BRIEF NOTE

A Variety of Human Hemoglobin with 4 Distinct Electrophoretic Components

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NATURALLY OCCURRING mixtures of electrophoretically different hemoglobin molecules within erythrocytes are a well known phenomenon. Some common examples of this are sickle cell trait (A-S), sickle cell-C disease (S-C), and C-trait (A-C). Since these hemoglobins are believed to be allelomorphs, it would not be possible for more than 2 of these genes to be inherited in an individual. However, there have been sporadic reports of simultaneous presence of 3 components. These have consisted of combinations of A (thalassemia), G and S [1] and A, S and Hopkins 2.[2] This would indicate that hemoglobin G and the reported Hopkins 2 component are not allelomorphic with hemoglobins A, S, C, etc.

During a recent screening of the blood of pregnant women for hemoglobinopathic anemia, a Negro woman was examined whose blood revealed a hemoglobin with four distinct electrophoretic components.

CASE REPORT

D. K. P., a 28 year old Negro woman seen in the prenatal clinic. Aside from a normal pregnancy of approximately 4 months there were no significant findings on physical examination. The blood revealed a considerable number of leptocytes (13.6 per cent of the red cells), moderate reticulocytosis (3.6 per cent) and slightly decreased osmotic fragility (patient: 0.45 to 0.20 per cent NaCl, control: 0.50 to 0.30 per cent NaCl). The hemoglobin was 12.35 Gm. per cent, RBC 4.49 million, hematocrit 37.8 per cent, MCV, 84 cu.μ, MCH, 27 $\gamma\gamma$, MCHC, 32 per cent. Hemoglobin paper electrophoresis with veronal buffer, pH 8.6, ionic strength 0.06, run for 5 hours at 0.6 ma/cm using Whatman #3MM filter paper and the pressure plate technic described by Smith and Conley,[3] revealed four distinct components, as seen in figure 1. The largest component was normal adult hemoglobin, readily identified by means of known controls. This made up 40 per cent of total hemoglobin present. The peak labeled C contains 20 per cent of total hemoglobin and has the same mobility of our hemoglobin C standard. The 2 remaining peaks are less easily identified. Component labeled G seems to coincide with G hemoglobin described by Edington and Lehmann[4] and comprises 24 per cent of the total. The fourth spot had an electrophoretic mobility of zero under stated conditions and made up the remaining 16 per cent. There was virtually no separation of components with paper electrophoresis using a phosphate buffer pH 6.1, ionic strength 0.1, run for 5 hours at 0.7 ma/cm, though all hemolysates moved toward the cathode. However, when the electrophoresis was carried out at

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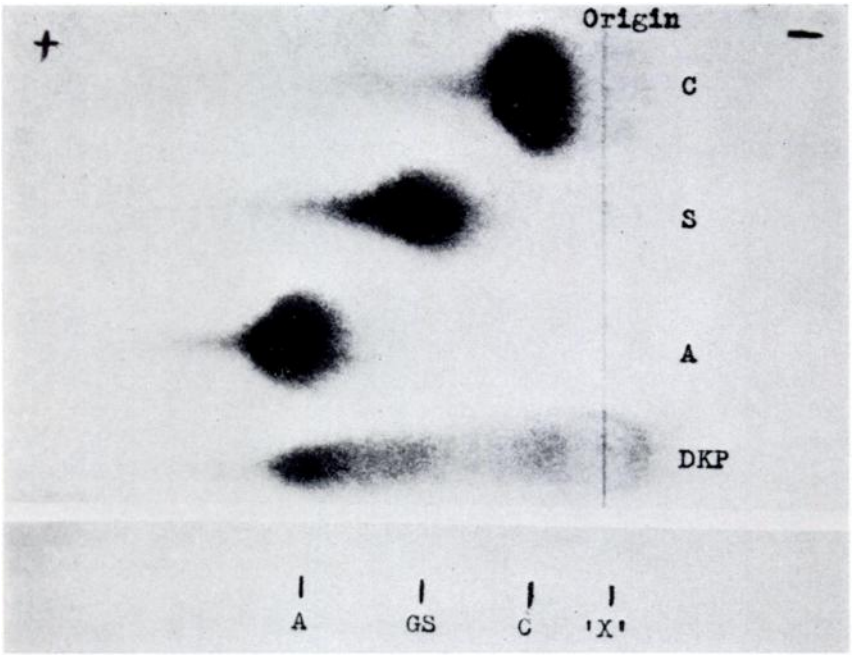

