dative phosphorylation nor optical density seems to be notably impaired, but the nitrogen content of mitochondrial suspensions decreases significantly. Therefore this second type of avitaminosis would appear to affect the mitochondrial population rather than energetic metabolism or structural integrity of the protoplasmic granules.

Is Alkali-Resistant Haemoglobin in Cooley's Anaemia Different from Foetal Haemoglobin?

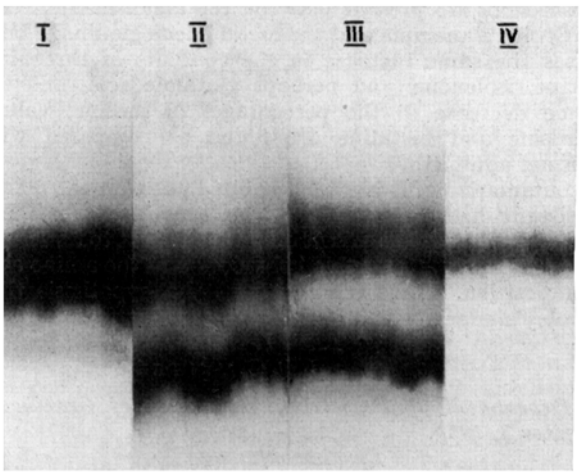
Recently PEROSA and BINI[1] reported in this Journal some differences between the alkali-resistant haemoglobin fraction in Cooley's anaemia and the foetal haemoglobin from cord blood. These differences were found in (1) the rates of denaturation of the carbon monoxide derivates both by alkali and by acids; (2) the rate of denaturation of the corresponding oxyhaemoglobins by acids, while (3) there were also indications that paper electrophoresis gives evidence of a different behaviour for both haemoglobins.

However, a number of other properties have been reported in the literature, in which this haemoglobin and the foetal haemoglobin behave identically: the ultraviolet spectral absorption[2], immunological behaviour[3], the denaturation rate of the oxyhaemoglobins by alkali[4] and the *N*-terminal residues[5]. Therefore it is not yet clear whether the abnormal haemoglobin in Cooley's anaemia is identical with the foetal haemoglobin from cord blood or not.

Some other techniques, by which differences between the normal adult Hb and the foetal Hb have been found, are: salting-out experiments[6], chromatographic analyses[7] and the estimation of the amino acid composition[8]. By applying these 3 methods to the alkali-resistant haemoglobin in Cooley's anaemia, it may be possible to contribute towards the resolution of the problem.

For the investigations reported here, a blood sample was used of a little boy suffering from Cooley's anaemia. Besides the normal adult Hb and the alkali-resistant fraction, the blood also contained a certain amount of the abnormal haemoglobin E[9]. As it was possible to separate the alkali-resistant fraction completely from the other 2 Hb's by means of a chromatographic procedure, the presence of the abnormal haemoglobin E did not influence the results obtained.

The salting-out experiments were carried out as described by DERRIEN[10] using a 3·5 molar phosphate buffer solution, pH 6·5. 2 haemoglobin samples were investigated: the haemoglobin of the patient (41% alkali-resistant fraction, measured by the method of BRINKMAN and JONXIS[11] and by a chromatographic technique[7]) and an artificial mixture, containing 41% of Hb–F and 59% of Hb–A. No differences were found. In both cases a fraction was present which was salted out at a somewhat higher phosphate concentration than the normal adult haemoglobin and the abnormal Hb–E that has the same solubility as Hb–A[9].



The chromatographic behaviour of the alkali-resistant haemoglobin in Cooley's anaemia.

I. Cooley trait (father of the patient studied; alkali resistant fraction 9%);
II. Cooley trait after addition of Hb–F.
III. Cooley's anaemia.
IV. Normal adult Hb.

Also the chromatographic behaviour of the alkali-resistant haemoglobin, present in the blood of the patient studied was identical with that of foetal haemoglobin. The Figure shows some results obtained with the "cuvette method" using the cation exchanger Amberlite IRC–50 (XE–64)[7]. It will be noted that Hb–A and Hb–E have practically the same rate of displacement.

The amino acid composition of a 48 h hydrolysate of the alkali-resistant fraction in Cooley's anaemia·

Amino Acid	Alkali-resistant fraction	Hb–F	Hb–A
Aspartic	10·9	10·25	10·5
Threonine	6·3	6·5	5·5
Serine	5·5	5·85	4·45
Glutamic	7·85	7·65	7·25
Proline	4·05	4·2	4·95
Glycine	4·3	4·6	4·55
Alanine	9·65	9·7	9·95
Cystine/2	0·95	0·95	1·00
Valine	9·35	9·5	10·9
Isoleucine	1·75	1·85	0·35
Leucine	15·1	15·3	15·15
Tyrosine	3·15	3·2	3·85
Phenylalanine	7·9	7·85	7·85
Lysine	9·7	9·85	9·9
Histidine	7·25	7·4	8·3
Arginine	3·3	3·35	3·35

The values are given as g/100 g protein.

