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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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

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by the method published previously[11] revealed some conspicuous differences. Particularly the percentages of eicosatetraenoate, octadecenoate and stearate vary considerably among erythrocytes of different species of animals (Table).

Although previous studies on a more limited number of animals suggested that there might be some relationship between the glycerol permeability and the fatty acid pattern of the erythrocytes, the present results do not permit the postulation of any clear correlation. In agreement with previous studies on rabbits[12] and rats[13], dietary induced changes in the fatty acid composition of erythrocyte lipids appeared not to be reflected by considerable variations in the penetration rate of glycerol (Table).

Phospholipids were separated by thin layer chromatography, phosphor analyses of the separated spots giving information about the proportions of the various classes. With respect to the content of choline phosphoglyceride the following percentages were recorded: guinea-pig 52.9%, rat 47.5%, dog 46.5%, rabbit 34.4%, cat 31.3%, man 29.7%, pig 28.4%, ox 6.2% and sheep 3.4%. In agreement with earlier observations[14] on a more restricted number of animal species, the erythrocytes having at low temperature a high permeability to glycerol contain a high percentage of lecithin. However, no simple correlation exists between time of hemolysis and the percentages of these phosphoglycerides, since there is a high lecithin level in erythrocytes from the cat and the dog as well. Besides the variations in lecithin we find significant differences in phosphatidyl ethanolamine phosphoglyceride. Low lecithin percentages are mainly compensated by an increase of sphingomyelin from which, however, phosphatidyl serine was not separated by the method used. A detailed computation of the charged groups of the lipid molecules and other compounds in these membranes is highly desirable. The importance of ionic forces in relation to the existence of polar pores is strongly suggested by the effect of pH on the permeability of glycerol (Figure 2). A lowering of the pH of the environment from 7.8 to 5.0 causes a great increase in hemolysis times of the rabbit red cell, giving it a temperature dependence which resembles that of the erithrocytes of the second group.

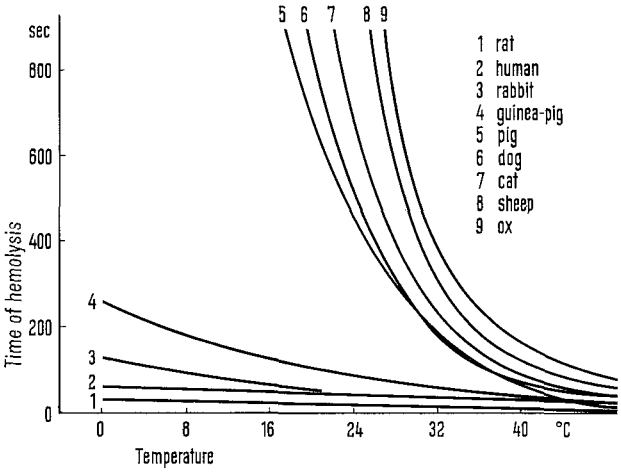

Fig. 1. Rate of hemolysis in isosmotic glycerol of erythrocytes from different mammals in relation to the temperature. Fresh citrated blood was diluted 1:10 with saline while stirring. 1 ml of this red cell suspension was added to 9 ml of 0.303 *M* glycerol (pH 7.0). The turbidity was followed with a colorimeter fitted with a red filter (625 mμ) until the scattering was equal to that of a standard 75% hemolysis.

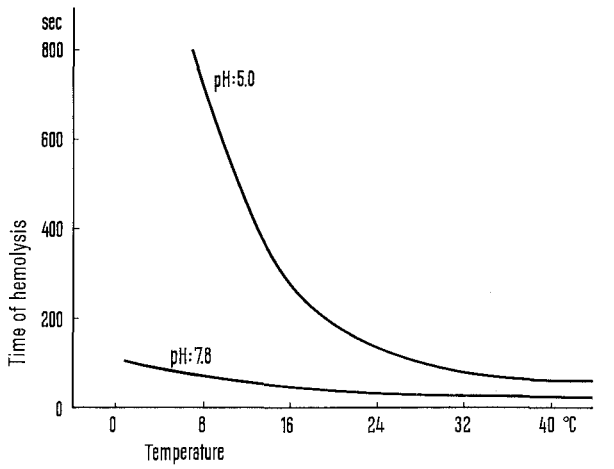

Fig. 2. Rate of hemolysis of rabbit red cells in phosphate buffer at pH 7.8 and 5.0 respectively. The red cells were washed with the buffer solution until the pH of the solution was constant. Measurements were carried out as described under Figure 1.

Fatty acid composition of red cell lipids from different mammals

Chain length and number of double bonds	16:0 (%)	16:1 (%)	18:0 (%)	18:1 (%)	18:2 (%)	20:3 (%)	20:4 (%)
Rat group 1[a]	28.7	1.8	12.1	14.5	6.4	Trace	33.8
Rat group 2[b]	19.6	2.7	9.7	35.3	9.5	7.0	12.5
Guinea-pig	16.1	2.6	26.6	12.3	15.7		16.1
Dog	16.9	1.7	19.0	14.2	12.9		30.8
Cat	20.1	2.6	17.8	11.0	21.5		18.5
Rabbit	22.3	3.3	10.5	11.8	32.0		6.6
Man	27.1	3.4	9.4	19.5	16.5		19.5
Pig	21.4	2.4	10.4	32.1	23.2		6.4
Ox	12.1	2.7	14.1	34.5	21.1		4.8
Sheep	15.7	1.6	9.6	52.3	14.6		2.9

[a] Group 1 was fed a normal diet; time of hemolysis at 18°C 15.6 ± 0.8 min. [b] Group 2 was fed an essential fatty acid deficient diet; time of hemolysis at 18°C 14.3 ± 0.7 min.

Zusammenfassung. Untersuchungen über die Unterschiede in der Lipoidzusammensetzung der Erythrocyten von neun Tierarten ergaben eine verschiedene Glyzerinpermeabilität dieser Zellen. Bei der einen Gruppe zeigen die Erythrocyten eine stark temperatur bedingte Glyzerinpermeabilität, bei der anderen eine hohe, kaum temperaturabhängige Permeabilität, die bei Erniedrigung des pH-Wertes im Medium stark abnimmt.

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