suppressor. This strain did not yield recombinants when crossed with the original A33 mutant. However, it differed from A33 in being sensitive to suppression by su3+ and in being revertible.

Tryptic peptide TP15, the peptide that contains the amino acid substituted by the A33 change, was isolated by column chromatography on Dowex 1-X2 resin (*12*). This peptide is one of the last eluted by this technique, appearing when the concentration of the eluting buffer is approximately 2*N* with respect to acetic acid. The peptide was further purified by paper electrophoresis at *p*H 3.7 prior to analysis of the amino acids. Table 4 shows the amino acid composition of TP15 from wild-type, mutant A33, and from mutant A33-X. It is evident that the su3+ gene selected the change from a methionine codon to a valine codon at position 48.

The ability to select for this change from methionine to valine as a single mutational event provides in vivo support for assigning AUG as the RNA codeword for methionine and GUG as a codeword for valine.

In the presence of two distinct suppressor genes we have been able to isolate CRM+ A mutants which have amino acid replacements in their A proteins predicted by the genetic code, but which are not found by ordinary reversion analysis because the amino acid replacements do not restore prototrophy. The new change in mutant A46 brings the number of amino acids that replace glutamic acid to four, out of a possible six predicted on the basis of the RNA codons.

As additional CRM+ mutants and suppressor genes are characterized, it will be possible to use our technique to extend the analysis of mutational relationships predicted by the genetic code.

HILLARD BERGER
CHARLES YANOFSKY
Department of Biological Sciences, Stanford University, Stanford, California 94305

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4. Abbreviations used are: A, adenine; G, guanine; C, cytosine; U, uridine; CRM+, mutants which produce an A protein which reacts with antibodies prepared with wild-type A protein (cross-reacting material); Arg, arginine; Asp, aspartic acid; Thr, threonine; Ser, serine; Glu, glutamic acid; Pro, proline; Gly, glycine; Ala, alanine; Val, valine; Met, methionine; Ile, isoleucine; Leu, leucine; Phe, phenylalanine; Trp, tryptophan; Cys, cysteine; Lys, lysine; Tyr, tyrosine; and His, histidine.
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Hemoglobin $\alpha_2\beta_2{}^{121\ \mathrm{Lys}}$ Chemical Identification in an Egyptian Family

Abstract. *Study of the amino acid composition and amino-terminal residue in the abnormal peptide of an electrophoretically slow hemoglobin revealed that the globin's structure was* $\alpha_2\beta_2{}^{121\ Lys}$ *(Oβ). This hemoglobin had been provisionally classified as Hb E or as a new hemoglobin. The clinical significance of this abnormality is not yet defined.*

We have chemically identified a hemoglobin variant located in the Nile Delta of Egypt, United Arab Republic. At *p*H 8.7 in starch-gel electrophoresis, this hemoglobin (Hb) moved to the zone of Hb E/A_2 and was tentatively classified as Hb E. However, a suggestion was made that the abnormal hemoglobin might be a new one because, unlike Hb E, the molecule was distinguishable from Hb A_2 in two-dimensional starch-gel electrophoresis (*1*).

The propositus and his family possessed the abnormality in a heterozygous form. The slow component was separated from hemoglobin A on starch gels and was eluted and purified (*2*). Polypeptide-chain hybridization with canine hemoglobin and with known abnormal human hemoglobin showed that the molecular abnormality

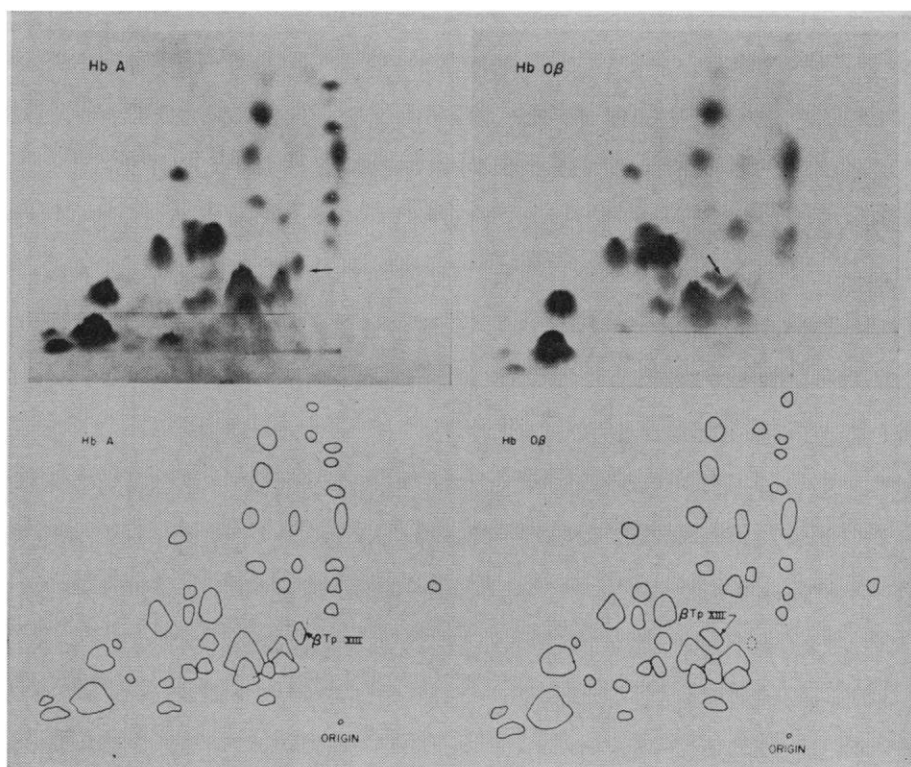

Fig. 1. Fingerprints and corresponding diagrams of trypsin digests of hemoglobins A and $\alpha_2\beta_2{}^{121\ Lys}$ (Oβ). Peptide β Tp XIII has moved farther toward the cathode on the left in the abnormal hemoglobin.