Fig. 1.—Paper electrophoresis with Veronal buffer, pH 8.6, ionic strength 0.06, showing the pattern of the propositus (D. K. P.) and those of known A, S and C bloods. Blood specimen drawn September 6th.

pH 9.0 (boric acid-Na0H buffer, ionic strength 0.6, run for 5 hours at 1.3 ma/cm), migration of the "X" component toward the anode was observed (fig. 2). Sickling tests were negative. Alkali resistant hemoglobin[5] and ferrohemoglobin solubility tests[6] were within normal limits. A sample of G hemoglobin similar to the G originally reported by Edington and Lehmann[4] was obtained from Dr. R. G. Schneider of Galveston, Texas. No separation of the known G hemoglobin and the component between A and C of our specimen was achieved (fig. 3). Attempts to separate the 4 components by Tiselius moving boundary electrophoresis using a cacodylate buffer, pH 6.5 with approximate ionic strength 0.1, produced only 3 peaks, but the A peak showed a shoulder component which may be the G component. The mobilities were reported as follows: The largest component (A plus G) 2.47; the middle peak (C) 3.07; and the third peak (X) 3.34. Starch block electrophoresis[7] run with a veronal buffer of pH 8.6, ionic strength 0.06, also separated four distinct components similar to the pattern attained by paper electrophoresis with the same buffer. The possibility that the "X" component represented denatured hemoglobin was ruled out by running several fresh specimens, processed at once, along with controls.

Family studies were undertaken on four of the siblings, a daughter and a nephew (Fig. 4). Both parents are dead. Three siblings demonstrated a pattern of C-trait (A-C). The daughter and nephew were normal. The remaining sibling, a sister, demonstrated an identical A-G-C-X pattern (fig. 5). She was asymptomatic and her blood had a hemoglobin of 12.4 Gm. per cent red cells of 4.4 million and a hematocrit of 34 per cent. There were target cells on the smear. The percentage of alkali-resistant hemoglobin was within normal limits, sickling tests were negative, and ferrohemoglobin solubility tests were in the normal range in all family members studied. The nephew referred to is the son of this sister.

Discussion

The inheritance of the hemoglobins in this patient and in her sister cannot be explained, if the hemoglobins are derived by single genes from each pa-

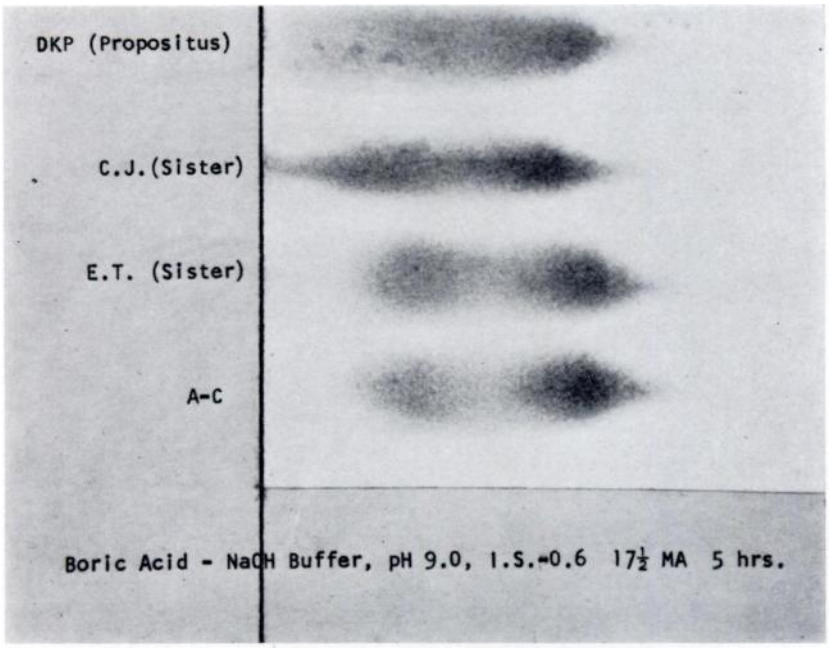

Fig. 2.—Paper electrophoresis at pH 9.0 showing that the slowest components of D. K. P. and her sister, C. J., move from the origin at this pH.

tient. Four electrophoretically distinct hemoglobins were present in both cases, and it appears that four genes must have been involved in the creation of this pattern.

