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;

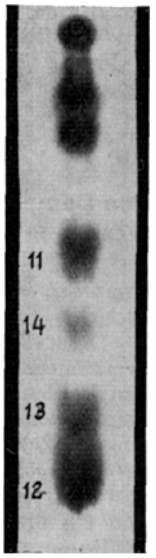


Fig. 3.–Hydrolysed crystalline human myoglobin in tertiary amyl alcohol. Key in the test.

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].

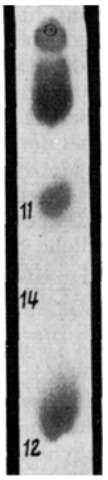


Fig. 4.–Hydrolysed crystalline human haemoglobin in tertiary amyl alcohol. Key in the test.

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.

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*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).

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[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).

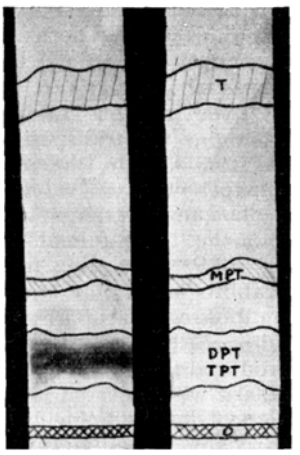
were subsequently kept in contact with a Ferrania X-Rays Film to demonstrate the presence of $P^{32}$(I).

Analysis and specific radioactivity of $ATP^{32}$

| Constituents | per cent |
|---|---|
| Hydrolysable phosphate (as P) | 7·65 |
| Total phosphate (as P) | 11·30 |
| Inorganic phosphate (as P) | 0·70 |
| ATP | 96·20 |
| ADP | 2·40 |
| AMP | 1·40 |
| **Material counted** | **Activity counts per min per micromole** |
| ATP before hydrol. | 345 |
| Labile P | 280 |
| Stable P | 60 |

P was determined according to FISKE and SUBBAROW[1]. Labile phosphate was hydrolysed in N HCl for 11 min at 100°. ATP, ADP, and AMP have been determined enzymatically according to KALCKAR[2]. $P^{32}$ was measured by means of a thin-mica window tube.

The Figure shows a typical radiochromatogram obtained from the liver of a rat killed 60 min after injection of $ATP^{32}$ and thiamine. The greatest amount of $P^{32}$ is localized in the spots corresponding to TPT and DPT,




I II

I Radiochromatogram and (II) chromatogram obtained from the liver of a rat killed 60 min after injection of $ATP^{32}$ and thiamine. TPT = triphosphothiamine; DPT = diphosphothiamine; MPT = monophosphothiamine; T = thiamine; O = Origin.

while no appreciable amount of $P^{32}$ is detectable in the spot corresponding to MPT. This suggests that $P^{32}$ is mainly localized in the two terminal phosphates of the phosphoric esters of thiamine.

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[1] C. H. FISKE and Y. SUBBAROW, J. Biol. Chem. *66*, 375 (1925).
[2] H. M. KALCKAR, J. Biol. Chem. *167*, 445 (1947).

*Riassunto*

Gli autori hanno preparato $ATP^{32}$ dai muscoli di coniglio trattato preventivamente con fosfato di sodio radioattivo. Iniettando per via endovenosa l'$ATP^{32}$ e tiamina a ratti albini è stato possibile mettere in evidenza, impiegando la tecnica cromatografica su carta, la formazione nel fegato di questi animali di $DP^{32}T$ e $TP^{32}T$. Questi risultati dimostrano che nell'animale vivente ha luogo una diretta trasposizione di gruppi fosforici dall'ATP alla tiamina.

## Die Wirkung von Demecolceinamiden an Zellen *in vitro*

Das von SANTAVY[1] aus den verschiedenen Organen der Herbstzeitlose (*Colchicum autumnale*) isolierte Demecolcin (Substanz F = Desacetyl-N-methylcolchicin[2]) wurde in unseren pharmazeutischen Laboratorien mit verschiedenen Aminen zu den den Colchiceinamiden entsprechenden Demecolceinamiden umgesetzt[3]. Diese haben wir auf ihre Wirkung auf Zellen *in vitro* geprüft.

*Prüfung an Hühnergefässfibroblasten:* Die Substanzen wurden in Tyrode gelöst den Kulturen 24 h nach der letzten Teilung während 8 h überschichtet. Für diese Versuche wurden 616 Kulturen verwendet.

Die Demecolceinamide führen wie Demecolcin[4] an Fibroblasten *in vitro* zu einem typischen colchicinartigen Effekt. Keiner der untersuchten Stoffe ist wirksamer als Colchicin. Das freie Amid ist weniger wirksam als das Mono-methyl- und das Mono-äthyl-amid, die die Wirksamkeit des Colchicins besitzen. Mit zunehmender Kettenlänge nimmt die colchicinartige Wirkung ab. Die disubstituierten Amide sind etwas weniger wirksam. LETTRÉ[5], der die entsprechenden Colchiceinamide an Fibroblasten prüfte, fand für das N-methyl-, N-äthyl- und N-dimethylderivat die stärkste Wirksamkeit, während N-propyl- und N-butylderivate einen Wirkungsabfall zeigten. Die von LETTRÉ mit der Colchiceinamidreihe und von uns mit der Demecolceinamidreihe gewonnenen Resultate zeigen in dieser Beziehung eine gute Übereinstimmung. Die mehrfach stärkere Wirksamkeit der Amide, die LETTRÉ bei der vergleichenden Prüfung von Colchicin und den Methyl- und Äthylcolchiceinamiden fand, konnten wir weder für Colchicein-monomethylamid noch für Demecolcein-monomethylamid nachweisen. Eine wesentliche Steigerung der Wirksamkeit an Fibroblasten tritt durch Einführung des Aminrestes in den Ring C des Colchicins bzw. Demecolcins nicht auf.

*Prüfung an Leukozyten in vitro:* 4 h nach Ansetzen der Leukozytenkulturen wurden die in Tyrode gelösten Substanzen während 12 h überschichtet[6]. Die Auswanderungsareale wurden planimetrisch ausgemessen und mit den Kontrollen verglichen. Die in Tabelle I angegebenen Werte sind Durchschnittszahlen aus 530 Einzelkulturen.

[1] F. ŠANTAVY, D. LANG und B. MALINSKY, Arch. int. Pharmacodyn. *84*, 257 (1950).
[2] F. ŠANTAVY, R. WINKLER und T. REICHSTEIN, Helv. chim. Acta *36*, 1319 (1953). – A. UFFER, O. SCHINDLER, F. ŠANTAVY und T. REICHSTEIN, Helv. chim. Acta *37*, 18 (1954).
[3] A. UFFER, Exper. *10*, 76 (1954).
[4] B. SCHÄR, P. LOUSTALOT und F. GROSS, Klin. Wo. *32*, 49 (1954).
[5] H. LETTRÉ, Angew. Chemie *63*, 421 (1952).
[6] R. MEIER und B. SCHÄR, Exper. *7*, 308 (1951).