des cristaux d'apatite débutait dans les fibrilles collagènes et se poursuivait dans la substance fondamentale.

Quant à nos observations sur la condensation de la substance fondamentale, elles sont semblables à celles de YAEGER[7] et de TAKUMA[11], qui concernent l'ossification de membrane et l'os primaire.

Enfin, la constatation par diffraction des rayons X de la nature apatitique des dépôts de substance minérale confirme les résultats obtenus précédemment par SELYE[12].

Summary. In the calcified deposits of the experimental cutaneous calcinosis, the apatite crystals measure approximately 5 nm in diameter and 50 nm in length; they are associated with the collagen fibrils but without any preferential orientation.

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[11] S. TAKUMA, Bull. Tokyo dent. Coll. *4*, 1 (1963).
[12] H. SELYE, Surg. Clin. N. Am. *43*, 1483 (1963).

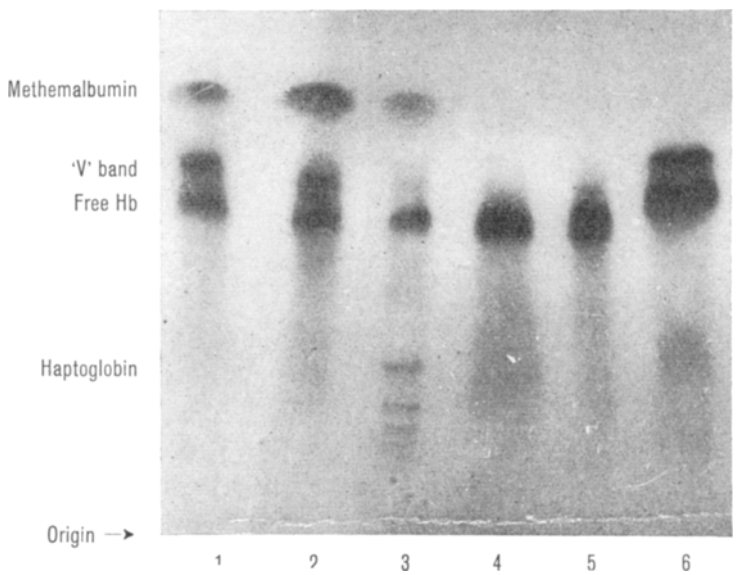
Formation of a Peculiar Heme-Protein Fraction in Plasmas Containing Hemoglobin[1]

In the course of our studies concerning the behaviour of some protein fractions (haptoglobin (Hp), methemalbumin (MetAlb), free hemoglobin (Hb)) in plasma of children (378 plasmas examined) in various physiological and pathological conditions (neonatal jaundice, thalassemia, favism), by means of starch-gel electrophoresis, we have observed in several instances the presence of a particular benzidine-positive band (referred to as 'V') which does not correspond to the above-mentioned fractions. The band is in a more advanced position towards the positive pole as compared to the free Hb band and is closer to the latter when compared to the MetAlb band (Figure).

The presence of the 'V' band appeared closely related to that of free Hb and was found more frequently in the cases without Hp (the difference, however, was not statistically significant: $0.5 > P > 0.3$). Its presence was almost constantly associated with that of the MetAlb band; when the 'V' band was absent, the MetAlb band was prevalently absent too (the difference was statistically significant: $\chi^2 = 48$; $P \ll 0.01$).

Both in full-term and in premature new-born infants presenting free plasma Hb, the 'V' fraction appeared more frequently in the first few days of life; and in this period it was found more frequently in the premature than in full-term infants (the difference, however, is not statistically significant: $0.5 > P > 0.3$). In nine children with thalassemia major the 'V' band was present only once out of five times with free Hb. In four cases of favism with hemolytic crises, the 'V' band was constantly present up to the fifth or sixth day following the crises.

[1] The presence of a fast hemoglobin fraction in the plasma and urine of adults with hemolytic anaemias has recently been reported[2]. A few years ago[3–5] the authors of the present article made analogous observations on the plasma of children with various hemolytic conditions. Their studies, however, were published in Italian and might have been overlooked. Thus it seemed worthwhile to report now in English the relevant data of these investigations together with some additional observations made on the subject during the last few years.
[2] H. R. MARTI, Exper. *21*, 199 (1965).
[3] E. BOTTINI, G. FILIPPI, and G. MAGGIONI, Arch. Ital. Ped. Puer. *22*, 368 (1962).
[4] E. BOTTINI, F. COZZI, and G. MAGGIONI, Boll. Soc. Ital. Biol. sper. *39*, 976 (1963).
[5] E. BOTTINI, F. COZZI, and G. MAGGIONI, Boll. Soc. Ital. Biol. sper. *39*, 979 (1963).



Plasma from 1-day-old premature infant. Starch gel electrophoresis in discontinuous system, stained with benzidine. 1, Plasma of the premature infant before exchange-transfusion. 2, The same plus dithionite. 3, Plasma of the premature infant after exchange-transfusion. 4, Hemolysate from the same premature infant. 5, The same plus dithionite. 6, Hemolysate from normal adult aged 8 days.

In a few cases of favism in which the analysis of urine was performed, a benzidine-positive fraction was found in a position intermediate between free Hb and MetAlb.

Preliminary experiments in the identification of the 'V' band have been carried out.