In 1957, Schwartz and co-workers[1] reported a family in which one member had both hemoglobin S and hemoglobin G in addition to the thalassemia gene. On the basis of careful examination of the whole family, the conclusion was reached that there were three separate hemoglobin genes, one responsible for hemoglobin G, one for hemoglobin S and one for thalassemia. The separate existence of genes for various hemoglobins has been supported by studies of the hemoglobin molecule which can be visualized in humans as an ellipsoid sphere made up of two identical half molecules, each containing two heme groups, and each containing two coiled polypeptide chains called the alpha chain and the beta chain.[8] Each of these polypeptide chains is made up of about 150 amino acids of 19 different kinds. In a number of chemical studies, Ingram[9-11] has succeeded in "finger-printing" these chains and pinpointing the chemical differences between normal and abnormal hemoglobins. Utilizing the classical technic of trypsin hydrolysis in dividing the polypeptide chains at the points where the amino acids lysine and arginine occur, Ingram[9] succeeded in breaking down the chains into smaller peptide fragments containing about a dozen amino acids each. These were then subjected to electrophoresis and chromatography resulting in a "finger-print" showing the sequence or character of amino acids in the peptide fragments from normal and abnormal hemoglobins. Further studies[12-14] have demonstrated that hemoglobins S, C, Dβ and E are characterized by alterations in the sequence of amino

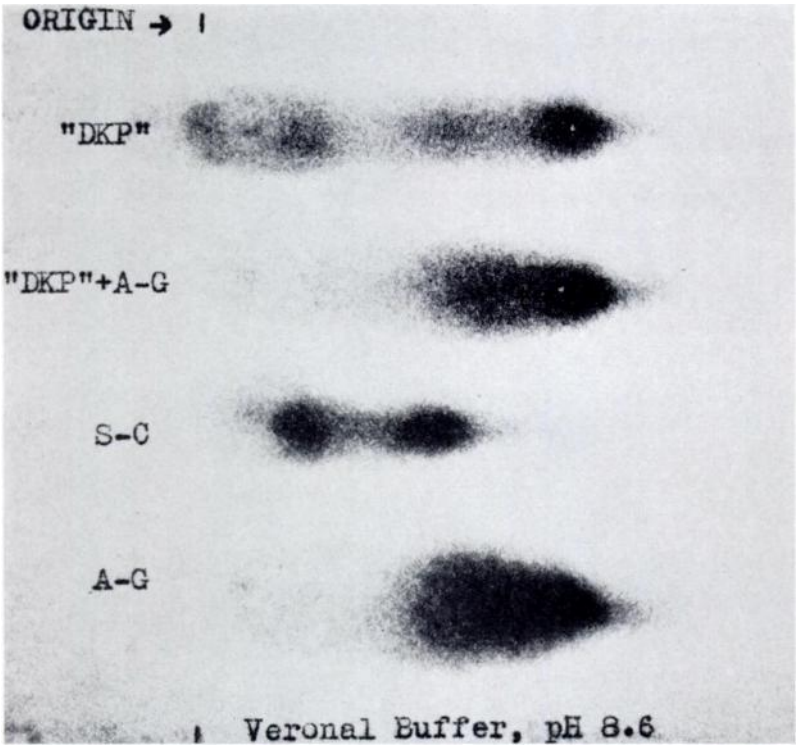

Fig. 3.—Paper electrophoresis with Veronal buffer, pH 8.6, ionic strength 0.06, showing the patterns of a known S-C hemoglobin, the hemoglobin A-G received from Dr. Schneider, the propositus and a mixture of the A-G blood with that of the propositus in equal parts. There appears to be no clear separation of the known G and the second component of the patient.

acids in the beta chain while hemoglobin Dα and I are characterized by abnormal alpha chains.

