medium was replaced by maintenance medium.

After being washed with Hanks balanced salt solution, cultures were inoculated with 0.25 ml of virus preparations. After incubation at 25°C for 1 hour to allow for virus adsorption, the inoculum was removed and cultures were fed with maintenance medium. Incubation was then continued at 35°C.

After inoculation, *p*H 7.2 was maintained with 1.4 percent $NaHCO_3$. Every 5 to 7 days, one-half of the fluid phase was replaced with fresh maintenance medium to prevent cell damage resulting from nutrient depletion.

Four to six cultures were used for each of the four virus strains studied in each experiment. Equal numbers of uninoculated cultures served as cell controls. Control cultures were processed in the same manner as those used for inoculation.

Uninoculated rabbit embryo cells form monolayers of densely packed elongated cells with a fibroblastic appearance. In cultures inoculated with rubella virus, the first indication of cytopathic effect is the loss of the dense arrangement of the monolayer in discrete areas. Infected cells lose their characteristic, elongated, spindle shape and become more rounded, and cytoplasmic threads become obvious. Finally the infected cells become condensed, degenerate, and detach from the surface, with discrete plaques remaining in the monolayer. On primary isolation, strains AE and LB produced foci by the 6th day after inoculation. Foci were noted by the 9th day after inoculation with the human amnion–adapted Bell strain and the monkey kidney–adapted M33 strain.

The cytopathic process progresses to involve 75 to 80 percent of the monolayer within 10 to 12 days after inoculation. Control monolayer cultures remain as sheets of densely packed elongated cells.

The four virus strains have been passaged in primary rabbit embryo cells four times without any loss of cytopathogenicity. The cytopathic changes produced by all four strains are comparable. Titers of virus in the supernatant fluids by the 8th to 9th day after inoculatioin reach 10^5 to 10^6 $TCID_{50}$ (tissue culture infective dose, 50 percent effective) per milliliter.

Neutralization studies were performed in rabbit embryo cell cultures with paired serums from three patients and with rabbit antiserum to the Bell strain. Previously titrated virus was diluted in Hanks balanced salt solution containing 0.1 percent peptone. Twofold serial dilutions of inactivated serum were prepared in Hanks balanced salt solution. Equal volumes of virus preparation and serum dilutions were mixed. Serum-virus mixtures and control virus preparations were held at 37°C for 1 hour before inoculation of 0.25-ml amounts into each of four cultures. Virus dilutions were prepared to contain 100 $TCID_{50}$ of virus per 0.25 ml of final virus-serum mixture. Inoculated cultures and serum, virus, and cell controls were held at 25°C for 1 hour, then 1 ml of maintenance medium was added to the inoculum, and incubation was continued at 35°C. Serums from the convalescent, but not acute, phase and rabbit antiserum to the Bell strain neutralized the cytopathic effects of all four virus strains. Cross-neutralization of our isolates with antiserum to the Bell strain indicated antigenic similarity. Neutralization studies have been performed with and without incorporation of fresh normal rabbit serum in the virus-antiserum mixture. The addition of fresh normal rabbit serum has not been a necessary requirement for consistent and reproducible neutralization of the cytopathic effects of rubella virus in this cell system.

Hemagglutination and hemadsorption tests, and also tests for the presence of bacteria and *Mycoplasma*, were negative.

Indications of the sensitivity of this technique for recovery of rubella virus from clinical materials are given by preliminary comparative studies of throat washings from 14 selected rubella patients. On primary isolation attempts, we recovered the virus from all of the 14 specimens tested in rabbit embryo cell cultures in contrast to recovery of the virus from 10 of 12 specimens tested in primary green monkey kidney cell cultures (*5*) and from 7 of 14 tested in primary human amnion cell cultures (*1*).

Definite and marked cytopathic effect of rubella virus in rabbit embryo cell cultures makes possible an efficient and reproducible direct technique for demonstration of virus and of specific neutralizing antibodies. In addition, growth of the virus in high titer in rabbit embryo tissue provides a convenient source of virus for other studies.