0.5% and 0.3% solutions of Hb (obtained from cord blood hemolysates) were incubated at 37°C for approximately 12 h with plasma obtained from a 1-day-old premature infant (0.6 kg), from the umbilical cord of a full-term infant, and from a normal adult, separately; the solutions were then examined by means of starch-gel electrophoresis. The 'V' band was present in the first case and was less apparent in the second case, whereas solutions containing adult plasma yielded no 'V' band at 0.3% and only a slight trace at 0.5% concentration. MetAlb was constantly present. Hemolysates without plasma did not present any band comparable to the 'V' band.

When the experiment was repeated with the ultrafiltrate of the above-mentioned plasmas, the band did not appear; but it was again observed when the concentrate of the plasmas was used.

Similar results were obtained by incubating normal adult Hb with umbilical cord plasma. We also incubated cord plasma with canine Hb; the latter is known to have a lesser electrophoretic mobility – at an alkaline pH – than Hb A. Electrophoresis revealed, besides a MetAlb band, a benzidine-positive band whose advanced position in respect to the canine Hb band was comparable to the advanced position of the 'V' band in respect to the HbA band.

After preserving the plasma for approximately 50 days at 4°C, the results were less apparent.

The results of our experiments in vitro seem to favour the theory that the 'V' band is to a large extent a result of the transformation of Hb (type HbA_3) and that in plasma there are non-ultrafiltrable factors capable of accelerating Hb transformation as compared to hemolysates; these factors appear to be more active in the first few days of life[6].

Riassunto. Nel plasma di neonati e di bambini in varie condizioni emolitiche è stata osservata una peculiare frazione proteica benzidino-positiva, elettroforeticamente più veloce dell'Hb libera. I risultati della casistica e di indagini sperimentali suggeriscono che questa frazione sia un prodotto di trasformazione dell'Hb (del tipo HbA_3) e che nel plasma esistano sostanze non ultrafiltrabili capaci di accelerare la trasformazione a paragone di quanto si osserva nell'emolizzato. Queste sostanze sembrano più attive nei primi giorni di vita, specie nell'immaturo.

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[6] Supported by 'Consiglio Nazionale delle Ricerche' (Italy).

Glycerol Permeability of Erythrocytes

Assuming the red blood cell to act as a perfect osmometer, JACOBS et al.[1,2] investigated the permeability of red cells by following the rate of hemolysis in isotonic solutions of non-electrolytes. Striking differences were found to exist between the erythrocytes from various species of mammals. A comparison between these variations in permeability behaviour and variations in certain lipid characteristics of a limited number of animals suggested a possible relation between these distinct characteristics[3]. In order to obtain further information these investigations have meanwhile been extended.

In Figure 1 the rate of hemolysis in 0.303 *M* glycerol is given as a function of temperature for erythrocytes from nine different species of animals. These results confirm the observations of JACOBS et al.[2] who already reported on the existence of two distinct groups of erythrocytes. The red cells of one group (rat, man, rabbit and guinea-pig) exhibited rapid hemolysis which was rather independent of the temperature. Those of the second group (pig, dog, cat, sheep and ox) exhibited slow hemolysis but revealed a significant increase in permeability at elevated temperatures. At 37°C or at higher temperatures the differences between the two groups are less pronounced. In order to account for the permeation of polar compounds such as glycerol which will not easily pass through a continuous lipid barrier, the existence of water-filled pores in the membrane has repeatedly been postulated. These channels could be formed by proteins[4], but if besides lamellar arrangement of the lipid layers also hexagonal patterns occur in biological interfaces[5–10], other possibilities for the existence of polar pores are apparent.

The temperature dependence of the glycerol penetration into the red cells of the second group of animals may be explained by minor alterations in the orientation of the lipid molecules. Considering the differences in the permeability behaviour of the erythrocytes between the two groups of animals it may be supposed that the properties of a membrane structure transforming between different lipid phases perhaps depend on the chemical composition of the lipids. Some lipid analyses of the red cells from the animals under discussion were carried out in chloroform-methanol extracts. Fatty acid analysis of the total lipids

[1] M. H. JACOBS, H. N. GLASSMAN, and A. K. PARPART, J. exp. Zool. *113*, 277 (1950).
[2] M. H. JACOBS, H. N. GLASSMAN, and A. K. PARPART, J. cell. comp. Physiol. *7*, 197 (1935).
[3] L. L. M. VAN DEENEN and J. DE GIER, *The Red Blood Cell* (Ed., C. BISHOP and D. M. SURGENOR; Academic Press 1964), p. 243.
[4] W. D. STEIN and J. F. DANIELLI, Disc. Faraday Soc. *21*, 238 (1956).
[5] A. K. PARPART and R. BALLENTINE, *Trends in Physiology and Biochemistry* (Ed. E. S. G. BARRON, Academic Press, 1952), p. 135.
[6] V. LUZZATI and F. HUSSON, J. Cell Biol. *12*, 207 (1962).
[7] F. S. SJÖSTRAND, Nature *199*, 1262 (1963).
[8] J. L. KAVANAU, Nature *198*, 525 (1963).
[9] J. A. LUCY, J. theoret. Biol. *7*, 360 (1964).
[10] B. ROELOFSEN, J. DE GIER, and L. L. M. VAN DEENEN, J. cell. comp. Physiol. *63*, 233 (1964).