From these studies Ingram suggests that there may be two genes which are responsible for the hemoglobin structure, as was first predicted by Itano.[15] One of these would control the amino acid sequence of the alpha chain peptides while the second would control the beta chain peptide structure. Mutations could occur in either or both of these genes, and it would then be possible to have from one to four different kinds of hemoglobin in one cell.

Additional support for this interpretation was provided recently by Itano and Robinson,[16] who found that hemoglobin could be dissociated into alpha and beta chains and that these chains would recombine in different patterns. Hemoglobin molecules were dissociated into two asymmetric units. When a mixture of abnormal hemoglobins with mutations in the alpha or beta chain were dissociated and then recombined, it was found that the alpha and beta chains combined at random with the formation of new hemoglobins. It was concluded that ". . . it is possible for an individual who is heterozygous for an abnormality in each chain to have four molecular species of adult hemoglobin in his red cells, provided the two types of chains are physically independent up to the time they associate to form complete molecules."[16]

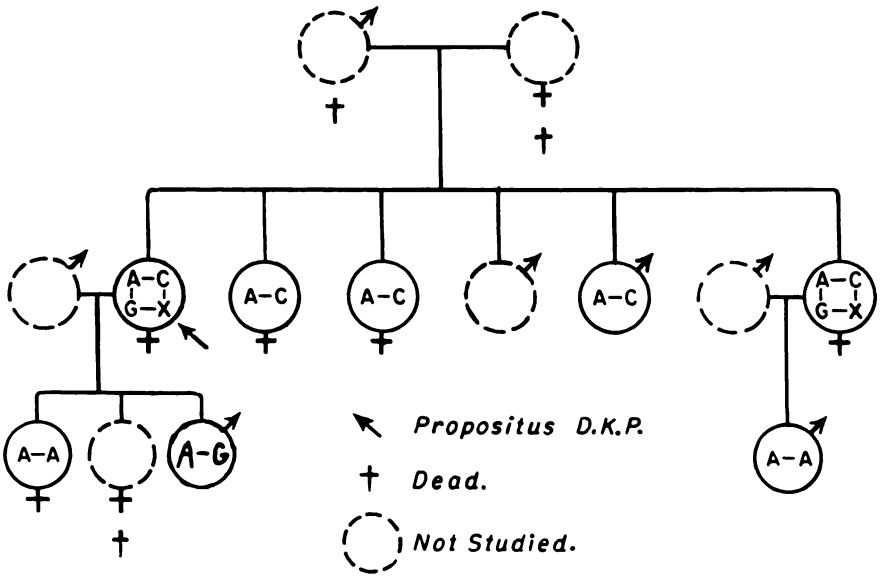

Fig. 4.—Pedigree of propositus (D. K. P.)

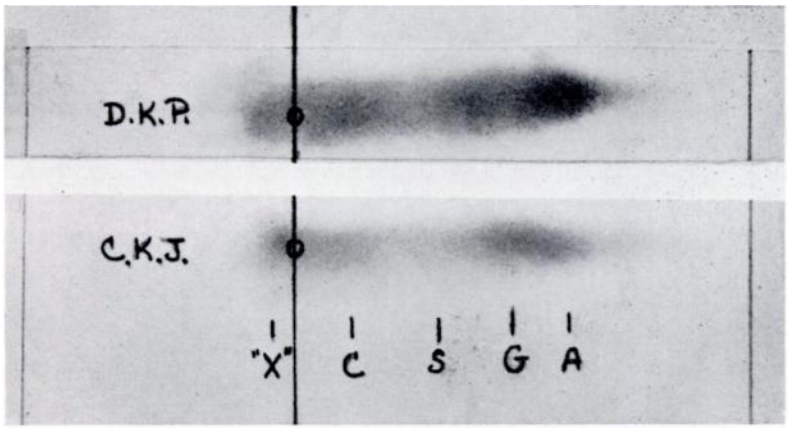

Fig. 5.—Paper electrophoresis with Veronal buffer, pH 8.6, ionic strength 0.06, showing a second blood specimen (September 13) of the propositus (D. K. P.) and the pattern of her sister (C. K. J.)