ANNE REDDICK
CATHERINE E. ROESEL
Department of Medical Microbiology and Public Health, Medical College of Georgia, Augusta

References and Notes

1. T. H. Weller and F. A. Neva, *Proc. Soc. Exp. Biol. Med.* **111**, 215 (1962); F. A. Neva and T. H. Weller, *Fed. Proc.* **22**, 208 (1963).
2. K. McCarthy, C. H. Taylor-Robinson, S. E. Pillinger, *Lancet* **1963-II**, 593 (1963); K. McCarthy and C. H. Taylor-Robinson, *Arch. Ges. Virusforsch* **16**, 415 (1965).
3. A. Günalp, *Proc. Soc. Exp. Biol. Med.* **118**, 85 (1965).
4. J. Leerhøy, *Science* **149**, 633 (1965).
5. P. D. Parkman, E. L. Buescher, M. S. Artenstein, *Proc. Soc. Exp. Biol. Med.* **111**, 225 (1962).
6. We thank Dr. F. A. Neva, Harvard School of Public Health, and Dr. E. L. Buescher, Walter Reed Army Institute of Research, for virus preparations and Dr. J. L. Sever, NIH, for antiserum. Supported in part by training grant T1-AI 248-01A1 and in part by NIH research grant AI 05245-02.
7. Medium 199 was obtained from Microbiological Associates, Bethesda, as 10-times concentrated stock and was diluted to proper concentration with water distilled in glass twice.

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Hemoglobin Sphakiá: A Delta-Chain Variant of Hemoglobin A_2 from Crete

Abstract. *A new variant of the normal minor component Hb A_2 has been detected in a family that lives in Sphakiá, Crete. Chemical studies of this abnormal hemoglobin, designated Hb A_2 δ Sphakiá, indicates a substitution of the histidyl residue number two of the δ-chain by an arginyl residue.*

Hemoglobin A_2 (Hb A_2), which was first described by Künkel and Wallenius (*1*), is a minor red-cell component which is present in all normal humans. It constitutes about 2.5 percent of the total hemoglobin of the red cell. In electrophoresis on paper, starch-gel, or starch-block, at alkaline *p*H, it migrates considerably more slowly towards the anode than Hb A and at approximately the same speed as Hb E. It consists of two α-chains of the same composition as those in Hb A (*2*) combined with two δ-chains which differ from the β-chain of Hb A in ten amino acid substitutions (*3*, *4*). Its structure can be written $\alpha_2\delta_2$.

Several electrophoretically separable, inherited variants of Hb A_2 have been found. Some of them are due to changes in the α-chain and occur in association with major α-chain variants. The first example of this type (*5*) was in a subject carrying the α-chain variant Hb G Ibadan only. This subject had no Hb A_2 but his red cells showed a slower minor component which had the constitution $\alpha_2^G\delta_2$ and a number of hemoglobins of this type have since been described (*6*). Two variants of the Hb A_2 due to

changes in the δ-chain are known. One of these, known as Hb A_2' or Hb B_2 (*7*), migrates considerably more slowly than Hb A_2 and occurs in approximately 1 percent of the American Negro population. Horton *et al.* (*8*) showed that the δ-chain was abnormal and A. O. W. Stretton (quoted by Baglioni, *9*) has indicated that the abnormality consists of substitution of glycyl for arginyl at position No. 16. This substitution has recently been confirmed by chemical studies of three different samples of Hb B_2 (*10*). A variant which is apparently the same as Hb B_2 has been reported in a Sicilian family (*11*) but, unlike the American Negro cases, it was associated with mild hemolytic disease. A second variant, Hb A_2 δ Flatbush, has been described (*12*). At alkaline *p*H its mobility is greater than that of Hb A_2 and it is therefore clearly distinguishable from Hb B_2. In Hb A_2 δ Flatbush, glutamyl is substituted for alanyl at position No. 22 of the δ-chain (*13*).

In the course of screening 171 hemolyzates from a population survey in Sphakiá, an isolated mountainous region of southern Crete, a single example of another Hb A_2 variant was detected in a 10-year-old girl. Its electrophoretic characteristics were briefly described (see *14*), and it was given the name Hb Sphakiá or Hb A_2 δ Sphakiá (*15*). When the hemolyzate was examined on starch-gel with a tris-ethylenediaminetetraacetate (EDTA) buffer system (*6*) and stained with *o*-tolidine–peroxide, which detects heme compounds, Hb A_2 was found to be somewhat weak, and an additional minor component was seen nearer the cathode. When the sliced gel was stained with amido black, this slow minor component appeared just behind (toward the cathode) the main nonhemoglobin protein band. The specimen was then compared on starch gels in parallel with hemolyzates containing Hb A_2 together with Hb B_2 (*16*). The Sphakiá variant was greater in mobility than Hb B_2 (Fig. 1). Its mobility was also greater than that of Hb B_2 in hemolyzates examined with the tris-citrate-borate buffer system of Poulik (*17*).

Because the specimen containing the Sphakiá variant showed no evidence of a second major component, slower than Hb A, it seemed probable that the abnormality was in the δ-chain.

