On the histamine-binding property of human serum

In 1968, Gecse, Karády & West reported that a non-protein fraction of rat serum and of human serum contains a substance which has the power *in vitro* to bind histamine. The active material was considered to be polypeptide in nature, with a molecular weight between 1000 and 5000. This finding was at variance with that of Guirgis (1967) who stated that the histamine-binding activity of human serum was in the relatively small molecular weight protein fraction which was linked in some way with both albumin and α- and β-globulins. We now report that the histamine-binding substance in human serum is attached predominantly to albumin from which it splits off during the process of coagulation or after papain digestion. Of even greater importance is the finding that serum from allergic patients lacks this histamine-binding property.

In our studies, blood was withdrawn from 30 healthy human volunteers (15 men and 15 women, aged 18–40 years) into glass tubes without anticoagulant. It was left at room temperature for 1 h to initiate coagulation and then warmed to 37° for another h to complete clot retraction. Serum was then obtained by centrifugation at 5000 *g* for 30 min at 0°. Serum (1 ml) was transferred to a Sephadex G 25 column (diameter 1 cm, length 47 cm) and this was eluted with a solution of sodium chloride (0·1M, pH 6·7, temperature 4°) passing at a rate of 10 ml/h. The eluate was collected in 1·5 ml samples and each was tested both for protein using agarose gel electrophoresis, the ninhydrin reaction, and light absorption at 280 nm, and for polypeptides by thin-layer chromatography, the ninhydrin reaction and amino-group halogenization with iodine vapour. After a dead space of about 15 ml (samples 1–10), the serum proteins were detected in the next 9 ml (samples 11–16). The next 4 samples were free of nitrogenous material (see Fig. 1) but polypeptides of low molecular weight (between 1000 and 5000) were eluted in the next 9 ml (samples 21–26), their peak concentration, as measured by the intensity of the ninhydrin reaction using light absorption at 510 nm, being at sample No. 23 (that is, after 35 ml of eluate had been collected).

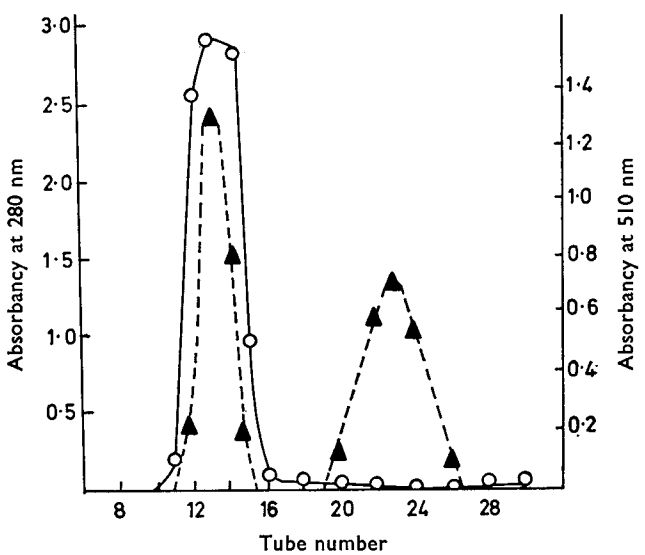

FIG. 1. Protein content (absorbancy at 280 nm) and polypeptide content (absorbancy of stabilized ninhydrin colour at 510 nm) of human serum after elution from Sephadex G 25. ○——○ protein content; ▲——▲ polypeptide content. Note that there are 2 peaks of ninhydrin-reactive material.

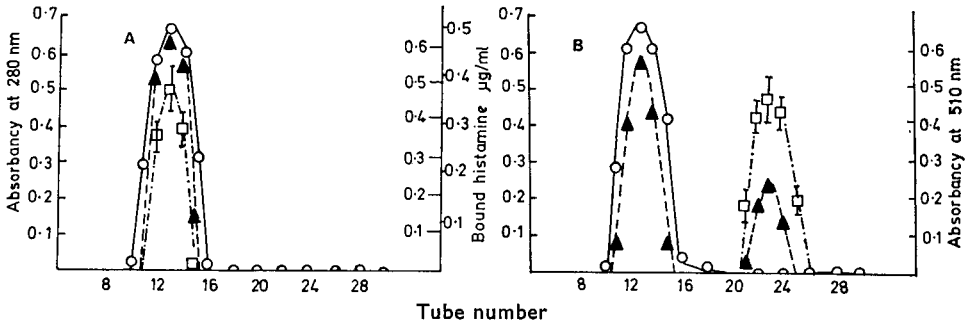

FIG. 2. Protein content (absorbancy at 280 nm), polypeptide content (absorbancy of stabilized ninhydrin colour at 510 nm), and histamine-binding activity of human plasma albumin (A) before, and (B) after papain digestion. ○——○ protein content; ▲——▲ polypeptide content; □——□ amount of bound histamine. Note that the histamine-binding activity of plasma albumin is lost after papain digestion and becomes associated with the low molecular weight polypeptide (samples 21–26), released from albumin.