In the case presented here four distinct hemoglobin components were seen. One was normal adult hemoglobin with 2 normal alpha chains and 2 normal beta chains ($\alpha_2^A \beta_2^A$). Another was hemoglobin C, which has been found to have 2 abnormal beta chains ($\alpha_2^A \beta_2^C$). The third was a hemoglobin with the same mobility (fig. 3) as the hemoglobin G described by Edington and Lehmann.[4] In order to explain the simultaneous occurrence of the 4 hemoglobins and their apparent segregation, this hemoglobin "Porter G" is *assumed* to be an abnormality only in the alpha chain ($\alpha_2^G \beta_2^A$).[17] Recent studies by Hill and Schwartz[18] have indicated that the hemoglobin described by Schwartz et al.[1] and also called G has an abnormality in the beta chain. However, the hemo-

globin G used here as a standard appears to be different from the hemoglobin G of Schwartz.[19] With the presence of α^A and α^G chains and β^A and β^C chains, it would then be possible to have a fourth kind of molecule (α_2^G β_2^C) corresponding to the hemoglobin X of our patient. It has been shown that if hemoglobin A has a charge of O, hemoglobins S and C must have a net charge of +2 and +4, respectively.[14] Hemoglobin G, which has a mobility close to S, is presumed to have a net charge of close to +2 also. A molecule with the configuration of α_2^G β_2^C would then have a net charge of approximately +2 plus +4 = +6, and the mobility should be close to zero on paper electrophoresis using a buffer of pH 8.6. The hemoglobin X found in this family was shown to have a mobility of zero at this pH and seems to fulfill the requirements for a hemoglobin with the configuration of α_2^G β_2^C.

Family studies reveal that the daughter of the propositus and the son of the sister with the same hemoglobin configuration have only normal adult hemoglobin (fig. 4). This would indicate that the genes for the alpha and beta chains probably are located on different chromosomes. If the alpha and beta chain genes were on the same chromosome (fig. 6,*1*), the children could not have had α_2^A β_2^A hemoglobin, since they would have had to inherit either an α^G β^A chromosome, forming G-hemoglobin molecules, or an α^A β^C chromosome, forming C-hemoglobin molecules. However, if the genes for the alpha and beta chains were on different chromosomes (fig. 6,2), the children could then inherit all combinations of the available alpha and beta genes, including the α^A β^A genes as in the case presented here.

There is however, the remote possibility that the abnormal genes for hemoglobin G and hemoglobin C are on the same chromosome in this patient (α_2^G β_2^C). If the propositus were heterogeneous for this molecule, the children would either be A-G-C-X or A-A-A-A, if the father is normal (fig. 6,*3*). In order to explain the fact that three siblings of the propositus were A-C, it has to be further assumed that the parent with the G-C chain was married to an individual with a C-trait hemoglobin component. Although unlikely, this possibility cannct be ruled out at the present time.*

In accordance with the recent practice of not identifying new hemoglobins by letter until all chemical and physical properties have been compared with existing components and those being studied at the present time in other laboratories, it was decided to refer to this new component by the name of the propositus—Porter. No abnormal clinical or laboratory findings could be attributed to the presence of this new component.

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*Since this paper was written, we have been fortunate to renew contact with the propositus. This has allowed us to study her blood again, her son's, age two years (fig. 4) and that of his father. The father was normal, but the boy's blood reveals a hemoglobin of 11.2 Gm. per cent, RBC 4.4 million and reticulocyte count of 1.1 per cent. No target cells were seen on the smear. On paper electrophoresis with the veronal buffer, pH 8.6, the boy's hemoglobin had an A-G pattern. This new information would now seem to eliminate schemes *1* and *3* in fig. 6 as possible genetic arrangements for the alpha and beta chains, since only scheme 2 (fig. 6) with separate chains for the A, G, and C components would produce the combination of siblings, one with only A hemoglobin and another with the A-G hemoglobin pattern.

