Influence of Heparin and Serotonin on Basophil Leucocytes of Rabbit Blood[1]

Both the tissue mast cell and the basophil leucocyte hold metachromatic mucopolysaccharide-containing granules. JORPES *et al.*[2] claimed that the mast cell granules contain heparin. TAUBERT[3] in 1951 extracted a substance with heparin-like activity from blood basophils of a patient suffering from chronic myeloid leukemia. PIETTE and PIETTE[4], using histochemical methods, suggested the presence of different stages of heparin synthesis in human and rabbit basophils.

Serotonin (5-hydroxytryptamine) has also been demonstrated in tissue mast cells of rats and mice, but not in man or rabbit[5]. No report concerning the presence of serotonin in the basophil leucocyte has been published.

In the present investigation, the influence of systemic administration of heparin and its antagonist protamine, as well as of serotonin and its releaser reserpine, on rabbit blood basophils has been studied.

The blood was diluted 1:10 with a modified[6] toluidine blue diluting fluid[7] for direct counting of basophils and all white blood cells in Fuchs-Rosenthal counting chambers.

Control counts were performed similarly, on the previous day in relation to injections of the solvent instead of the test-agent in the *same* animals.

Results.—Heparin injection was followed by a significant fall ($P < 0{\cdot}02$) in the number of basophils within 6 h, while protamine sulphate did not produce any significant change (Fig. 1).

6 h after administration of serotonin, an increase in the number of circulating basophils was observed, though statistically non-significant. This basophilia, however, seems to be part of a generalized leucocytosis (Fig. 2). In the other group of 10 rabbits, 24 h after serotonin injection, the basophil count proved to be significantly higher than that of the mean control count ($0{\cdot}02 < P < 0{\cdot}05$), the increase in the total leucocyte count was non-significant. The basophil and the total white blood cell count then returned to the control level within 3 days

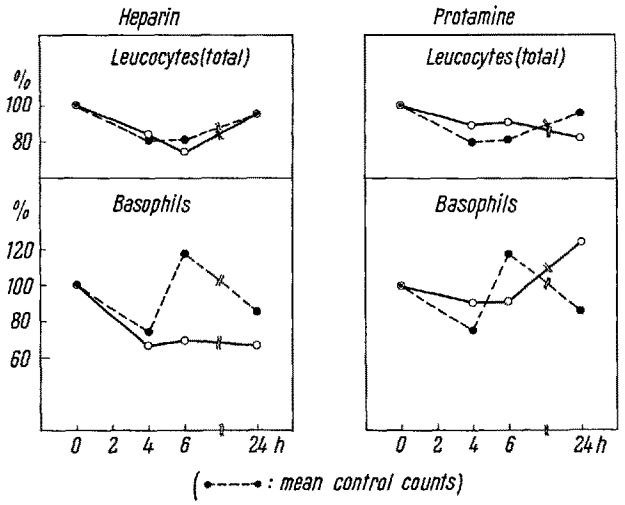

Fig. 1.—Effect of intravenous injection of heparin (1·5 mg/kg) and protamine (0·8 mg/kg) on basophil and all white cell counts of rabbit blood (mean for 10 rabbits).

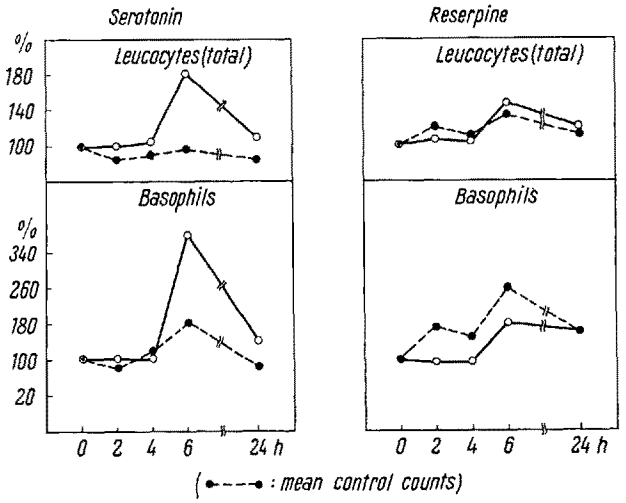

Fig. 2.—Effect of intravenous injection of serotonin (15 mg/kg) and reserpine (5 mg/kg) on basophil and total white cell counts in rabbit blood (mean for 5 rabbits).

Methods.—Male white rabbits of 2000–2500 g body weight were caged separately and not used until they had become accustomed to handling. *Heparin* (Leo, Copenhagen): 1·5 mg/kg, *protamine* (Roche, Basel): 0·8 mg/kg, *serotonin* (Nutritional Biochemical Corp., Ohio): 15 mg/kg, and *reserpine*: 5 mg/kg, were injected slowly at about 9 a.m. into ear veins of groups of 10, 10, 5, and 5 rabbits respectively. Blood samples were obtained immediately before as well as 2, 4, 6, and 24 h after injection. In another group of 10 rabbits (injected in the same way with serotonin), the counts were performed every morning at 9 a.m. for 4 successive days before and 3 days after a single intravenous serotonin injection.

(Fig. 3). Reserpine, on the other hand, did not induce any change in the basophil or the total leucocyte count (Fig. 2).

Discussion.—Following intravenous administration of heparin in *man*, ANGELI *et al.*[8] reported an increase in the number of circulating basophils, reaching a peak in 2 h, coincident with a rapid decrease in the anti-coagulant activity of the plasma. They attributed this effect to a specific regulatory action where the basophils 'fix' the circulating heparin. However, EIBER and DANISHEFSKY[9] reported that intravenously injected radioactive heparin is cleared from the blood of *man* within 3–7 h. Moreover, BOSEILA and ASBOE-HANSEN[10] did not find any increase

[1] This work was supported by grants from the Danish Rheumatism Association, and Eli Lilly and Co., Indianapolis, Indiana, U.S.A., to Dr. ASBOE-HANSEN.

