is missing entirely in the preparation from Hb A_2 δ Sphakiá. Amino acid analyses after hydrolysis for 22 hours at 110°C in 6*N* HCl were carried out on each tryptic peptide from the variant. Several zones required further chromatography on columns of Dowex 50W-X2 in order to obtain single peptides. Accurate analyses of such small amounts of peptides were achieved by modifying a Spinco amino acid analyzer to include a long-path photometer which increased sensitivity tenfold (*23*).

The analyses of peptides indicated that the composition of all of the tryptic peptides from δT-2 through δT-16 are normal. No normal δT-1 peptide could be detected; instead three abnormal peptides appear to be related to δT-1. One of the peptides was present in the zone marked by the first arrow in Fig. 2 and had the composition (Leu,Thr,Pro,Glu_2,Lys) (*22*). A second abnormal peptide was found in the zone indicated by the fourth arrow in Fig. 2. Its composition was (Val,Arg). A small amount of the αT-10 peptide (Leu,Arg) was also present in this zone, as is evident from the chromatogram of normal aminoethyl δ-chain. This represents contaminating α-chain not completely removed by the countercurrent distribution procedure. A third abnormal peptide was found in the zones marked by the third arrow in Fig. 2. This peptide was isolated by rechromatography and had the composition (Val,Arg,Leu,Thr,-Pro,Glu_2,Lys). Because of the lack of material, further sequence studies were not possible. Considering the hydrolytic specificity of trypsin and comparing the composition of these abnormal peptides with the sequence of the normal δT-1 peptide, we think it reasonable to conclude that the histidine which is normally present as the second residue of the δ-chain has been replaced by an arginine in Hb A_2 δ Sphakiá. Except for the unlikelihood of inversions of amino acids within single tryptic peptides, or of inversions of tryptic peptides within the whole δ-chain, the results of these chemical studies indicate that Hb A_2 δ Sphakiá differs from Hb A_2 by only this substitution of an arginyl residue for the histidyl residue at position No. 2 from the amino terminus of the δ-chain. The formula of this abnormal hemoglobin may therefore be designated as $\alpha_2\delta_2{}^{2Arg}$.

The relative mobilities of Hb A_2, Hb B_2, and Hb A_2 δ Sphakiá would appear to be explainable simply on the basis of a glycyl-to-arginyl change compared to a histidyl-to-arginyl change. Because of the partial protonation of histidyl at the *p*H's used for electrophoresis, the glycyl-to-arginyl substitution of Hb B_2 would result in a greater change in the net positive charge of the molecule than the histidyl-to-arginyl substitution of Hb A_2 δ Sphakiá.

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22. Nomenclature of tryptic peptides includes designation of hemoglobin chain (in this case the δ-chain), the type of peptide (in this case T for tryptic peptide), and the position of the peptide from the amino terminal end of the polypeptide chain. Designation of position is by arabic numerals. The abbreviations for amino acid residues are as follows: Arg, arginine; Val, valine; Leu, leucine; Thr, threonine; Pro, proline; Glu, glutamic acid; Lys, lysine.
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Antibodies Affecting Metabolism of Chicken Erythrocytes: Examination of Schizophrenic and Other Subjects

Abstract. *Human plasma contains an antibody which produces a complement-linked lysis of chicken erythrocytes and an associated marked stimulation of the cells' aerobic glycolysis. This appears to account for reported alteration in chicken erythrocyte metabolism produced by the plasmas of some schizophrenic patients.*

Incubation of chicken erythrocytes in modified Krebs-Ringer solution containing glucose and the plasmas or serums of some schizophrenics results in a higher ratio of lactate to pyruvate than similar incubation with the plasmas or serums of healthy controls (*1*, *2*). This observation has been used as the basis for the biochemical identification of some schizophrenics in "blind" studies of two separate populations (*2*). It has been postulated that these findings reflect a possible biochemical abnormality in schizophrenia.

