Short communications

Effect of cellulose sulphate on serum complement

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Cellulose sulphate 50–600 μg/ml reduces complement titres in human serum. This effect is, in contrast to the clot-promoting and plasma kinin forming action of cellulose sulphate, not mediated by clotting factor XII.

Rothschild (1968) reported that injection of cellulose sulphate induces in rats intense plasma kinin formation resulting in hypotensive responses and eventual depletion of plasma kininogen. *In vitro* analyses with human plasma (Kellermeyer & Kellermeyer, 1969) have shown that cellulose sulphate achieves these effects by activating clotting factor XII (Hageman factor), an enzyme known to trigger intrinsic blood clotting, fibrinolysis and plasma kinin formation (Esnouf & Macfarlane, 1968). Recent evidence suggests that factor XII may also activate the reaction sequence of the complement (C′) system, possibly through plasma kallikrein (Donaldson, 1968; Gigli, Mason, Colman & Austen, 1970). The effects of cellulose sulphate on serum complement were therefore studied.

Methods.—Hexadimethrine bromide was obtained from Abbott Laboratories, soya bean trypsin inhibitor (SBTI) from Koch and Light Ltd., and aprotinin (Trasylol) from Bayer Ltd. Cellulose sulphate was prepared by the method of Astrup, Galsmar & Volkert (1944).

Complement (C′) titres were assayed by measuring the 50% haemolytic units (C′H50; Osler, Strauss & Mayer, 1952). C′1 esterase activity was measured by following the hydrolysis of 0·025 M N-acetyl-L-tyrosine ethyl ester monohydrate (ATEe) at pH 7·2 and 37° C in an automatic titrator (Radiometer). Margolis's (1958) "kaolin clotting time" method was adapted to measure the clot-promoting effect of cellulose sulphate. Plasma kinin formation was assayed by applying activated serum directly to the isolated rat uterus. Kininogen levels in serum or plasma were assayed by measuring the kinin released by trypsin 250 μg/ml in presence of EDTA; SBTI was added immediately before samples were tested. To remove clotting factor XII, plasma serum was treated with celite 30 mg/ml for 1 h, then separated and left at room temperature for 2 h until all generated enzyme activity had decayed.

Results.—Figure 1 shows that incubation of 1 ml of serum with 50–600 μg of cellulose sulphate for 10 min greatly reduced haemolytic C′ titres. The final reduction in titre produced by a given concentration of cellulose sulphate was attained within 1–2 min. The reduction appeared to be due to consumption of C′ by activation, and subsequent inactivation, rather than to true inhibition, since cellulose sulphate activated the esterase of the first component of C′ (C′1 esterase). This enzyme was measured by recording the hydrolysis of ATEe. The method is far less sensitive than haemolytic assays of C′ factors (Donaldson, 1968), which may explain the finding that cellulose sulphate induced detectable esterolytic activity only in higher concentrations (1–10 mg/ml) and in serum lacking the α_2-globulin C′1 esterase inhibitor (hereditary angio-oedema serum).

Optimal acceleration of plasma clotting was produced by 1–5 μg/ml of cellulose sulphate. The time course of this effect was slower, the shortest clotting times being attained only when plasma had been incubated with cellulose sulphate for 8–12 min before recalcification. With higher concentrations (10–50 μg/ml) the described (Kellermeyer & Kellermeyer, 1969) anticoagulant effect of cellulose sulphate was seen.

Activation of the plasma kinin-forming system required higher doses of cellulose sulphate; 500 μg/ml induced slight and 5 mg/ml moderate kinin formation. Even higher doses reduced the plasma kininogen level only by 15–25%. Kinin formation induced by kaolin led to similar depletion. These findings agree with reports that the enzymes in human plasma attack only one-quarter to one-third of the kininogen digested by trypsin or tissue kallikreins (Margolis & Bishop, 1963).

The role of factor XII in the activation of complement by cellulose sulphate was further examined by the following experiments:

Potent activation of factor XII with kaolin or glass for 2 or 10 min did not reduce the C′ titre, although it produced considerably more clot-promoting and kinin-forming activity than did cellulose sulphate.

