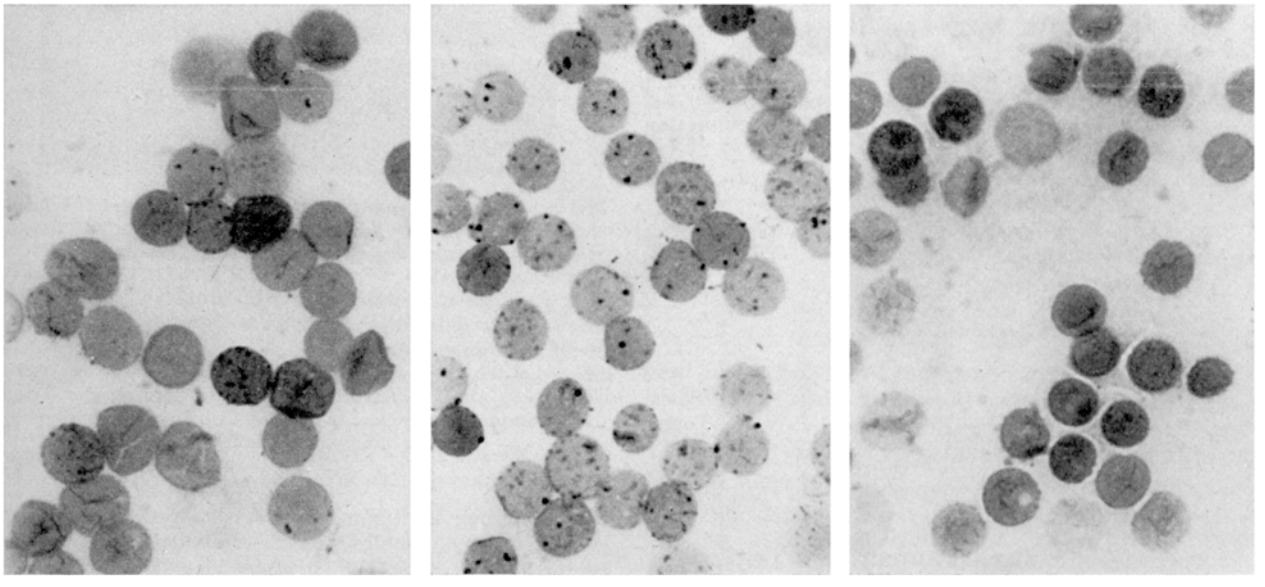
Effect of the Methemoglobinemie on Heinz Body Formation

It has been shown in a previous paper that the methemoglobinemic phase of acute nitrobenzone poisoning precedes the processes founded on peroxidative oxidation (Heinz body and verdoglobin formation)[1]. It seemed possible that it is the methemoglobinemia which inhibits the Heinz body formation. This action of the methemoglobinemia was investigated in an experiment presented in the present paper. Our experimental method was founded on the cyanide-peroxide test[2].

