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The Histamine-binding property of serum

SIR,—Parrot & Laborde (1953) found that human and rat serum *in vitro* bind histamine and they suggested that γ -globulin is probably responsible for this action. We now find that the non-protein fraction of serum also contains a substance which has the power to bind histamine.

Rats of the R-Amsterdam strain of either sex (170-190 g) were stunned and bled from the carotid arteries into collecting test tubes and the blood was allowed to clot for 2 hr at room temperature. After separation of the serum by centrifugation, 7 ml was transferred to a Sephadex G-25 column (diameter 3 cm, length 60 cm), which was eluted with a solution of sodium chloride (0.1 M, pH 6.7, temperature 4°) passing at a rate of 30 ml/hr. The eluate was collected in 5 ml samples and tested both for protein, using paper electrophoresis, paper chromatography, and light absorption at 280 m μ , and for polypeptides by thin-layer chromatography. The serum proteins were detected in the first 9 samples (45 ml) but the next 8 samples were free from nitrogenous material. Polypeptides of low molecular weight (1,000-5,000) were eluted in the next 8 samples, the peak concentration, as measured by the intensity of the ninhydrin reaction, being in sample No. 20 (that is, after 100 ml of solution had been collected).

Each sample was subsequently tested for its ability to bind histamine. This was determined by mixing aliquots of 1 ml with a solution of histamine (1.2 μ g base) and incubating the mixture at 37° for 20 sec. The free histamine was then estimated on the atropinized guinea-pig ileum using a four-point assay procedure, and the amount of histamine combined with the eluate sample was calculated and expressed as a percentage of that added. The first 17 samples, of which the earlier ones contained the free serum proteins, did not bind histamine but much of that present in the samples containing the polypeptides (Nos 18-25) was not free. The sample with the peak concentration of polypeptides (usually No. 20) contained the highest amount of combined histamine (up to 40% of that added). All the eluates from the columns had no effect by themselves on the isolated ileum and hence contained no kinin. The possibility that an antihistamine-like substance derived from serum was present in these samples cannot entirely be ruled out for hydrolysis of the samples before incubation with histamine removed most, but not all, of the power to bind histamine.

When fresh normal human serum was treated in a similar way, only the polypeptide fractions had the ability to bind histamine. The pathophysiological significance of this finding may be important in the field of allergy.

Institute of Pathophysiology,
University School of Medicine,
Szeged, Hungary.

A. GECSE
S. KARADY

British Industrial Biological Research Association,
Woodmansterne Road,
Carshalton, Surrey, England.
June 13, 1968

G. B. WEST

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