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in the number of metachromatically granulated leucocytes following incubation of *rabbit* blood *in vitro* with heparin. So, it seems more likely that the observed changes in the number of circulating basophils (increase, or decrease as in the present experiment) reflect a generalized systemic reaction to heparin administration.

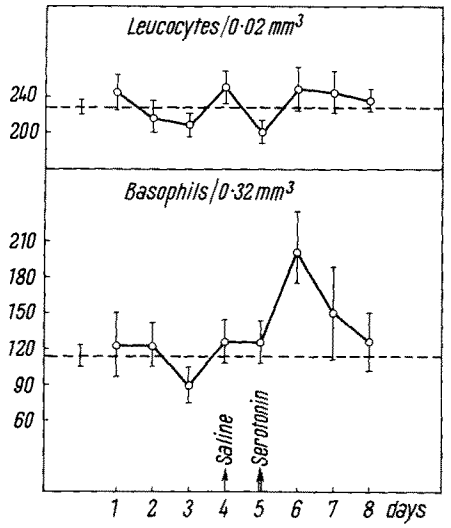

Fig. 3.—Effect of intravenous injection of serotonin (15 mg/kg) on morning counts of basophil and all white cells in rabbit blood (mean for 10 rabbits; standard error of the means indicated by vertical lines; ---- indicates the mean count of 4 control days).

Serotonin injection caused an increase in the number of circulating basophils in rabbit blood, significant only after 24 h, but it did not produce a specific, quick, and highly significant basophilia comparable to that occurring within 4 h as a response to histamine administration in the rabbit[11]. Whereas in the rabbit, histamine is a highly potent edema-producing factor, acting by an increase of capillary permeability, serotonin produces but a negligible increase in tissue water in this species[12]. This difference may explain the non-conformity between the actions of the two agents on the blood basophils in the rabbit.

Under the conditions of the present experiment, neither protamine nor reserpine seems to induce a measurable change in the number of circulating basophils of rabbit.

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Connective Tissue Research Laboratory, Institute of Medical Anatomy, Copenhagen (Denmark), May 8, 1959.

Résumé

Chez le lapin, l'injection intraveineuse d'héparine a été suivie d'une diminution significative du nombre des basophiles du sang au cours de 6 h. La sérotonine a produit pendant ce temps une augmentation du nombre total des leucocytes, et au bout de 24 h, une augmentation spécifique du nombre des basophiles.

Les basophiles n'ont pas changé de nombre après l'injection de protamine ou de réserpine.

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Studies on a Possible Method of Separation of γ-Casein by High-Speed Centrifugation and its Identification by Agar Electrophoresis

By high-speed centrifugation, various portions of casein separate out, according to their contents in the milk and the applied centrifugal force. HEYNDRICKX and VLEESCHAUWER[1,2] subjected milk, and its sera obtained by centrifugation, to moving boundary electrophoresis. From the results of investigation on centrifuged and non-centrifuged milk, the authors concluded that three caseins occur in milk, and identified the α- and β-components. They made no reference to γ-casein, but mentioned that the component which settled down faster and showed a decrease from 9·3 to 3·6% in the milk serum was also of a casein character.

The present report provides additional information as to the nature of the casein fraction that settles down on high-speed centrifugation at low temperature. Using a suitable concentration of the sediment, it has been possible to demonstrate by agar electrophoresis[3] the presence of a distinct band of γ-casein, and also three bands of casein fractions, in a mixed solution of the sediment and of the caseins precipitated from the clear serum.

Method.—Milk was dialysed in the cold in cellophane bags for 72 h against veronal buffer at pH 8·6 and ionic strength of 0·1. An aliquot of 20 ml of dialysed milk was centrifuged at 10,000 r.p.m. in the cold (2°C) for 30 min. This separated the fat out as a solid mass at the top leaving a clear serum, and a compact sediment at the bottom.

Treatment of the sediment: After removal of the layer of fat, the clear serum was decanted off completely. The sediment was washed twice with a solution of *N*/50 acetate buffer of pH 6·0 and then with cold ether. The sediment was finally dissolved in 1·0 ml of KCl-borate buffer solution of pH 10·0 (Solution 1).

Treatment of the serum: The casein fractions in the clear serum were precipitated at pH 4·6 with dilute acetic acid and centrifuged. The precipitate from 5·0 ml of clear serum was washed with cold ether and dissolved in 2·0 ml of KCl-borate buffer solution (Solution 2).

Solution 3 is a mixture of equal volumes of solution 1 and Solution 2 and, therefore, contained all the casein fractions present in milk. The concentration of the casein fraction that separated as a sediment on centrifugation was at a higher level in solution 3 as compared to that present in the original milk.

The solutions were subjected to agar electrophoresis for 6 h at 250 volts and a current strength of 8 mA. The electrophoretic diagrams are shown below in the following Figures.

Discussion.—Figure 1 shows a distinct band due to the casein fraction present in the sediment. In Figure 2, the same band is seen against two distinct bands due to the casein components present in the clear serum. The single band at the top having the lowest mobility is presumably due to γ-casein, while the two lower bands probably correspond to β- and α-caseins in the order of increasing mobility.

Figure 3 depicts the three bands due to α-, β-, and γ-caseins in the mixed solution, the band of γ-casein being

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