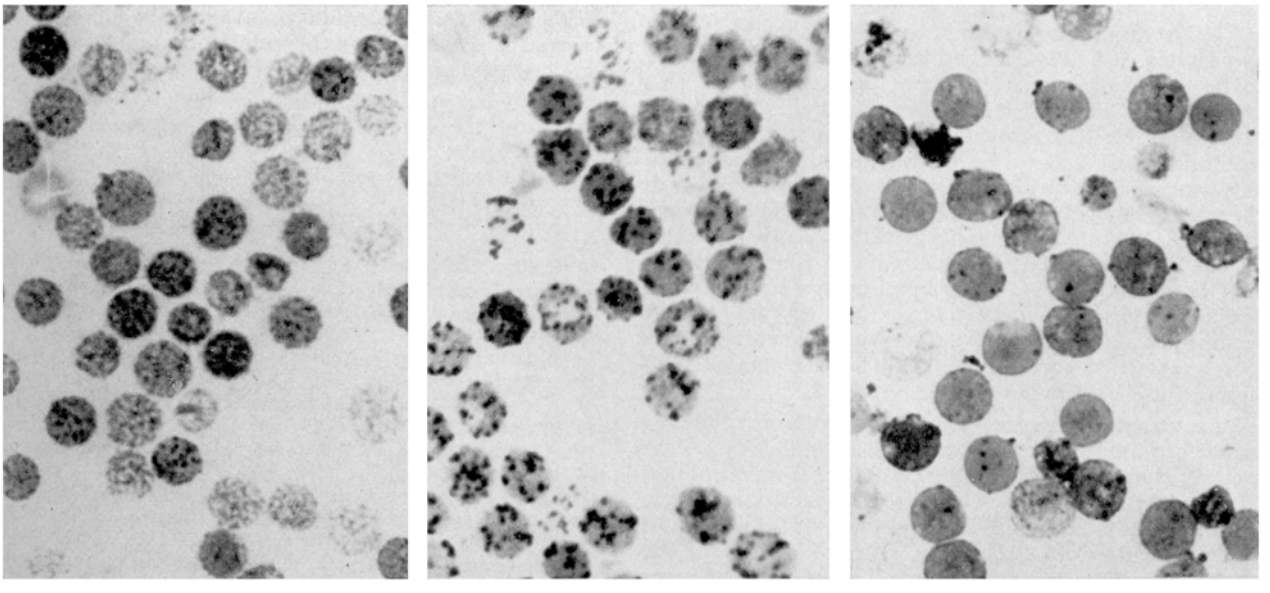
Citrate plasma of 5·0 ml human blood was separated by centrifugation and 15 ml of 2% sodium nitrite was added to the blood cells. After 15 min at room temperature, the red cells were washed with physiological NaCl twice, and after washing were complited with citrate plasma to 5 ml. The control sample was treated with physiological saline instead of nitrite. To 1 – 1 ml treated (methemoglobinemic) and control blood 1 – 1 ml 3×10^{-1}, 3×10^{-2}, and 3×10^{-3} potassium cyanide and after 5 min 1 ml of 1% hydrogen peroxide were added. This was carried at 37°C. Hydrogen peroxide was dissolved in $M/15$ phosphate buffer of pH 7·0. Solutions of lower concentrations prepared from 3×10^{-1} M of KCN were diluted with the same buffer solution.

The number of Heinz bodies in methylviolet preparations (supravital technique) were counted on samples taken after 90 min. It was found at every concentration

[1] L. Magos and M. Sziza, Acta haemat. *22*, 51 (1959).
[2] L. Magos, Exper. *12*, 264 (1956).



Ia Ib Ic



IIa IIb IIc

Heinz bodies produced by cyanide-peroxide test in normal (IIa, IIb, and IIc) and methemoglobinemic red blood cells (Ia, IIb, and IIc)

of KCN that the Heinz body formation was inhibited in red cells containing methemoglobin, as compared with control series. A typical experiment is presented in the Figure.

The question is: how the methemoglobin inhibits the Heinz body formation? The cause of the inhibition may be that the methemoglobin binding all the KCN, the breakdown of hydrogen peroxide is caused by the uninhibited catalase instead of peroxidatic utilization. This possibility was also examined. 0·1 ml KCN-blood mixture was added to 10 ml of $M/60$ phosphate buffer, pH 6·6, and the optical density was determined at 635 mμ before and after adding a drop of neutralised sodium cyanide. There has been no decrease of the optical density in the first (3×10^{-1} M KCN) and in the second sample (3×10^{-2} M KCN). It seems certain that all the ferric ions of the methemoglobin molecules, and likely that all the ferric ions of the catalase molecules, were bound to the cyanide in the second sample and there must be some excess cyanide in the first sample. Another possibility is that the methemoglobin, binding all the hydrogen peroxide, causes a hydrogen peroxide deprivation for the catalase and in this way inhibits the peroxidatic function of catalase.

L. MAGOS

State Institute of Occupational Medicine, Department of Industrial Hygiene, Budapest, September 1, 1959.

Zusammenfassung

Die methämoglobinisierten (mit Nitrit vorbehandelten) roten Blutkörperchen sind *in vitro* der Heinz'sche Körperchen bildenden Wirkung des Zyanid-Peroxyd-Testes gegenüber resistenter als normale Erythrozyten.