The younger brother of the proposita was included in the survey and did not have the variant. On a subsequent occasion small blood samples were obtained by finger-prick from the father, mother, and elder brother of the proposita. This material was examined in Athens on a tris-EDTA-borate gel a few days later; the variant was present together with Hb A_2 in the mother and elder brother but not in the father. The variant was thus present in the mother and in two of her three children who were tested. The fourth member of the sibship, a daughter, was not examined.

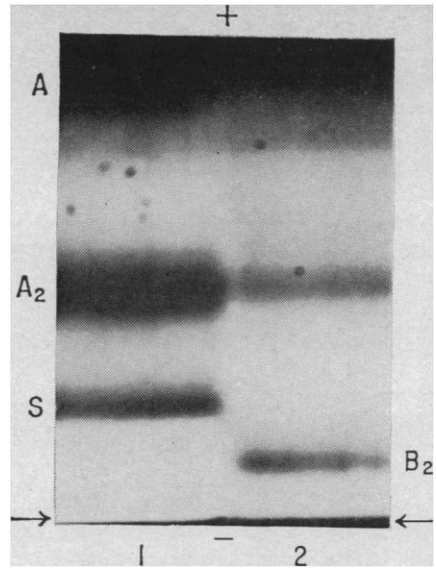

Fig. 1. Starch gel electrophoresis. Sample 1 demonstrates Hb A_2 and Hb Sphakiá, and sample 2 demonstrates Hb A_2 and Hb B_2. Only the cathodal edge of Hb A is shown. The origin is marked by arrows; tris-EDTA borate buffer at *p*H 8.6; staining was with *o*-tolidine and hydrogen peroxide.

Stained blood films from the proposita and from her father, mother, and elder brother were examined but none of these subjects showed appreciable abnormalities of the red cells.

A sample of approximately 20 milliliters of heparinized whole blood was obtained by venipuncture from the proposita and dispatched to London in an iced insulated container. The variant Hb A_2 δ Sphakiá was isolated and purified by chromatography on carboxymethyl Sephadex followed by starch-grain electrophoresis as described for Hb A_2 (*18*). The purified hemoglobin was sent to Portland, Oregon, in ice, where it was converted to globin (10-mg yield) by removing the heme group with a mixture of cold acid and acetone (*19*). The α- and δ-chains of the globin were separated by countercurrent distribution by a modification of the procedure of Hill *et al.* (*20*); 4.5 mg of δ-chain was obtained and treated with ethylenimine yielding the *S*-aminoethyl derivative (*4*). The aminoethyl δ-chain (AEδ) was hydrolyzed with trypsin (Worthington, twice crystallized, free of salt) in an aqueous solution of trimethylamine (*21*). The tryptic peptides, corresponding to 3.5 mg of aminoethyl δ-chain, were separated by automatic peptide chromatography on a column (0.9 × 17 cm) of Spinco type 15A resin and a linear gradient of pyridine–acetic acid developers (*4*).

The chromatograms of tryptic peptides from the aminoethyl δ-chain of the variant hemoglobin and normal aminoethyl δ-chains were compared (Fig. 2). The second arrow points to the region of the chromatogram where δT-1 and δT-8 (lysine) are normally eluted as a double peak (*22*). Although δT-8 is present, normal δT-1

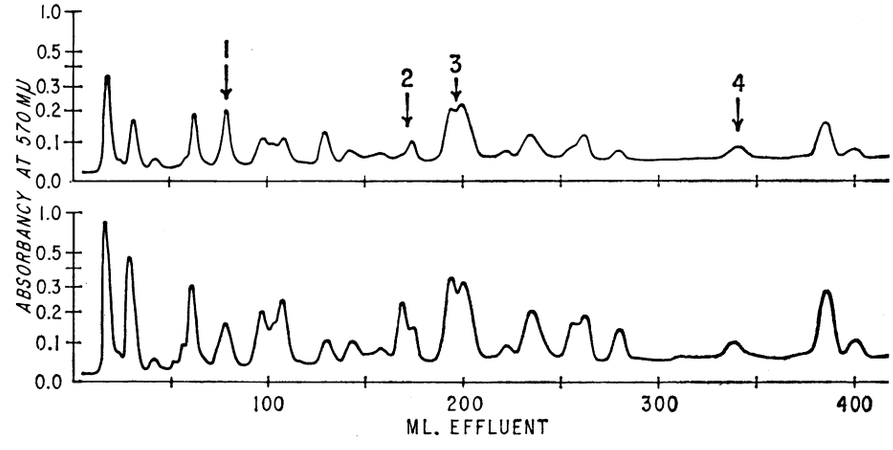

Fig. 2. Peptide patterns of tryptic hydrolyzates of the aminoethylated δ-chains from Hb A_2 δ Sphakiá on top and normal Hb A_2 on the bottom. The absorbance of the reaction products of ninhydrin and peptides was measured continuously at 570 mμ.

