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The Vasoconstrictor Factor of Platelets

RAND and REID[1], studying the source of RAPPORT's serotonin[2], obtained active preparations only in cases in which the serum was made from whole blood or platelet rich plasma. On the basis of these results they conclude that "the appearence of RAPPORT's serotonin in serum depends on the presence of the lighter elements of the blood (probably platelets) at the time of clotting". The possible identification of the platelet vasoconstrictor factor with serotonin is questioned also by QUICK[1] and STEFANINI[3]. With the intention of solving the problem, we prepared platelets, free of plasma and other blood elements, by means of fractional centrifugations in a refrigerated centrifuge. From ethanol extracts of platelets, obtained from rabbit and sheep blood, it has been possible to separate by chromatographic procedure a fraction, which with EHRLICH's reagent gives the same color as serotonin, and which with diazotized sulphanilic acid develops a pink color not changing to orange following exposure to hydrochloric acid vapors. We used WHATMAN paper n° 1, employing as a solvent a mixture of ethanol, water and chloroform, in the ratio 80:20:50.

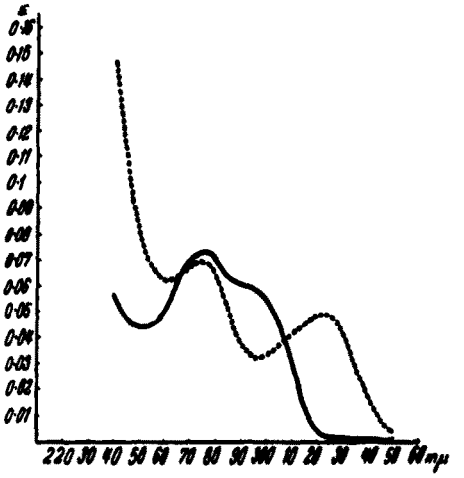

Fig. 1.–Extract of 0·02 cm³ of fresh platelets of rabbit. Eluate of the paper section giving violet color with EHRLICH's reagent: ultraviolet absorption spectrum in water at pH 7·3 (solid line) and pH 12 (dotted line).

The eluate of this section of the chromatographic strip gives in neutral water an ultraviolet absorption spectrum identical with that of RAPPORT's serotonin. Following alkalinization, the spectrum undergoes the same changes

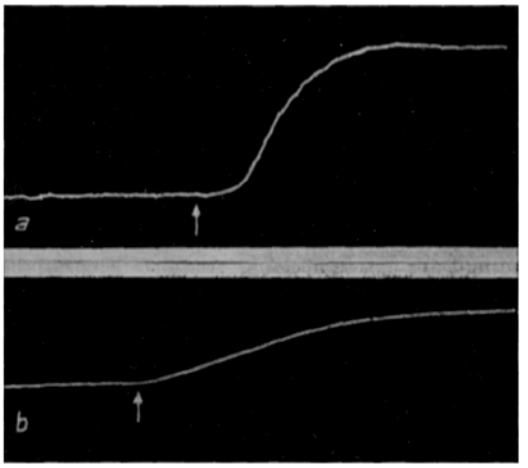

Fig. 2.–Extract of 0·005 cm³ of fresh platelets of rabbit. Eluate of the paper section giving violet colour with EHRLICH's reagent: (*a*) activity on isolated guinea pig gut suspended in 10 cm³ of TYRODE's solution; (*b*) activity on isolated rat uterus suspended in 10 cm³ of TYRODE's solution.

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as serotonin does (Fig. 1). Adding picric acid in water solution, typical crystals in shape of yellow-red rosettes are formed. This fraction of the platelet extract is very active, producing: (*a*) contraction of isolated guinea pig gut (Fig. 2*a*), even when neo-antergan is added to the medium; (*b*) contraction of the isolated rat uterus (Fig. 2*b*); (*c*) constriction of small vessels in rat meso-appendix preparation; (*d*) marked shortening of the bleeding time, even when animals are previously injected with heparin. The eluates of the remaining strip have none of the spectrophotometric properties and none of the biologic activities described above; histamine is found, but only in very small amounts.