Each sample was subsequently tested for its ability to bind histamine (Gecse & others, 1968). The earlier samples containing serum proteins did not bind histamine but those containing the polypeptides removed histamine from solution, the peak concentration (sample No. 23) binding up to 35% of that added. All the eluates from the columns had no action on the guinea-pig isolated ileum, rat uterus or rat duodenum, and hence contained no kinin.

Blood samples were later withdrawn from healthy volunteers into polythene tubes containing citrate (1/10 volume, 4%) and the plasma proteins were fractionated by Cohn's method. Fraction V containing 96% of albumin (as determined by gel electrophoresis) was then transferred to the Sephadex G 25 column and eluted with sodium chloride solution. Nearly all the histaminopexic activity was found in the protein eluate (samples 11–16, with a peak at No. 13). This is shown in Fig. 2 A and indicates that the albumin in some way binds histamine. All other fractions of the plasma had no such activity. When Fraction V was digested with papain (2 h, pH 8·0, 37°) and then passed through Sephadex G 25, the histamine-binding activity of plasma moved to the polypeptide region (samples 21–26, with a peak at No. 23). This elution volume corresponded to that required for eluting low molecular weight peptides such as kinins. The peptide did not bind acetylcholine, bradykinin or 5-hydroxytryptamine and lost its power to bind histamine when treated with strong alkali. Blood from all healthy volunteers behaved in a similar manner, there being no differences between young or old men and women. In all cases, the low molecular weight polypeptide was released from albumin during the process of coagulation or by papain digestion.

Plasma albumin or serum peptide derived from blood of 8 allergic patients (generalized dermatoses) lacked histamine-binding activity. Thus, the lack of a histaminopexic substance may play a role in the pathogenesis of allergic diseases.

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Anti-acetylcholine, and adrenaline-potentiating activity of oxotremorine on guinea-pig isolated vas deferens

Oxotremorine produces a sharp but transient fall in blood pressure, as does acetylcholine or carbachol, which is prevented both by atropine methylbromide and atropine sulphate. On the guinea-pig and rat isolated ileum and on the rat isolated urinary bladder, oxotremorine causes contractions similar to those elicited by acetylcholine or carbachol (Haslett, 1963; György, Pfeifer & Kenyeres, 1970). According to results reported by Cox & Hecker (1971), oxotremorine has the same activity on the guinea-pig isolated intestine as has acetylcholine.

We have examined some effects of oxotremorine on the guinea-pig isolated vas deferens.

Vasa deferentia, freshly removed from the guinea-pig and desheathed, were suspended at 31° in 15 ml of Locke solution with oxygen bubbled through it. Contractions were recorded kymographically on a smoked drum. The test compounds were added to the bath fluid either in a single concentration or cumulatively, always doubling the preceding concentration. The concentrations stated refer to the salts of the following compounds: oxotremorine oxalate, physostigmine salicylate, atropine sulphate, carbachol chloride, acetylcholine chloride.

At $5 \cdot 10^{-7}$ to $5 \cdot 10^{-5}$ g/ml oxotremorine produced contractions of the vas deferens in about 60% of the experiments. Oxotremorine (10^{-6} g/ml) added to the bath fluid at not less than 10 min intervals induced contractions of the same magnitude, but at 2 to 5-min intervals, or a higher concentration (10^{-5} g/ml), the contractions became smaller. Physostigmine enhanced, atropine methylbromide (10^{-8})—even at low concentrations—inhibited oxotremorine (10^{-6})-elicited contractions, and phentolamine (2 and 5×10^{-7}) had no effect. Oxotremorine was in all experiments less effective in eliciting contractions than was acetylcholine: in eight experiments the mean value for contractions elicited by acetylcholine at 10^{-7} g/ml concentration was $51 \cdot 6 \pm 3 \cdot 9$ mm as against $49 \cdot 6 \pm 3 \cdot 1$ mm for contractions induced by oxotremorine at 10^{-6} g/ml concentration in nine experiments. Accordingly, oxotremorine exhibited an about ten times lower activity on this preparation than did acetylcholine. The maximum contractions obtainable were all lower with oxotremorine than with acetylcholine or carbachol. Measured on the same piece of vas deferens, the mean value (23 experiments) for maximum contraction produced by oxotremorine was only $23 \cdot 6 \pm 6 \cdot 3$% of that obtained with either of the other two compounds, whose maxima were always identical. At $5 \cdot 10^{-7}$ to $5 \cdot 10^{-5}$ g/ml, oxotremorine reduced

Table 1. *Effects of oxotremorine (OT) on carbachol-induced contractions in guinea-pig isolated vas deferens.*

Oxotremorine concentrations g/ml	n	ED50 of carbachol μg/ml		ED50 after OT
		ED50, controls	ED50 after OT	ED50, controls
$5 \cdot 10^{-7}$	6	$3 \cdot 2 \pm 1 \cdot 4$	$6 \cdot 5 \pm 1 \cdot 5$	2·0
$5 \cdot 10^{-6}$	8	$4 \cdot 4 \pm 1 \cdot 1$	$51 \cdot 4 \pm 35 \cdot 9$	11·7
$5 \cdot 10^{-5}$	5	$2 \cdot 7 \pm 0 \cdot 4$	$178 \cdot 3 \pm 65 \cdot 5$	66·0

± = s.e.