A smooth muscle-stimulating substance in bovine plasma

We have investigated the properties of smooth muscle-stimulating substances present in plasma of man, guinea-pig, chicken, pig, ox and rat. Plasma (3 ml) obtained by centrifuging heparinized blood at 4,000 *g* for 15 min at 0° was transferred to a Sephadex G 25 column (diameter 2 cm, length 100 cm) which was then eluted with a solution of sodium chloride (0·1M, pH 6·7, temperature 4°) passing at a rate of 30 ml/h. The eluate was collected in 5 ml samples and tested on the rat isolated uterus (30°, de Jalon fluid) and the isolated guinea-pig ileum (37°, Tyrode solution) using bradykinin and histamine respectively as standard spasmogens. Only those samples which contained protein (detected by gel electrophoresis) were active on both preparations and showed dose-response relations. Therefore, the spasmogens are probably not compounds of low molecular weight like kinins. Furthermore, their activities were not modified by antihistamine or anti-5-hydroxytryptamine drugs or by atropine, and incubation with chymotrypsin also did not reduce activity.

The plasma proteins of ox were then fractionated by Cohn's method and freeze-dried. Fraction IV-1 with more than 50% α_2-globulin contained nearly all the active material and this fraction potentiated both the action of bradykinin on the uterus and that of histamine in the ileum. Fraction IV-1 was then transferred to a Sephadex G 200 column (diameter 2 cm, length 30 cm) which was eluted with Tris-buffer (pH 8·05) passing at a rate of 18 ml/h. Samples of eluate (3 ml) were collected and tested for protein by measuring light absorption at 280 nm and by studying gel electrophoresis. Three peaks of absorbancy were identified but only the first (contained in samples 6–14 and therefore in 18–42 ml of eluate) possessed the smooth muscle-stimulating activity on both the uterus and the ileum. This contained most of the α_2-globulin (over 85%) and had a carbohydrate moiety of about 8% and a molecular weight about 800,000. The second peak (samples 15–18 or 45–54 ml of eluate) consisted mostly of β-globulin and γ-globulin whilst the third (samples 19–24 or 57–72 ml of eluate) was predominantly albumin (see Fig. 1).

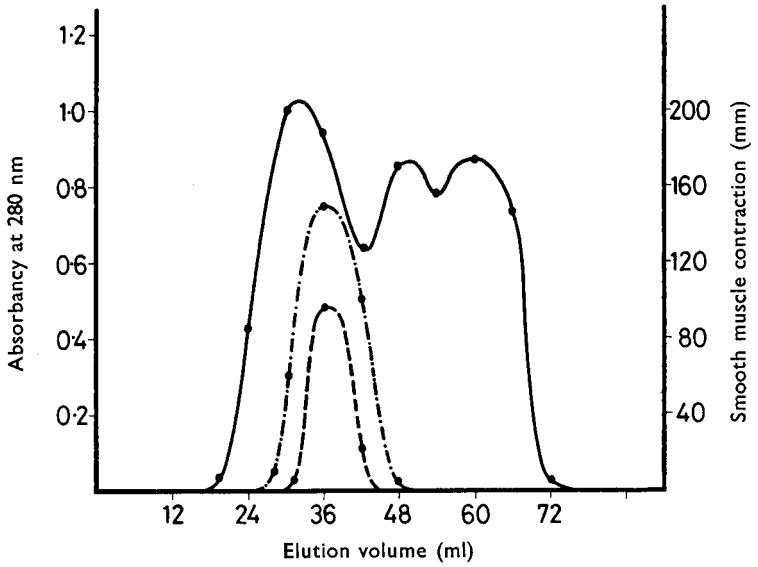

FIG. 1. Protein content (absorbancy at 280 nm) and biological activity of bovine plasma Cohn fraction IV-1 after elution from Sephadex G 200. ●——● protein content; ●— · —● activity on rat uterus; ● — — ● activity on guinea-pig ileum. Note that there are 3 peaks of protein but only the first possesses activity on both preparations.

Since the plasma level of α_2-globulin in man is often raised in conditions of inflammation or stress, this finding may be of pathological importance.

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Gastrointestinal absorption of two polymorphic forms of aspirin

Two forms of aspirin have been prepared and characterized (Tawashi, 1968). Form II dissolved half as fast again as form I from the planar surface of compressed tablets. Methods based on solubility-temperature dependence (Nogami, Nagai & others, 1969) failed to establish a thermodynamic difference between the two forms, apparently because of the thermodynamic instability of form II and its rapid reversion to form I in solution. Reversion to form I takes place within minutes with ultrasonic energy.

The thermodynamic relation between the two forms was studied by differential thermal analysis (DTA) and thermal gravimetric analysis (TGA). The analyses were made on 10 mg samples, in a dynamic flow of Argon, at 4°/min with alumina as a reference material in a Mettler recording vacuum thermoanalyser. Conditions of particle size, packing of the sample and rate of heating were examined. Fig. 1A shows the temperature curve, DTA and TGA diagrams for forms I and II. The differences in thermal behaviour and mass effects were clearly observed in both forms. From the area of the endothermic peak (of the DTA curves) the heat of fusion was measured, after calibrating the instrument with a material of a known heat of fusion (Barshad, 1952; Garn, 1965). Comparing the endothermic peak areas of both forms, with that of benzoic acid (10 mg), analysed under the same conditions, form I gave a heat of fusion of 29·1 cal/g and form II gave 36·9 cal/g.

Therefore, it was of interest to determine the rate of gastrointestinal absorption of the two different forms in normal human subjects, by measuring the serum salicylate concentration after the oral ingestion of 600 mg of aspirin. After an overnight fast, each subject was given form II crystals dispersed in 50 ml water (room temperature) followed by another 50 ml of water used to wash the containing vessel. The time between the addition of the aspirin to water and the administration was 3 min. Blood samples were taken 10, 20, 30, 45 and 60 min after the oral ingestion, allowed to clot, and the serum separated by centrifugation. The total salicylate was determined by the method of Trinder (1962). Form I was given after 1 week to the same subjects under the same conditions, and with both forms of about the same particle size. Each point on the salicylate concentration-time curve (Fig. 1B) represents the average of 6 determinations within $\pm$4·1 as standard error. The data obtained are in agreement with the previous dissolution rate studies of the two forms, and with differences obtained in the thermal analysis.

In this study Form II increased the salicylate concentration 70% above the value obtained by Form I for the same period of time.