We are reporting studies of the mechanism by which human plasmas and serums influence the metabolism of chicken erythrocytes. It has been observed that intact chicken erythrocytes demonstrate no aerobic glycolysis. An antibody has been found in the blood of all subjects tested, which, in the pres-

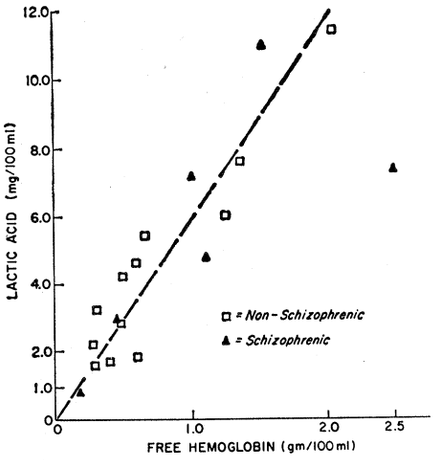

Fig. 1. Relation of lactic acid production to hemolysis. Each point represents the values at the end of a 60-minute incubation of chicken erythrocytes with the plasma of a different subject.

ence of complement, stimulates aerobic glycolysis of chicken erythrocytes accompanied by progressive lysis.

Schizophrenic and nonschizophrenic subjects were chosen by two psychiatrists from the population of Rockland State Hospital, Orangeburg, New York. All subjects were in good physical health and were not on medication.

Human serum or plasma and chicken erythrocytes were obtained by the methods described previously (*1*, *2*). When plasma was used, whole blood was treated with heparin to give a final concentration of three to four units per milliliter. Consistent with the reported anticomplementary effect of heparin, (*3*) low concentrations of heparin partially inhibit the plasma effects, and heparin concentrations of 12 to 15 units per milliliter completely block the action of human plasma on chicken erythrocytes.

Guinea pig serum was used as the complement source in some of the experiments (*4*). Because the guinea pig serum had a considerable quantity of antibody for chicken erythrocytes, the serum was absorbed four times on packed chicken erythrocytes at 0°C for 10 minutes.

For the routine assays of plasma activity, 1.0 ml of plasma was mixed with 4.0 ml of a 33 percent suspension of chicken erythrocytes in a modified Krebs-Ringer phosphate medium, *p*H 7.4. After incubation at 37°C for 60 minutes, a sample was taken to determine free hemoglobin (*5*). The protein was precipitated in the remainder of the incubation mixture, and after centrifugation, the supernatant was assayed for lactate, pyruvate, and glucose (*6*). In an identical mixture that was not incubated, the protein was precipitated at 0°C, and the concentrations of metabolites were determined. In several experiments free potassium in the medium was determined with a flame photometer. In these experiments the incubation mixture was first centrifuged to remove intact cells and cell debris.

When human plasma or serum was omitted from the incubation medium and replaced with an equivalent volume of the modified Krebs-Ringer phosphate solution, intact chicken erythrocytes did not use glucose and did not accumulate lactic or pyruvic acid. However, when human plasma or serum was added to the incubation mixture, aerobic glycolysis occurred whether or not the donor was schizophrenic. The rate of aerobic glycolysis was closely correlated with the amount of hemoglobin released during the incubation (Fig. 1). (Spearman rank order correlation coefficient, r_s, was 0.916, with $p < .001$.) Similarly, after incubation, the ratio of lactate to pyruvate was correlated closely with the net change in lactate ($r_s = 0.917$; $p < .001$) and with the incubation hemolysis ($r_s = 0.911$; $p < .001$). Net change in pyruvate was not significantly correlated with hemolysis.

Table 1. Effects of mechanical disruption on glycolysis of chicken erythrocytes. The cells, a 33-percent suspension of erythrocytes, were disrupted by exposure to radiation from a Branson sonifer (#LS-75) for 1 minute, or by successive freezing at −70°C and thawing (twice).

