heavy chains were only slightly active; isolated FAB fragments produced by papain were more active.

Seven of the cold agglutinins which reacted with antiserum B.M. (Table 1) showed both I and i red-cell specificity. Four showed only I specificity and one (Myr.) showed primarily i specificity. Four showed an additional reactivity with rabbit red cells at 37°C. The cold agglutinins isolated from serums of patients with atypical pneumonia did not react with the antiserum B.M.

Efforts were made to study the effect of combination with red cells on the specific antigenic determinants. Two isolated cold agglutinins when attached to red cells were considerably less effective in absorbing out the specific reactivity of antiserum to B.M. than an equivalent amount of free cold agglutinin.

These studies indicate that there are a group of antigens characteristic of macroglobulins with cold agglutinin activity. The term "cross specificity" has been applied to this antigenic similarity, distinguishing it from the "individual antigenic specificity," which is readily observed with cold agglutinins as well as with some antibodies produced by direct immunization (*5*). Previous studies on various other antibodies (*5*, *7*) failed to reveal cross specificity of the type observed for the cold agglutinins. However, these antibodies lacked the homogeneity of the cold agglutinins.

The major question raised by the above observations is whether the specific antigens characterizing cold agglutinins relate directly to the presumed antibody-combining site of these proteins. A complete answer is not yet available, and the main evidence depends simply on direct correlation. The fact that these antigens are at least partially blocked by combination of the cold agglutinin with its antigen favors such a concept. However, no clear relation was apparent between these antigens of the cold agglutinins and the exact specificity of the cold agglutinins for red cells of different types.

Alternatively the γM cold agglutinins might belong to a minor subgroup of the macroglobulins which includes antibodies of other specificities. There is precedence for such a concept in the case of antibodies to dextran where the vast majority belong to the minor γG2 subgroup (*10*). Against this view is the finding of an assortment of different specific antigens in cold agglutinins, whereas subgroup antigens are usually extremely similar from one protein to another. A third possibility is that some unusual antigenic material is linked to these macroglobulins that is involved in their cold agglutinin property. Studies on another group of homogeneous antibodies such as the antibodies to γ-globulins should help decide between these alternatives. Regardless of the outcome of further studies, the determination of the chemical basis for the various antigens should be feasible and should prove of considerable interest.

RALPH C. WILLIAMS, JR.
Department of Medicine,
University Hospitals,
Minneapolis, Minnesota
HENRY G. KUNKEL
J. DONALD CAPRA
Rockefeller University, New York

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Hemoglobin Variant Common to Chinese and North American Indians: $\alpha_2\beta_2^{22\ Glu\rightarrow Ala}$

Abstract. *An electrophoretically slow hemoglobin variant, in which the structural change involves the replacement of a glutamyl residue by alanyl at position beta-22, was reported in two groups of North American Indians: hemoglobin-G Coushatta, in Alabama-Coushatta Indians in Texas; and hemoglobin-G Saskatoon, in descendants of Santee Indians living in Canada. Hemoglobin-G Hsin-Chu, found in Taiwan in a Chinese from the northern Chinese province of Liaoning, is now shown to have the same structural anomaly.*

An electrophoretically slow variant typical of G-type hemoglobins was found in Taiwan in 1964 during a survey of presumably normal Chinese (*1*); one Chinese male had the G variant and normal hemoglobin-A in approximately equal amounts. Study of the structure of the hemoglobin, which was named G Hsin-Chu, now shows that the structural anomaly occurs at the beta-22 position where the glutamyl residue is replaced by an alanyl residue: $\alpha_2\beta_2^{22\ Glu\rightarrow Ala}$. Thus this variant appears to be identical with two others whose structures were reported recently in North American Indians: hemoglobin-G Coushatta (*2*) and hemoglobin-G Saskatoon (*3*).

The studies of structure used reported procedures (*4*). The purified mixture of A+G hemoglobin was subjected to tryptic digestion, and the resultant peptides were examined by peptide-mapping procedures (*5*) on Whatman 3MM paper (*6*). A new arginine-positive (*7*) peptide was present near the normal βT3 peptide from hemoglobin-A; its electrophoretic mobility

Table 1. Amino acid composition of abnormal βT3 peptide from hemoglobin-G Hsin-Chu.

Amino acid residue	Normal βT3 molar ratio	Observed for the abnormal βT3 peptide		
		Micro-mole	Molar ratio	Nearest integer
Arg	1	0.033	1.0	1
Asp	2	.064	1.9	2
Glu	2	.042	1.3	1
Gly	3	.094	2.9	3
Ala	1	.066	2.0	2
Val	3	.095	2.9	3
Leu	1	.035	1.1	1

toward the anode was lower and its chromatographic mobility was higher than that of the βT3 peptide.

For further analysis the abnormal peptide was separated from other peptides by high-voltage paper electrophoresis, first at *p*H 5.4 (*8*) and again at *p*H 1.9 (*9*); it was eluted with 5.7*N* hydrochloric acid and hydrolyzed for 18 hours in a sealed, evacuated tube at 110°C. Then quantitative amino acid analysis of the resultant hydrolysate was made by automated chromatography (*10*). The results appear in Table 1 (*11*).