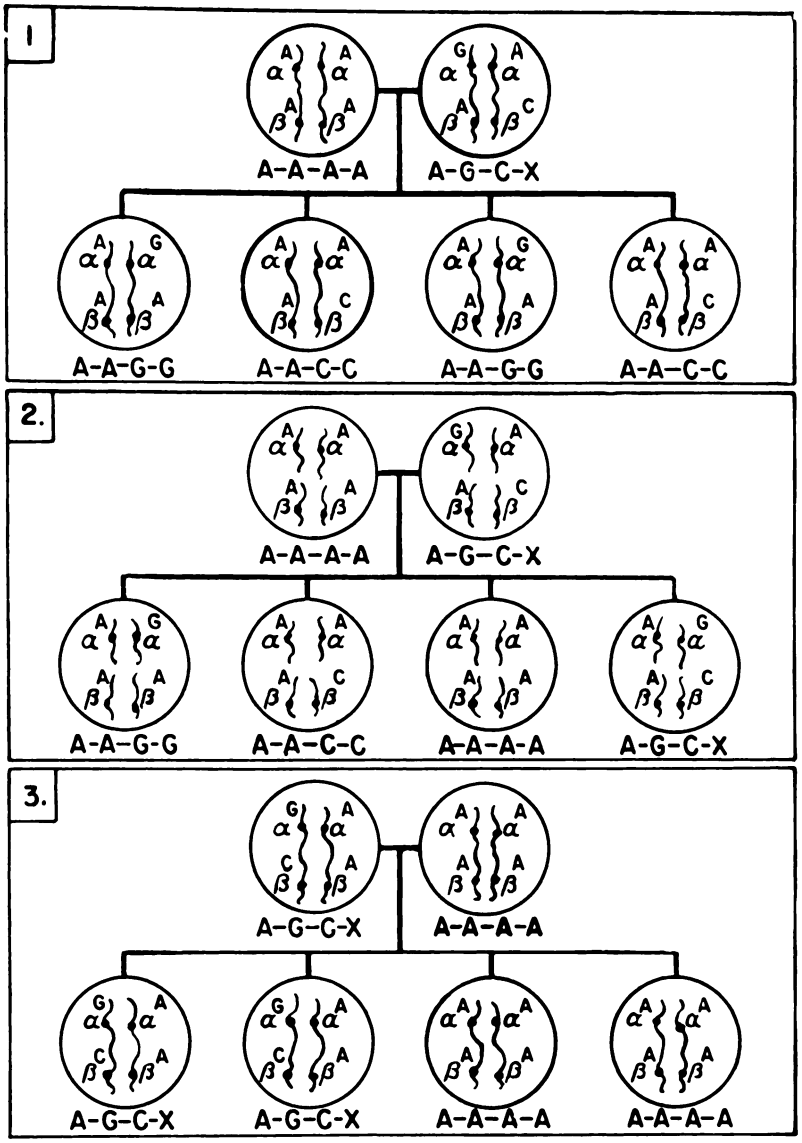

Fig. 6.—Proposed pedigree showing possible combinations of genotypes. The corresponding phenotypes are indicated below each circle.

1. If the α and β chain genes were located on the same chromosomes.

2. If the α and β chain genes were located on different chromosomes.

3. If the two abnormal components (G and C) were on the same chromosome.

Summary

A human hemoglobin with four distinct electrophoretic components was observed in an individual with leptocytosis and reticulocytosis. Three of the components were the previously reported hemoglobins A, C and G. The fourth (Porter) with a mobility of zero on conventional paper electrophoresis in

alkaline buffer of pH 8.6, appears to be a new component arising as a result of the random association of the other two abnormal components.

Summario in Interlingua

Un hemoglobina con quatro distincte componentes electrophoretic esseva observate in un patiente con leptocytosis e reticulocytosis. Tres del componentes esseva le previemente reportate hemoglobinas A, C, e G. Le quarte (Porter), con le mobilitate zero in electrophorese conventional a papiro con tampon alcalin de pH 8,6, pare esser un nove componente que resulta del association aleatori del altere duo componentes anormal.

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