Disruption method	Lactate accumulation (mg/100 ml)
*No additions**	
Intact cells	− 0.20†
Sound treatment	+31.77
Freezing	+ 2.56†
With additions‡	
Intact cells	− 0.17
Freezing	+36.11

* Incubation of 5.0 ml of a 33 percent suspension of chicken erythrocytes in modified Krebs-Ringer medium. † Average of two experiments. ‡ 2 m*M* Adenosine triphosphate, 0.2 m*M* diphosphopyridine nucleotide, and 20 m*M* nicotinamide were added to the incubation medium.

The relation between the structural integrity of chicken erythrocytes and the rate of aerobic glycolysis was investigated by several techniques. Simple mechanical disruption of cells, in the absence of human serum or plasma, induced active glycolysis (Table 1). Disruption of the erythrocytes by high frequency sound resulted in more ac-

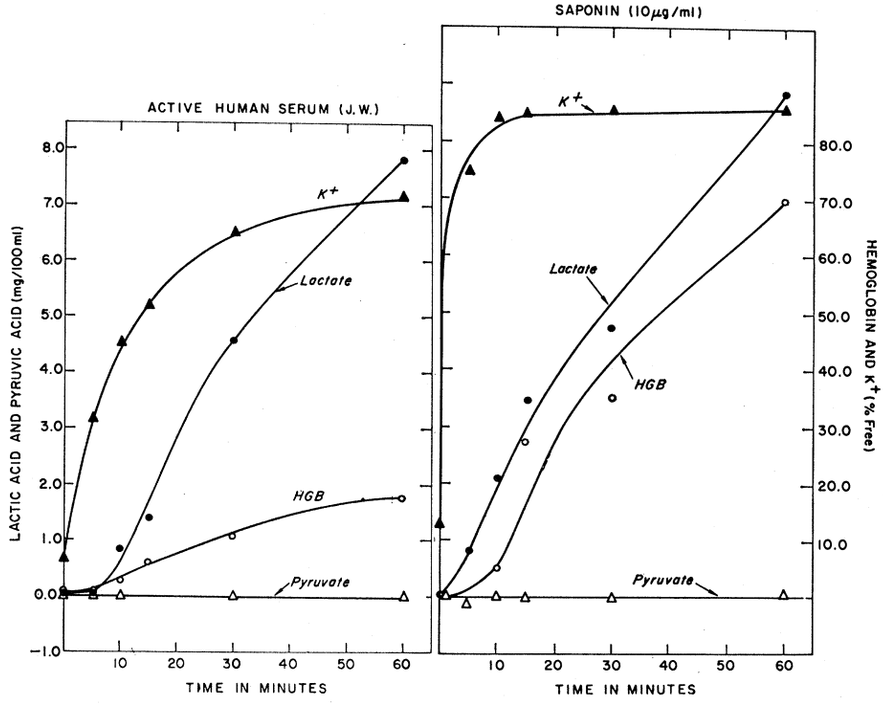

Fig. 2. Time course of serum and saponin effects on chicken erythrocytes. Hemoglobin and K^+ liberation are expressed as percentage of total cell disruption. (*a*) Effects of active human serum. (*b*) Effects of saponin (10 μg/ml).

Table 2. The effects of immune lysis on the aerobic glycolysis of chicken erythrocytes. "Sensitized" cells were first incubated with an active human serum in the presence of 0.01*M* EDTA. "Native" cells were first incubated in the absence of active human serum but in the presence of 0.01*M* EDTA. GPS, guinea pig serum.

Addition	Free hemoglobin (g/100 ml)	Lactate change (mg/100 ml)
Native cells		
None	0.02	−0.35
GPS (undiluted)	.00	+0.46
Sensitized cells		
None	0.01	+0.06
GPS (undiluted)	.47	+4.08
GPS (diluted 1 : 2)	.43	+2.15
GPS (diluted 1 : 3)	.34	+1.33

tive glycolysis than did disruption by rapid freezing in the absence of added nucleotides. If the incubation mixtures were fortified with adenosine triphosphate, diphosphopyridine nucleotide, and nicotinamide, freezing and thawing exerted as great a stimulatory effect as the sound treatment did.