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.

R. T. JONES, B. BRIMHALL
Division of Experimental Medicine and
Department of Biochemistry,
University of Oregon Medical School,
Portland

E. R. HUEHNS
Medical Research Council Group in
Hemolytic Anemia, University College
Hospital Medical School, London

N. A. BARNICOT
Department of Anthropology,
University College, London

References and Notes

1. H. G. Künkel and G. Wallenius, *Science* **122**, 288 (1955).
2. E. R. Huehns and E. M. Shooter, *Nature* **189**, 918 (1961); V. M. Ingram and A. O. W. Stretton, *Ann. N.Y. Acad. Sci.* **190**, 1079 (1961).
3. C. J. Mueller and J. H. P. Jonxis, *Nature* **188**, 949 (1960); V. M. Ingram and A. O. W. Stretton, *Biochim. Biophys. Acta* **62**, 456 (1962); ———, *ibid.* **63**, p. 20; R. J. Hill and A. P. Kraus, *Fed. Proc.* **22**, 597 (1963).
4. R. T. Jones, *Cold Spring Harbor Symp. Quant. Biol.* **29**, 297 (1964).
5. E. M. Shooter, E. R. Skinner, J. P. Garlick, N. A. Barnicot, *Brit. J. Haematol.* **6**, 140 (1960).
6. E. R. Huehns and E. M. Shooter, *J. Med. Genet.* **2**, 1 (1965).
7. R. Ceppellini, in *Symposium on Biochemistry of Human Genetics*, G. E. W. Wolstenholme and C. M. O'Connor, Eds. (Churchill, London, 1959), pp. 133–138; T. H. J. Huisman and C. A. Meyering, *Clin. Chim. Acta* **5**, 103 (1960).
8. B. Horton, R. A. Payne, M. T. Bridges, T. H. J. Huisman, *Clin. Chim. Acta* **6**, 246 (1961).
9. C. Baglioni, in *Molecular Genetics*, J. H. Taylor, Ed. (Academic Press, New York, 1963), part 1, p. 444.
10. R. T. Jones, F. W. Boerma, T. H. J. Huisman, *Amer. J. Human Genet.* **17**, 511 (1965); and unpublished studies of R. T. Jones, B. Brimhall, and T. H. J. Huisman.
11. E. Silvestroni, I. Bianco, S. I. Magalini, *Lancet*, p. 1384 (1963).
12. H. M. Ranney, A. S. Jacobs, R. B. Bradley, Jr., F. A. Cordova, *Nature* **197**, 164 (1963); T. H. J. Huisman and R. C. Lee, *Blood* **26**, 677 (1965).
13. R. T. Jones, B. Brimhall, T. H. J. Huisman, *Clin. Res.* **14**, 168 (1966).
14. N. A. Barnicot, C. Krimbas, R. B. MacConnell, G. R. Beaven, *Human Biol.* **37**, 274 (1965).
15. The designation Hb A_2 δ Sphakiá is consistent with the nomenclature of hemoglobins agreed upon at the 10th International Congress of Haematology, Stockholm, 1964.
16. These specimens were kindly provided by T. H. J. Huisman and P. Barkhan.
17. M. D. Poulik, *Nature* **188**, 949 (1960).
18. E. R. Huehns and E. M. Shooter, *J. Mol. Biol.* **3**, 257 (1961).
19. M. L. Anson and A. E. Mirsky, *J. Gen. Physiol.* **13**, 469 (1930).
20. R. J. Hill, W. Konigsberg, G. Guidotti, L. C. Craig, *J. Biol. Chem.* **237**, 1549 (1962).
21. C. Baglioni, *Biochim. Biophys. Acta* **97**, 37 (1965).
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.
23. R. T. Jones and G. Weiss, *Anal. Biochem.* **9**, 377 (1964).
24. We are indebted to the family in which the variant occurred for their cooperation. We thank M. Isichakis, Drs. N. and D. Pimbli, A. Zervos, Mrs. Zoe Zanudaki, and Mrs. Mary Skulaki of the Red Cross, Chania, Crete, and S. Tsacas (Department of Genetics, College of Agriculture, Athens) for assistance, and Dr. C. Krimbas (Department of Genetics, College of Agriculture, Athens) for providing facilities for some of the laboratory work; and Miss Marie Duerst for amino acid analyses. Fieldwork supported by a grant from the Wenner-Gren Foundation. Chemical characterization supported by PHS grant CA-07941.

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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-