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.

M. BRACCO and P. C. CURTI

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].

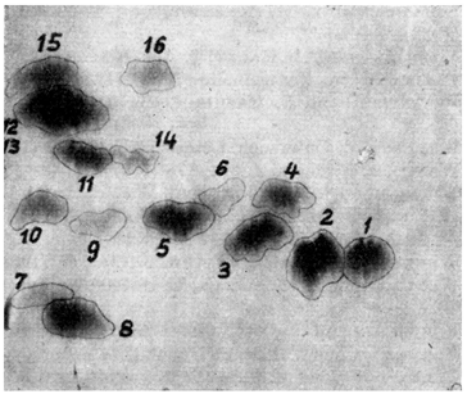

Fig. 1.–Two-dimensional chromatogram of hydrolysed crystalline human myoglobin (oxydized). Key for amino acids in the test.

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

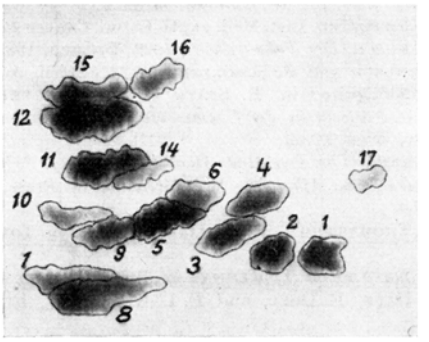

Fig. 2.–Two-dimensional chromatogram of hydrolysed crystalline human haemoglobin (oxydized). Key for the amino acids in the test.

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

[1] A. ROSSI-FANELLI, Arch. Sci. Biol. Napoli *26*, 244 (1940).
[2] A. ROSSI-FANELLI, Science *108*, 15 (1948).
[3] A. ROSSI-FANELLI, *Haemoglobin* (Butterworths Sci. Publ., London, 1949), p. 115.
[4] D. L. DRABKIN, J. Biol. Chem. *185*, 231 (1950).

[1] R. CONSDEN, A. H. GORDON, and A. J. P. MARTIN, Biochem. J. *41*, 590 (1947).
[2] C. E. DENT, Biochem. J. *43*, 169 (1948).
[3] E. WORK, Biochem. J. *42*, 49 (1948).

acid; 3, glycine; 4, serine; 5, alanine; 6, threonine; 7, arginine; 8, lysine; 9, histidine; 10, proline; 11, valine; 12, leucine; 13, isoleucine; 14, methionine; 15, phenylalanine; 16, tyrosine. In human haemoglobin we found: 1, aspartic acid; 2, glutamic acid; 3, glycine; 4, serine;

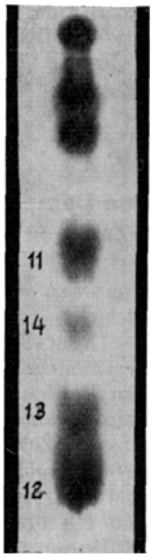

Fig. 3.–Hydrolysed crystalline human myoglobin in tertiary amyl alcohol. Key in the test.

5, alanine; 6, threonine; 7, arginine; 8, lysine; 9, histidine; 10, proline; 11, valine; 12, leucine; 14, methionine; 15, phenylalanine; 16, tyrosine; 17, cystine. Tryptophane has not been detected owing to its destruction during the HCl hydrolysis; its occurrence in both myo- and haemoglobin has, however, been confirmed[1].

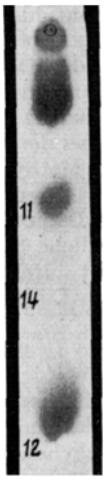

Fig. 4.–Hydrolysed crystalline human haemoglobin in tertiary amyl alcohol. Key in the test.

Besides the quantitative differences of several amino acids, to which we have already called attention elsewhere[2], haemoglobin differs from myoglobin because the former contains cystine but lacks of isoleucine, while the latter lacks cystine but contains isoleucine. The same differences have been found in the myo- and haemoglobin crystallized from the horse, with the exception that very small traces of a cystine-like substance appeared in myoglobin.

The origin of it will be investigated later.

These findings emphasize the deep discrepancies which exist in the chemical composition of the two proteins.

A. Rossi-Fanelli, D. Cavallini, and P. Merucci

Institute of Biological Chemistry, University of Rome, August 18, 1953.

Riassunto

È stata eseguita l'analisi cromatografica su carta degli aminoacidi costituenti la molecola della mioglobina ed emoglobina umana cristallizzate. I due pigmenti differiscono dal punto di vista qualitativo per il contenuto in cistina ed isoleucina. Il primo aminoacido si trova nella emoglobina e non nella mioglobina, il secondo solo nella mioglobina.

Risultati analoghi sono stati ottenuti con mioglobina ed emoglobina di cavallo.

On the Phosphorylation of Thiamine in the Living Animal

It is known that ATP is necessary for the phosphorylation of thiamine *in vitro* both with yeast (Lipton[1], Weil-Malherbe[2]) and animal tissues (Ochoa[3], Lipschitz[4], Leuthard[5]). Leuthard *et al.*[6] have recently isolated a liver fraction catalyzing the phosphorylation of thiamine by transposition of a pyrophosphoric radical from ATP to thiamine.

In the present paper we give evidence that a direct transposition of phosphoric groups takes place from ATP to thiamine in the living animal. For this purpose we have prepared ATP^{32} according to Dounce *et al.*[6] from muscles of rabbits killed 60 min after intravenous injection of 15 million counts of P^{32} as sodium phosphate[7]; the results of the analysis of the ATP so obtained are reproduced in the Table.

White rats of 200 g were injected intravenously with 30 mg ATP^{32} and 20 mg thiamine hydrochloride per kilogram of body-weight. The animals were killed 30 and 60 min after the injection and the liver contents of thiamine and of its mono-, di- and tri-phosphoric esters were determined by the chromatographic technique previously described[8]. The chromatograms (II) were sprayed with an alkaline terricyanide solution in order to localize the spots of thiamine and of its esters, and

[1] A. Rossi-Fanelli, *Haemoglobin* (Butterworths Sci. Publ., London, 1949), p. 115.

[2] A. Rossi-Fanelli, Arch. Sci. Biol. Napoli *26*, 244 (1940); Science *108*, 15 (1948).

[1] M. A. Lipton and C. A. Elvehjem, Cold Spring Harbor Symp. quant. Biol. *7*, 184 (1939).

[2] M. Weil-Malherbe, Biochem. J. *33*, 1997 (1939).

[3] S. Ochoa, Biochem. J. *33*, 1962 (1939).

[4] M. A. Lipschitz, V. R. Potter, and C. A. Elvehjem, Biochem. J. *32*, 474 (1938).

[5] F. Leuthard and H. Nielsen, Helv. chim. Acta *35*, 1196 (1952).

[6] A. L. Dounce, A. Rothstein, G. T. Beyer, R. Meyer, and R. M. Freer, J. Biol. Chem. *174*, 361 (1948).

[7] Supplied by the Atomic Energy Research Establishment, Harwell, Didcot-Berks.

[8] A. Rossi-Fanelli, N. Siliprandi, and P. Fasella, Science *116*, 711 (1952).