Cellular loss of potassium began immediately upon mixing human serum with chicken erythrocytes at 37°C (Fig. 2*a*). The early, rapid leak of potassium also suggests that the initial lesion occurs at the plasma membrane.

Saponin causes the formation of discrete plasma membrane lesions (*7*). In a concentration that causes progressive, though not complete, hemolysis of a 33-percent suspension of chicken erythrocytes during a 1-hour incubation, saponin induced loss of potassium ion, hemolysis, and glycolysis qualitatively similar to that induced by active human serum (Fig. 2*b*).

In order to determine whether complement was required for hemolysis (*8*), chicken erythrocytes were incubated with human serums that had been heated at 56°C for 30 minutes to inactivate complement. Under these conditions, the serums did not induce K^+ loss, hemolysis, or glycolysis. However, activity could be partially restored by the addition of guinea pig complement. In other experiments, complement was inhibited by carrying out the incubation in the presence of 0.01*M* ethylenediaminetetraacetate (EDTA) (*11*). Under these conditions, hemolysis and glycolysis did not occur. The antibody, however, appeared to be active because the cells agglutinated. The agglutinated cells, washed free of serum and EDTA, could be hemolyzed by the addition of guinea pig complement. In the presence of glucose, hemolysis was accompanied by active glycolysis (Table 2). Complement had no effect on cells not previously sensitized.

The appearance of active aerobic glycolysis after damage to tissues capable of the oxidative metabolism of glucose has been interpreted to be release from the Pasteur effect (*10*). Intact chicken erythrocytes are impermeable to glucose, and one might postulate that the appearance of glycolysis after damage to the plasma membrane is a "permissive" release of glycolysis rather than true stimulation or activation (*11*). Our data suggest that human plasma or serum damages the cell membrane of chicken erythrocytes and subsequently causes increased permeability to glucose. It appears likely that these effects are due to the reaction of a heterogenetic antigen of chicken erythrocyte plasma membrane with an antibody of human serum and complement. The distribution of the antibody in populations of mental patients and control normal subjects is still undetermined (*12*).

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"Respirable" Dust

In "Airborne particulates in Pittsburgh: association with *p,p'*-DDT" (*1*), Antommaria and co-workers refer to a paper of mine (*2*) as a source for the term "respirable" dust. According to their account, they measured by a two-stage sampler the dust which could penetrate and deposit in the lower respiratory tract (terminal airways and alveoli) and thereby that which constituted the greatest risk to health. This dust, they say, corresponds to the "respirable" fraction. Their use of this expression does, in fact, conform to the original use by the British Medical Research Council in discussing the risk of pneumoconiosis from coal dust (*3*); however, the MRC description cannot be applied to dust generally.

The study of *p,p'*-DDT aerosol is a case in point. If DDT, alone or associated with a vector aerosol, is absorbed reasonably well from mucous membranes and has important systemic toxic effects—and both these characterizations appear appropriate (*4*)—then the deposition site of the dust becomes relatively unimportant, because all the DDT will be absorbed and potentially injurious.

The authors do not discuss the toxicity of DDT aerosols, and it is conceivable that they have evidence of an important pulmonary effect; nevertheless, it must be stressed that "respirable" dust and "respirable" fraction are ambiguous and changeable terms. At best, they are used to describe the dust which constitutes the greatest risk to health; the site of the injury can vary, as will concern over the sites of dust deposition. The terms, therefore, connote different things for different dusts. To complicate matters further, occasionally the expressions will pertain to the dust which is literally respirable, that is, the dust which accompanies the inspired air into the respiratory tract.

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