The amino acid composition of the abnormal peptide indicated that it contained one additional alanyl residue and one less glutamyl residue than did the normal βT3 peptide, so that one of the two glutamyl residues normally present in the βT3 section of the β-chain, at positions β-22 and β-26, must have been replaced by alanyl in hemoglobin-G Hsin-Chu. The normal amino acid sequence for βT3 peptide is

-Val-Asn-Val-Asp-Glu-Val-Gly-Gly-
18 22
Glu-Ala-Leu-Gly-Arg-
26 30

To determine which glutamyl position was affected, analysis by stepwise degradation, by a modified Edman degradation procedure (*12*), was applied to 1-μM quantities of the abnormal βT3 peptide; for comparison, parallel degradation studies were made of the βT3 peptide from normal hemoglobin-A. Descending paper-chromatographic procedures (*13*) were used for identification of the phenylisothiohydantoin–amino acid derivatives released at each cycle of the degradation. The initial eight sequences in both peptides were determined.

The results were the same in both peptides for the first four positions; in the fifth cycle, glutamic acid was found in the normal βT3 peptide, whereas alanine was found in the βT3 peptide from the G-hemoglobin. Three additional cycles were run on both peptides, and the normal amino acid derivatives were obtained. These results were considered sufficient to determine that the anomaly in hemoglobin-G Hsin-Chu involved replacement of a glutamyl residue by one of alanine at position β-22.

Occurrence of an alanyl residue at position β-22 is one of the changes characteristic of the δ-chain (*14*). Therefore, as in the earlier study (*2*), it was necessary to determine whether any other δ-like substitutions were present such as those found in the hybrid δ-β chains that replace normal β-chains in the Lepore hemoglobins (*15*). Accordingly the peptides corresponding to βT2 and βT5 were isolated by a combination of high-voltage electrophoresis (*8, 9*) and chromatography, hydrolyzed, and analyzed for their amino acid compositions. In both peptides the amino acid distributions were those expected for the β-chain peptides and not for the δ-chain; the only change found was that described above at the β-22 position.

The first subject having hemoglobin-G Hsin-Chu had migrated to Taiwan from mainland China; his parents had come from the northern Chinese province of Liaoning. Several other subjects from northern provinces carry similar, electrophoretically slow, hemoglobin variants of which some presumably will prove to be G Hsin-Chu.

Hemoglobin-G Coushatta was found (*2*) in several Alabama-Coushatta Indians now living in eastern Texas. Hemoglobin-G Saskatoon was found (*3*) in several North American Indians of apparently mixed tribal ancestry, now living in Canada, whose recent forebears included Santee Indians who lived in the Dakotas. Final evaluation of the ethnologic significance of the occurrence of this G-hemoglobin, $\alpha_2\beta_2^{22\ Glu\rightarrow Ala}$, in such widely differing groups as northern Chinese and North American Indians awaits further large-scale studies for more precise determination of the patterns of occurrence and incidences of hemoglobin variants in various ethnic groups.

R. QUENTIN BLACKWELL*
CHEN-SHENG LIU
HUNG-JU YANG, CHENG-CHANG WANG
JEANETTE TUNG-HSIANG HUANG
Department of Biochemistry, U.S. Naval Medical Research Unit No. 2, Taipei, Taiwan

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6. Electrophoresis was carried out first for 1.5 hours at 40 volt/cm with pyridine-acetate buffer at *p*H 5.4. Descending chromatography was run overnight with the solution of pyridine, butanol, acetic acid, and water in the respective volume proportions of 10:15:3:12 [R. L. Hill, R. T. Swenson, H. C. Schwartz, *Blood* **19**, 573 (1962)].
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10. Technicon Autoanalyzer equipment was used according to standard procedures, with norleucine internal and external standards.
11. Common abbreviations are used for amino acid residues: Arg, arginine; Asp, aspartic acid; Asn, asparagine; Glu, glutamic acid; Gly, glycine; Ala, alanine; Val, valine; and Leu, leucine.
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* Mailing address: Box 14, NAMRU-2, APO San Francisco, Calif. 96263

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Obsidian Sources Characterized by Neutron-Activation Analysis

Abstract. *Concentrations of elements such as manganese, scandium, lanthanum, rubidium, samarium, barium, and zirconium in obsidian samples from different flows show ranges of 1000 percent or more, whereas the variation in element content in obsidian samples from a single flow appears to be less than 40 percent. Neutron-activation analysis of these elements, as well as of sodium and iron, provides a means of identifying the geologic source of an archeological artifact of obsidian.*

Obsidian artifacts are often recovered from archeological sites considerably removed from geologic sources of the mineral. Occurrence of obsidian on Hopewell sites in the Illinois and Scioto valleys of the eastern United States is only one example; the nearest obsidian flows are in Mexico, New Mexico, and Yellowstone National Park, and on the Pacific Coast. In studies of prehistoric trade patterns, it is desirable to know the true sources.

Recent analysts of prehistoric obsidian artifacts and sources in the Near East (*1, 2*) and the Aegean (*3*) have attempted to demonstrate that each obsidian source may be characterized by elemental analyses. Studies of the elemental content of obsidian have since been extended to New Zealand (*4*) and