By complete elution on an Amberlite IRC–50 (XE–64) column, it was possible to isolate the alkali-resistant

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fraction free from the other components. This fraction was used for the estimation of the amino acid composition. The analyses were carried out in the manner described earlier[8]. The results obtained are given in Table I and compared with those described for both normal adult and foetal haemoglobin. No significant differences are present between the alkali-resistant Hb in Cooley's anaemia and the foetal haemoglobin. In both cases the same increase in the contents of threonine, serine, isoleucine and perhaps glutamic acid, and the same decrease in the percentages of proline, valine, tyrosine and histidine are found as compared with normal adult Hb.

Summarizing, it has been pointed out that the alkali-resistant haemoglobin in Cooley's anaemia does not differ from foetal haemoglobin, with respect to its solubility, the chromatographic behaviour and the amino acid composition. These data support the hypothesis that Cooley's anaemia and foetal Hb's are identical.

T. H. J. HUISMAN, H. K. PRINS, and P. C. VAN DER SCHAAF

Department of Pediatrics, University of Groningen, October 3, 1955.

Zusammenfassung

Es wurde festgestellt, dass sich das alkaliresistente Haemoglobin bei Coaleys Anaemie und fötales Haemoglobin gleich verhalten in bezug auf ihre Löslichkeit, ihre chromatographische Auftrennung und die Zusammensetzung ihrer Aminosäurebausteine. Die Resultate stützen die Hypothese einer Identität der beiden untersuchten Haemoglobinarten.

Changes in Red Cells Metabolism in Presence of Incomplete Antibodies

Although the incomplete antibodies largely account for the haemolysis in erythroblastosis foetalis and in acquired haemolytic anaemias, no definite knowledge has so far been gained as to their action mechanism. It can readily be demonstrated that the red cells have adsorbed incomplete antibodies on their surfaces[1], but in which way the antibody-coated erythrocytes are destroyed is still a problem to be solved.

The incomplete antibodies display *in vitro* agglutinating power only when special devices, such as the use of bovine albumin media[2] or trypsinized erythrocytes[3], are employed, but it is doubtful whether these types of serological reactions can be compared with the agglutination determined by complete antibodies. It can be shown, for example, that normal sera may also, under certain conditions, agglutinate the trypsinized erythrocytes[4], but in no case does the complement addition result *in vitro* in haemolysis of incomplete antibody-coated red cells.

The *in vitro* behavior of incomplete antibodies does not correspond to an actual agglutinating power *in vivo*: the presence of sludged blood as shown by WASASTJERNA *et al.*[5] in the conjunctival vessels of patients with haemolytic anaemia and positive Coombs test, cannot be considered as definite evidence of intravascular agglutination since such phenomenon can also be found in a wide variety of diseases[6], whenever the blood sedimentation rate is increased[7], thus suggesting that the blood sludge formation is a product of rouleaux aggregation rather than an immunological reaction.

The purpose of this study is to test the possibility that the red cell metabolism (and especially the glycolytic and the cholinesterase activities) may be affected by incomplete antibodies. It is known that in mammalian red cells the energy available as a result of metabolic activity arises chiefly from glycolysis[8] and that the electrolyte balance inside the cell is maintained by this energy[9]. Nevertheless, a glycolysis inhibition, however it is achieved, brings about a loss of K and an accumulation of intracellular Na with increase of cell water[1], thus causing the swelling of the erythrocyte.

Cholinesterase also interferes with red cell cation exchange[10], and moreover a close relationship exists between glycolysis and cholinesterase activity[11].

There is some evidence that a part at least of the enzymes involved in these enzymatic systems is located at the cell surfaces[12], so that the incomplete antibodies, when adsorbed on to red cell membrane, might exert a direct damaging action.

In this report we will examine the effect of incomplete antibodies upon erythrocyte glycolysis.

Materials and Methods. Our experiments were carried out upon total defibrinated blood and/or red cells washed and suspended in Tyrode solution; in some cases, Armour bovine albumin was added to the sensitized red cells to achieve agglutination. The technical details can be found in the Tables. As a source of incomplete antibodies, an anti-D serum, whose isoagglutinins were previously carefully adsorbed, was used. The incomplete agglutinins titre of this serum was 1:256 (+) in bovine albumin or with trypsinized erythrocytes. Its effectiveness was controlled in all experiments by carrying out the Coombs test on red cells after glycolysis estimation. The red cells were obtained from normal D-positive donors.

The initial glucose level was regulated in the samples at approximately 2‰ and then resupplied with an additional intake when the experiment exceeded 6 h. The blood samples were incubated and continuously shaken in a water bath at 37°C for a period of 3–12 h. Glycolysis, measured as glucose consumption, was determined by taking hourly samples (always double) for sugar titration. Glucose was estimated by Hagedorn-Jensen method.

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