Autoradiographs of Human Sera Tagged with Radioactive Thyroxine and Investigated in Immunoelectrophoresis

The thyroxine circulating in the blood is mainly bound to proteins. It has been found that an α-globulin is likely to be the physiological carrier of the hormone[1–3]. This globulin has been referred to as the thyroxine-binding protein (TBP). If the blood concentration of thyroxine is increased, there is also a loose attachment to the albumin. Titration of the unutilized binding capacity of TBP can therefore be done by adding labelled thyroxine to plasma, using the albumin as a reference[2,3]. Normally, one third of the binding capacity is utilized[4]. Further studies of TBP have revealed that it has a molecular weight of about 50000, an iso-electric point below pH 4·5, and that it seems to be a glycoprotein[5,6]. Since thyroxine is relatively loosely attached even to the TBP, further characterization of the TBP has not been determined.

In contrast with ordinary electrophoretic methods, by means of immunoelectrophoresis[7] it is possible to obtain a closer characterization of precipitating components of, for example, human sera. Therefore, radioactive synthetic thyroxine[8] was added in varying amounts to plasma of a healthy euthyroid adult male. Pairs of samples of the plasma were then separated by means of agar-gel electrophoresis on object slides[9]. One of them was fixed in acetic acid, dried, and autoradiographed. On the other slide, the electrophoretically separated components were allowed to diffuse against a rabbit anti-human serum[7,9,10]. This slide was then dried and autoradiographed. The development revealed arc-shaped lines, the position of which were identical with certain precipitates in the agar-gel (Fig.). Two radioactive spots were obtained in the agar-gel electrophoretic pattern and two distinct arcs appeared in the radioautograph of the other slide when the concentration of the added thyroxine did not exceed 1 μg/ml plasma. The corresponding precipitates have been tentatively characterized as the α_1-glycoprotein[11] and the albumin. A higher concentration of labelled thyroid hormone gave a diffuse pattern in the agar-gel radioautogram but not any new arcs.

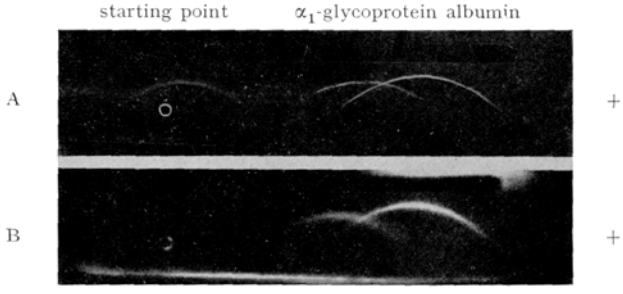


Fig. A: Immuno-electrophoretic pattern of a sample of human plasma to which labelled thyroxine was added (1 μg/ml). Starting point, the position of the α_1-glycoprotein and the albumin precipitates as indicated at the top. Negative pole was on the left, positive on the right. Slight retuch of the α_1-glycoprotein arc. On the original slight at least 14 precipitates were seen

Fig. B: Radioautograph of the plate shown in A. Although the radioactivity of the α_1-glycoprotein precipitate was weaker than that of albumin arc, the specific activity of the former was higher because the glycoprotein was present in the plasma in much lower concentration than the albumin

The finding of distinct radioactive precipitates from plasma to which small amounts of labelled thyroxine had been added, permits the following conclusions. It is highly probable that the TBP is at least partly identical with an α_1-glycoprotein; this is in agreement with previous assumptions in the literature[3,5,6]. We cannot exclude the existence of other TBP components not developed by the immune serum used, but they must occupy closely identical electrophoretic positions. No radioactivity could be

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[4] E. D. ALBRIGHT, F. C. LARSON, and W. P. DEISS, J. clin. Invest. *34*, 44 (1955).

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[7] P. GRABAR, and C. A. WILLIAMS, Biochim. biophys. Acta *10*, 193 (1953).

[8] Labelled thyroxine from Amersham, England (I^{131}, approximately 5 mC per mg l-thyroxin; stock solution contains 0·2 mg thyroxin in 50% aqueous propylene glycol).

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[10] J. J. SCHEIDEGGER, Int. Arch. Allergy *7*, 103 (1955).

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