Sulphuric Esters of Polysaccharides as Activators of a Bradykinin-forming System in Plasma

PREVIOUS work[1] has shown that intravenous injection of agar, a naturally occurring sulphuric ester of polygalactose, leads to an appreciable reduction in bradykininogen concentration as well as to a significant increase of *p*-tosylarginine methyl ester (TAMe) esterase activity in the plasma of the rat. Other results indicate that, besides agar, synthetic sulphuric esters of certain polysaccharides are potent activators of the bradykinin forming system in rat or human plasma *in vitro*. In the experiments demonstrating this activity, 0·2 per cent oxalated plasma was collected, avoiding contact with glass, and treated with 1 mg/ml. of 8-hydroxy quinoline (8HQ), an inhibitor of enzymes which destroy bradykinin[2]. Kinin formed was assayed on isolated, atropinized guinea-pig ileum which had been treated with antihistamine, using synthetic bradykinin as the standard. Powdered cellulose, glycogen from shellfish, inulin, soluble starch and agar were added to rat plasma, either as such, or after conversion to water soluble sulphuric esters by treatment with chlorosulphonic acid in pyridine[3]. The effects of carrageenin, a sulphuric ester of polygalactose, extracted from Irish moss, as well as of heparin, were also examined. When incubated at 37° C with rat plasma, all sulphuric ester polysaccharides caused stimulation of smooth muscle equivalent to, on average, 1·0 μg of bradykinin/ml. The active material formed had the characteristics of bradykinin; it was rapidly destroyed by plasma in the absence of 8HQ, as well as by chymotrypsin. Maximum activation was reached 1 min after the addition of 10^{-4} or 10^{-5} g of cellulose sulphate to 1 ml. of plasma (Fig. 1). Larger concentrations (10^{-3} g/ml.) of this activator were less effective during the early phase of the incubation, which suggests an inhibitory effect of excess sulphopolysaccharide on bradykinin production. Ten parts by weight of hexadimethrine bromide, previously shown to inhibit kinin formation[4], inactivated one part of cellulose sulphate. None of the non-sulphated, native polysaccharides induced kinin formation; heparin, a sulphated derivative of polyglucosamine and glucuronic acid, previously shown[5] to be an activator of certain kinin-forming agents, was much less active than the other sulphopolysaccharides examined. The amounts of bradykinin formed by optimal concentrations of cellulose sulphate in rat plasma were the same as those resulting from the exposure of plasma to powdered glass[6]. Either treatment led to a comparable depletion of plasma-kinin precursor, so that pre-incubation of plasma with glass in the absence of 8HQ, followed by incubation with cellulose sulphate and quinoline, did not result in kinin activity while, reciprocally, pre-incubation of plasma with cellulose sulphate in the absence of 8HQ, rendered it unresponsive to subsequent activation with glass in the presence of 8HQ.

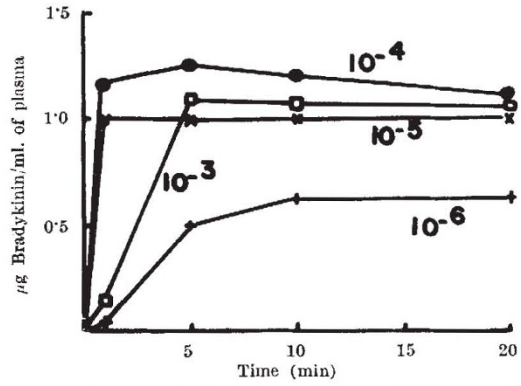

Fig. 1. Effect of concentration (w/v) of cellulose sulphate on the formation of bradykinin in oxalated rat plasma.

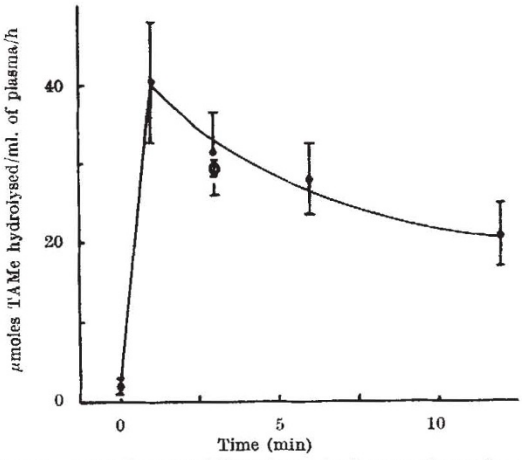

Fig. 2. Activation of TAMe esterase in human plasma by cellulose sulphate and glass powder. (Esterolytic activity was determined by the method of Brown[7].) ●, Cellulose sulphate, 1 mg/ml.; ○, glass powder, 500 mg/ml.

The bradykinin-forming activities of cellulose sulphate, of agar and of heparin were also tested on oxalated human plasma. Their efficiency was approximately the same as those obtained in rat plasma, heparin being again the least active of the series.

Kinin formation by polysaccharide sulphates may involve activation of a proteolytic process. This was indicated by results showing that (*a*) soy-bean trypsin inhibitor (40 μg/ml. of plasma) prevented the action of every active polysaccharide tested, and (*b*) TAMe esterase activity in human plasma was markedly enhanced by cellulose sulphate (Fig. 2). Maximum activity, obtained after 1 min of incubation, was significantly decreased after 12 min. The nature of this inactivating effect is unknown but deserves further investigation. For comparison, Fig. 2 shows that an equivalent amount of esterolytic activity was present in plasma incubated for 3 min, either with glass powder or with cellulose sulphate.

Polysaccharide sulphuric esters are prepared under comparatively mild conditions which do not cause changes in structure other than the introduction of sulphate substituents at hydroxyl groups[3]. This fact, as well as the high activity of this class of compounds, may make them useful tools in the study of the relationship between structure and activity as well as of chemical details of the kinin-forming process. These compounds, by decreasing bradykininogen stores *in vivo*, could, in addition, yield information about the participation of bradykinin in certain physiological or pathological processes[1].

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Polyprenols of Wood and Leaf Tissue of the Silver Birch, *Betula verrucosa*

GREEN leaves of *Aesculus hippocastanum* and of *Ficus elasticus* contain mixtures of castaprenol-10, -11, -12 and -13 and ficaprenol-10, -11, -12 and -13 respectively[1,2]. The wood of these plants contains no polyprenols, but that of