Previous removal of clotting factor XII by celite did not alter serum C′ titres, or prevent their reduction by cellulose sulphate. The clot-promoting action of kaolin and cellulose sulphate was nearly abolished.

Actions of inhibitors. The antiheparin agent hexadimethrine bromide inhibits the activation and activity of clotting factor XII (Ratnoff & Miles, 1964; Eisen, 1964). The activation of C′ by cellulose sulphate was also inhibited. Hexadimethrine 150 μg/ml serum reduced the C′-depleting effect of cellulose sulphate by 75%, and 1 mg/ml by 99%. Whilst the C′ titres in serum samples not treated with cellulose sulphate were not lowered by these concentrations of hexadimethrine, higher concentrations (3–6 mg/ml) did reduce C′ titres. Hexadimethrine may thus be a weak inhibitor of the activation of C′ by the antigen-antibody interaction, on which the C′ assay is based.

Hexadimethrine appeared to counteract cellulose sulphate at least partly at the stage of activation of C′1 esterase, since it effectively inhibited the ATEe hydrolysis induced in serum from patients with hereditary angio-oedema.

Soya bean trypsin inhibitor (200–500 μg/ml), which inhibits plasma kallikrein and plasmin but not factor XII or C′1 esterase, did not interfere with the depletion of C′ by cellulose sulphate.

High concentrations of aprotinin (1,000 u./ml) slightly inhibited C′ depletion by cellulose sulphate, but not the titre in control samples of serum.

Discussion.—Most of the present findings argue against the view that factor XII is normally an important activator of complement. Cellulose sulphate greatly reduced C′ titres, but only moderately accelerated clotting. Silicates promoted clotting far more intensely, without detectably consuming C′. Previous removal of factor XII did not impair the mobilization of C′ by antigen-antibody interaction, nor the effect of cellulose sulphate on C′. The exact mechanism and serum factors through which cellulose sulphate acts on C′ require further study. Hexadimethrine may suppress this effect not by inhibiting factor XII but by combining with cellulose sulphate in the same way as it neutralizes

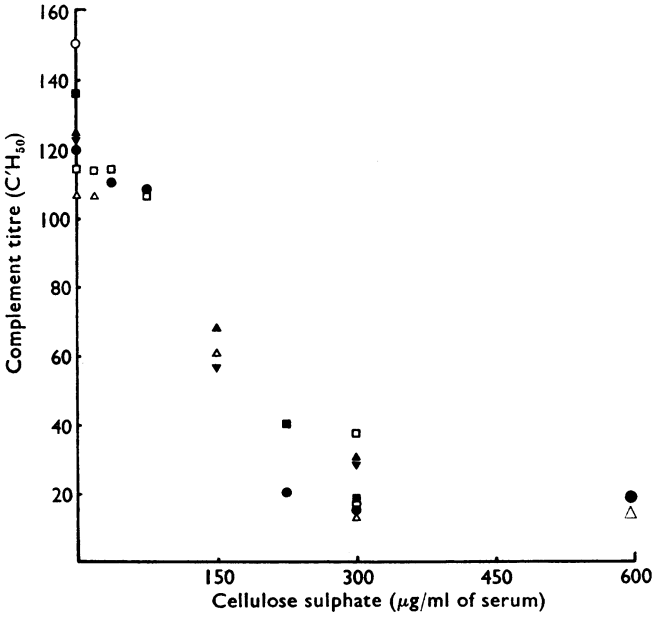

FIG. 1. Effects of incubating serum with cellulose sulphate for 10 min on complement titres. Symbols represent individual sera.

another sulphated polysaccharide—heparin.

The action of cellulose sulphate on C′ *in vivo* is being studied. The described *in vitro* effects raise the possibility that the depressed inflammatory responses found after cellulose sulphate treatment (Leme, Schapoval & Rocha e Silva, 1967) cannot be attributed solely to reduced plasma kinin formation. Depletion of C′ also reduces inflammation (Willoughby, Coote & Turk, 1969).

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