PHYSIOLOGY

Anticomplementary Action of Aspirin

THE role of complement (C') in allergy[1,2], anaphylaxis[3,4], and the possible role of antigen–antibody reactions, and thus also of C'[5] in rheumatism and general fever phenomena, induced us to determine whether classical antidotes against these diseases, like aspirin (acetyl-salicylic acid)[6], salicylic acid, quinine and cortisone, have any anticomplementary action.

For this purpose the influence was studied of solutions of these agents on the hæmolysis normally provoked by the action of guinea pig's serum (C') on sheep erythrocytes (E) sensitized with rabbit anti-sheep erythrocytes serum (A): $EAC' \rightarrow E^*$. Standard hæmolysis tubes were filled with 0·5 ml. C', diluted 1/100; 0·1 ml. E, diluted 1/50, and 0·1 ml. A, diluted 1/100, and incubated at 37° C. during 30 min. With the standard C', A and E reagents obtained from the Pasteur Institute, Paris, complete hæmolysis was always obtained in these circumstances. The dilutions were made in Michaelis's barbital buffer, $p\mathrm{H} = 7{\cdot}4$, $\mu = 0{\cdot}15$, containing $1{\cdot}5 \times 10^{-4}$ M Ca^{++} and 5×10^{-4} M Mg^{++}. The different dilutions of aspirin, etc., tested were also made in this buffer, and the solutions thus obtained then served to dilute the C'. In this way the total volume of the reagents was kept constant, and the material to be tested for its action on C' was brought into contact with C' before any other reagent. The results are presented in Table 1.

Table 1. THE ANTICOMPLEMENTARY EFFECT OF ASPIRIN, SALICYLATE, QUININE AND CORTISONE

	Concentration (weight/volume) giving 50 per cent hæmolysis in 30 min.	Smallest concentration completely inhibiting hæmolysis in 30 min.
Aspirin	4×10^{-4}	5×10^{-4}
Sodium salicylate	no inhibition	
Quinine	$> 5 \times 10^{-4}$	slight delay of hæmolysis
Cortisone acetate	$2{\cdot}5 \times 10^{-4}$	$3{\cdot}3 \times 10^{-4}$

It is clear from Table 1 that aspirin is the only compound tested which has a strong anticomplementary action within the normal therapeutical doses, at least so far as guinea pig serum is concerned (which contains more C' than human serum). Sodium salicylate seems to have no action at all, which seems strange, as it is still much in use in many countries, instead of aspirin. Its beneficent action (if any[7]) may perhaps be explained by an acetylation during its metabolism. Quinine, which has only a slightly retarding action on C' hæmolysis, probably owes its beneficent action to a different mechanism altogether. Cortisone acetate is only anticomplementary in rather stronger than therapeutical doses: the mechanism of its action must be complex.

We have also determined which of the C' factors is inhibited by aspirin. We suspected that the inhibited factor is C_3', because of the work of Osler *et al.*[3], and we were supported in this suspicion when $EA + (C' + \text{aspirin})$, after centrifuging three times and washing with buffer (to remove the aspirin), gave rise to hæmolysis when fresh C' + ethylenediamine disodium acetate (EDTA) was added[8]. This hypothesis was confirmed by using C_4' (prepared by heating the serum at 56° C. for 20 min. and then treating it with ammonium[9]), instead of $C' + \text{EDTA}$, because this C_3' also caused the $[EA + (C' + \text{aspirin})]$, washed three times, to hæmolyse.

We have not up to now determined whether the inhibition of C_3' by aspirin is irreversible or reversible[10]. We have been unable to determine which C' factor cortisone acetate inhibits, for, although we have chosen this form of cortisone for its relatively good water solubility, it could not be satisfactorily removed from the sheep erythrocytes by centrifuging and washing. But we have assured ourselves that the hæmolysis-preventing action of cortisone acetate is due to its action on C', and is not an aspecific protection against hæmolysis (as is the case with cholesterol[11]). Cortisone acetate, aspirin, sodium salicylate and quinine do not protect sheep erythrocytes against hæmolysis by saponin, digitonin, or dodecyl sulphate.

Work on the action of aspirin and other substances on human C' *in vitro* and *in vivo*, and on guinea pig C' *in vivo*, is still in progress; but it can already be concluded, in the light of the results mentioned, and in view of the work of Osler *et al.*[3], that the action of aspirin on C' can quite satisfactorily explain its multiple therapeutical benefits in various pathological states such as allergy, rheumatism and fever. Thus, quantitative estimations of this type of therapeutical activity of a great many existing and new compounds may quite easily be obtained through simple complement-hæmolysis reactions.

We are indebted to the frequent and fruitful discussions we had with Dr. A. Eyquem of the Pasteur Institute, Paris.

Note added in proof. 4×10^{-4} aspirin has since proved active against much higher C' concentrations *in vitro*. This anti-complementary action, and the lack of it in the case of salicylate, has been confirmed *in vivo*.

C. J. VAN OSS
J. C. FRIEDMANN
M. FONTAINE

Laboratory of Physical Biochemistry,
National Veterinary College,
Alfort (Seine).

[1] Schwab, L., Mole, F. C., Hall, T., Breen, H., Kirk, M., van Zandt-Hawn, C., and Haneway, C. A., *J. Exp. Med.*, **91**, 505 (1951).
[2] Zanussi, C., and Invernizzi, F., *Boll. First Sieroterap. Milan*, **37**, 192 (1958).
[3] Osler, A. C., Hawrisiak, M. M., and Ovary, Z., *Fed. Proc.*, **16**, 428 (1957).
[4] Büsing, K. H., *Allergie und Asthma*, **3**, 15 (1957).
[5] Schmidt, H., "Die Konglutination; Das Komplement", 31 (Dr. Dietrich Steinkopff Verlag, Darmstadt, 1959).
[6] Gross, M., and Greenberg, L. A., "The Salicylates" (Hillhouse Press, New Haven, 1948).
[7] Roskam, J., *Acta Clin. Belg.*, **3**, 305 (1948).
[8] Mayer, M. M., Levine, L., Rapp, H. J., and Marucci, A. A., *J. Immunol.*, **73**, 443 (1954).
[9] Kabat, E. A., and Mayer, M. M., "Experimental Immunochemistry", 123 (C. C. Thomas, Springfield, Ill., 1958).
[10] Work on more or less related compounds by Mills, S. E., and Levine, L., *Immunology*, **2**, 368 (1959), has recently come to our notice; in the light of this work a reversible inhibition of C_3' appears more likely.
[11] Ponder, E., *J. Gen. Physiol.*, **28**, 349, 357 (1945).

Possible Sources of Tartronate Deficiency in Man

SINCE exogenous tartronate (hydroxymalonate) is probably essential for the nutrition of rats[1], it has been suggested[2] that prolonged deficiency of available tartronate might result also in metabolic disturbances in man. Tartronate, which is produced only by plants[3], has chemical and physiological properties which could result in its becoming deficient in human diets. These properties are: (*a*) easy decarboxylation in hot, slightly acid solution; (*b*) complete