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.

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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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Table I.—Effect of the anti-D incomplete antibodies upon glycolysis of the D-positive red cells. Value are expressed as % of their respective controls.

Number of experiments executed	Experimental conditions (see also footnote)	First sample. Direct Coombs test: + + +	Second sample. Direct Coombs test: + — —
4	Defibrinated blood plus anti-D serum (1)	73 ± 8	77 ± 11
4	Red cells washed and suspended in Tyrode plus anti-D serum (2)	76 ± 9	81 ± 4
5	Red cells sensitized with anti-D serum, then rewashed and suspended in Tyrode (3) . . .	101 ± 15	99 ± 12

Composition of samples: (1) First sample: defibrinated blood, 4 cm³; anti-D serum, 0·30 cm³. Second sample: defibrinated blood, 4 cm³; anti-D serum, 0·06 cm³. (2) First sample: washed red cells suspended in Tyrode solution, 4 cm³ (2 cm³ of packed red cells and 2 cm³ of Tyrode solution); anti-D serum, 0·30 cm³. Second sample: washed red cells suspended in Tyrode solution, 4 cm³; anti-D serum, 0·06 cm³. (3) First sample: 4 cm³ of red cells washed and suspended in Tyrode solution (2 cm³ of packed red cells and 2 cm³ of Tyrode solution) are incubated at 37°C for 1 h with 0·30 cm³ of anti-D serum, then rewashed and resuspended in Tyrode. Second sample: as the first, except that the incubation was made with 0·06 cm³ of anti-D serum.

Results. Our findings are reported in 2 Tables and may be summarized as follows: (1) The addition of anti-D serum to defibrinated blood results in a certain inhibition of glycolysis: a decrease of about 25–30%. (2) Similar results, even if slighty less conspicuous, are obtained when anti-D serum is added to red cells washed and suspended in Tyrode solution. (3) If, however, the washed red cells are incubated at 37°C for 1 h with anti-D serum and then rewashed before resuspending them in Tyrode solution for glycolysis estimation, the resulting values do not significantly differ from the controls. The reason for this behavior remains obscure at this stage, although it can be hypothetically assumed that the presence in the medium of free antibodies or simple serum is needed for glycolysis inhibition. Further work is in progress to elucidate this point.

It is very interesting to note that agglutination *per se* does not affect erythrocyte glycolysis. In fact, the addition of albumin to a sample containing anti-D serum and red cells washed and suspended in Tyrode solution, does not modify the inhibition percentage (Table II). Taking this observation as a starting point, we have also examined the influence of complete antibodies (iso-agglutinin anti-A) on erythrocyte glycolysis, finding no trace of inhibition, whatever the intensity of the agglutination. In some experiments, moreover, the glucose uptake of agglutinated red cells overcomes that of the controls.

Table II.—Influence of agglutination on the inhibition caused by incomplete antibodies on red cells glycolysis. Values are expressed as % of their respective controls.

Number of experiments	First sample (1) Agglutination: — — —	Second sample (2) Agglutination: + + +
First experiment (duration: 3 h)	87	86
Second experiment (duration: 12 h)	77	80

Composition of the samples: (1) Red cells washed and suspended in Tyrode solution, 4 cm³ (2 cm³ of packed red cells and 2 cm³ of Tyrode solution); anti-D serum, 0·30 cm³. (2) Red cells washed and suspended in Tyrode solution containing bovine albumin 20%, 4 cm³ (2 cm³ of packed red cells and 2 cm³ of Tyrode-albumin); anti-D serum, 0·30 cm³.

It remains, of course, to be seen what importance the observed enzymatic inhibition may assume in the pathogenesis of incomplete antibody hemolysis. Our findings, however, may suggest a working hypothesis about the genesis of acquired spherocytosis which may be due to electrolyte unbalance resulting from lowered availability of energy. The observed results are completely independent of the agglutination and are not reproducible with complete antibodies. To the apparent differences between the 2 types of antibodies[13], may be added the capacity possessed only by the incomplete ones to interfere in the red cell energetic metabolism, a difference which may indicate a fundamental diversity in the action mechanism.

It is interesting to remember at this point that cortisone, which, as is generally known, is successfully employed in the therapy of acquired haemolytic anaemias, produces an evident rise of the erythrocyte glycolysis[14].

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Zusammenfassung

Die unvollständigen Antikörper verursachen eine Hemmung der Erythrozytenglykolyse. Dieser Effekt ist völlig unabhängig von der Eigenschaft der sensibilisierten Erythrozyten, nach Zusatz von Rinderalbumin zu agglutinieren. Die Glykolyse wird durch Agglutinine (vollständige Antikörper) nicht gehemmt. Die Bedeutung dieser Befunde für die Pathogenese der Hämolyse wird kurz diskutiert.

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Thrombozytose und Eosinopenie bei Ratten nach einmaliger 5-Oxytryptamininjektion

In Fortsetzung experimenteller Untersuchungen von Hedinger und Langemann[1] über die endokrine Aktivität der Karzinoide und ihrer 5-Oxytryptaminausscheidung wird bei weissen Ratten der Einfluss einer hohen Einzeldosis von 5-Oxytryptamin auf die Thrombozyten- und Eosinophilenwerte des Blutes untersucht. Als Versuchstiere dienen 180–230 g schwere weisse Ratten einer langjährigen Inzucht des Pathologischen Institutes der Universität Zürich. 5 Tieren wird 250 µMol/kg Körpergewicht 5-Oxytryptaminhydrochlorid[2] in einmaliger Dosis subkutan injiziert. Die Thrombozyten des der Schwanzspitze entnommenen Blutes werden ungefärbt direkt in der Zählkammer mit Hilfe des Phasenkontrast-

[1] Chr. Hedinger und H. Langemann, Schweiz. med. Wschr. *85*, 368 (1955).

[2] Der Firma Dr. A. Wander AG. verdanken wir die zum Versuche verwendeten Mengen von 5-Oxytryptaminhydrochlorid.