The results show, beyond doubt, that the vasoconstrictor factor, contained in rabbit and sheep platelets, is identifiable with 5-hydroxy-tryptamine or serotonin of RAPPORT. We did not find this substance in blood elements others than platelets or in other tissues. From human platelet extracts it is possible to separate a fraction with the same biological properties as serotonin. We were not able, however, by means of chromatographic and spectrophotometric procedures, to identify the vasoconstrictor factor of human platelets with 5-hydroxytryptamine.

Addendum. In later investigations we succeeded to isolate also from human and dog platelet extracts, a fraction with all the spectrophotometric, chemical, and biologic properties of 5-OH-tryptamine. This has been made possible by using paper electrophoresis after complete removal of lipids from extracts.

The content of 5-OH-tryptamine is in the range of 100 γ per 1 g of fresh platelets in man and 4–5 times greater in rabbit.

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Central Laboratory, Villaggio Sanatoriale di Sondalo, and Institute of Medical Pathology, University of Siena, July 14, 1953.

Riassunto

Il fattore vasocostrittore delle piastrine di uomo, cane, coniglio e montone viene identificato, per mezzo di un'indagine combinata cromatografica e spettrofotometrica, con la 5-idrossitriptamina.

Amino Acid Composition of Crystallized Human Myoglobin and Haemoglobin

In a previous paper we have reported that myoglobin differs from haemoglobin by the chemical nature of the globin component[1].

Amino acid composition of myoglobins of a number of animal species (horse, ox, etc.) has been studied in several laboratories; however, to our knowledge, there is only the study of one of us[2] on the N partition, sulphur and iron content and amino acid composition of human myoglobin. We have now extended our previous research and have studied the qualitative composition of amino acid of human myoglobin and haemoglobin by filter paper partition chromatography.

Human myoglobin was crystallized according to the method suggested by one of us[3]; human haemoglobin was obtained in the crystalline form following the technique of Drabkin[4]. Samples of the crystallized proteins, with the iron content corresponding to the values reported in the literature, were hydrolysed in sealed test tubes with 6 N HCl at 120° for 6 h. The hydrolysed material was dried many times to remove HCl; it was then dissolved in a few milliliters of water and desalted with the Dent modification of the CONSDEN, GORDON, and MARTIN desalting apparatus[1]. A sample of this solution was used for the N determination, an another sample containing 150–180 μg of N was used for the paper two-dimensional chromatography on n. 4 WHATMAN filter paper. The solvent were phenol and collidine-lutidine. Cystine and methionine were identified after oxydation with ammonium molibdate and hydrogen peroxyde[2].

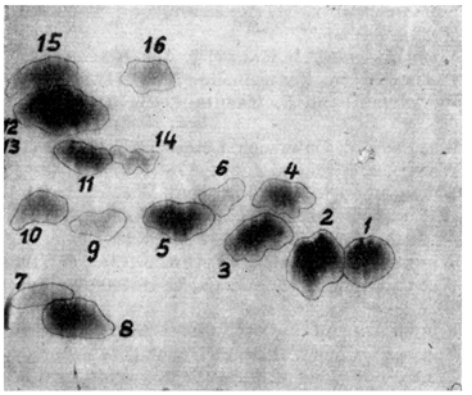

Fig. 1.–Two-dimensional chromatogram of hydrolysed crystalline human myoglobin (oxydized). Key for amino acids in the test.

Leucines were resolved by one-dimensional chromatography running for three days in tertiary amyl alcohol according to WORK[3].

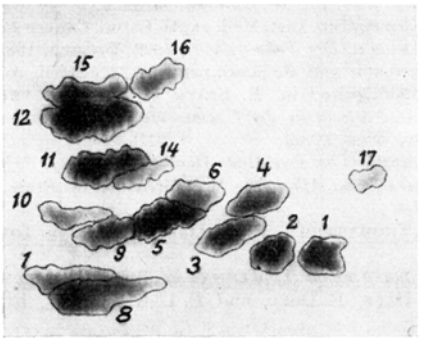

Fig. 2.–Two-dimensional chromatogram of hydrolysed crystalline human haemoglobin (oxydized). Key for the amino acids in the test.

In Figures 1 and 2 are reported the chromatograms of human myoglobin and haemoglobin (oxydized) run in phenol and collidine-lutidine.

Figures 3 and 4 represent the one-dimensional chromatograms of human myo- and haemoglobin run in tertiary amyl alcohol.

We may conclude that in human myoglobin the following amino acids are present: 1, aspartic acid; 2, glutamic

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