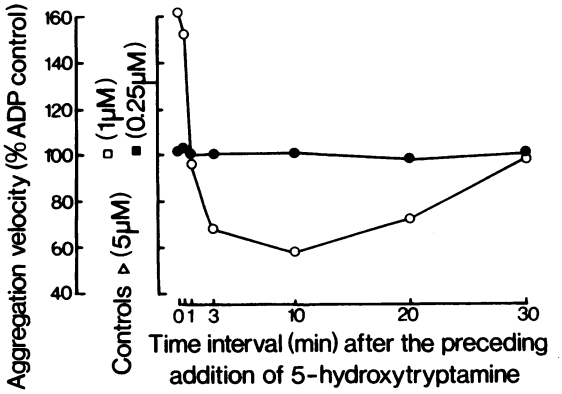

Figure 1 Aggregation velocity of human platelets (expressed as percentage of ADP control) produced by 1 μM ADP at different intervals after preceding addition of 5 μM 5-HT in the presence (●) and the absence (○) of 0.25 μM methysergide (MeS). For comparison aggregation velocities of 5-HT (△) and ADP alone (□) and ADP in the presence of methysergide (■) are indicated on the left of the figure. Methysergide was added 5 min before 5-HT or ADP; all samples were incubated for 30 min at 37° C before the addition of the aggregating agent.

Further evidence that both potentiation and inhibition of aggregation velocity by 5-HT are independent of the uptake of 5-HT was obtained with 5-methoxy-α-methyltryptamine (5 μM) which is not taken up by platelets (Born *et al.*, 1972) yet produced both potentiation and inhibition just like 5-HT itself. Therefore these effects of 5-HT are unrelated to its uptake by platelets but rather to a reversible effect of 5-HT on the plasma membrane.

M. Motamed is in receipt of a fellowship from the Wellcome Trust. F. Michal is a Senior Research Fellow of the British Heart Foundation.