Table 1. Acid and neutral amino acids of peptides β Tp XIII of Hb A and Hb $\alpha_2\beta_2^{121\,Lys}$. Because of the specific cleaving action of trypsin on arginine and lysine, and since staining for arginine was negative on these peptides, lysine is assumed to be present also.

Amino acid	Hb A β Tp XIII (molar ratios)	Abnormal Hb β Tp XIII (molar ratios)
Threonine	1.00	0.98
Glutamic acid	2.94	2.11
Proline	1.81	1.93
Alanine	2.01	2.08
Valine	0.93	1.08
Tyrosine	.83	0.71
Phenylalanine	.96	.97

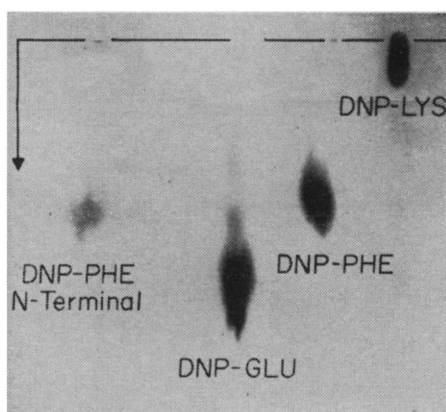

Fig. 2. Chromatogram in 1.5*M* phosphate buffer *p*H 6.0, showing DNP-phenylalanine as the NH_2-terminal amino acid of abnormal peptide β Tp XIII and some other DNP-amino acids as controls.

was in the β-chain. An abnormal α-chain substitution, like that in Hb O Indonesia, which shares similar electrophoretic properties with the above hemoglobin (*3*), was thus excluded.

Tryptic digests of Hb A and Hb E were made as standards to compare with similar preparations of our unknown. High-voltage electrophoresis was done on a sample applied to Schleicher and Schuell filter paper No. 598, cut to a dimension of 58 by 10 cm. The buffer used was *p*H 4.4 (water, pyridine, and acetic acid, 94:2:4) and a potential gradient along the length of the paper was 35 volt/cm held for 75 minutes. After electrophoresis of individual tryptic digests of Hb A, Hb E, and the present unknown, staining with ninhydrin and for arginine revealed that our unknown was not the classic Hb E.

Second-dimension peptide patterns (fingerprints) were formed by using a chromatographic solvent system of butanol, acetic acid, and water (4:1:5), in a recycled ascending procedure. The peptides were more adequately resolved after the second chromatographic run. Figure 1 shows a resulting fingerprint of the abnormal hemoglobin compared to one of Hb A. The peptide of interest, β Tp XIII, was identified by differential staining. It will be noted that this peptide had moved from a relatively uncharged position in Hb A to a less acidic point on the "fingerprint" of the unknown trypsin digest. This shift was suggestive of the abnormality Hb $\alpha_2\beta_2^{121\,Lys}$ ($O\beta$) sometimes referred to as Hb O Arab, described by Baglioni *et al.* (*3*).

The corresponding peptides were eluted from the chromatograms of digested β-chains (*4*) and further purified by additional electrophoresis in *p*H 3.6 buffer (pyridine, acetic acid, and water; 1:10:90). The isolated peptides were submitted to amino acid analysis (*5*). Comparison of the amino acid compositions revealed a decrease in glutamic acid residues in β Tp XIII of the abnormal hemoglobin (Table 1). The NH_2-terminal amino acid of the abnormal β Tp XIII (*6*) was found to be phenylalanine since its dinitrophenyl (DNP) derivative obtained a similar R_F value as that of standard DNP-L-phenylalanine in 1.5*M* phosphate buffer *p*H 6.0 descending chromatography (Fig. 2). Fingerprints of chymotryptic digests of trypsin-resistant core showed no difference from those of Hb A.

In accordance with Baglioni and Lehmann (*3*), it was now possible to establish that glutamic acid, normally at position 121 in the β-chain, had been replaced by lysine, which was in turn cleaved by trypsin, leaving phenylalanine at the NH_2-terminal end of β Tp XIII. We have demonstrated a tyrosine yield in the mutant peptide quantitatively equal to the normal control (Table 1). This result was different from a corresponding value reported by Baglioni and Lehmann (*3*). Heterozygous carriers of this hemoglobin manifest no clinical symptoms or signs (*1*). However, since no homozygotes have been reported, speculation cannot be made on its pathological effect, if any. We have discussed the anthropological implications of this hemoglobin variant (*7*).

K. A. KAMEL
K. C. HOERMAN
U.S. Naval Medical Research Institute, Bethesda, Maryland 20014
A. Y. AWNY
Clinical Pathology Department, Ain-Shams University Medical School, Cairo

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Seed Dormancy: Breaking by Uncouplers and Inhibitors of Oxidative Phosphorylation

Abstract. *When 2,4-dinitrophenol and carbon dioxide were applied together to dormant seeds of* Trifolium subterraneum *L. (subterranean clover), 2,4-dinitrophenol did not disturb the breaking of dormancy which carbon dioxide usually induces in legume seeds. On the contrary, on its own, it promoted germination in a substantial proportion of seeds; a similar effect was produced by other uncouplers or inhibitors of oxidative phosphorylation.*

The mechanism by which CO_2 acts in breaking the dormancy of small-seeded legumes, especially subterranean clover remains unknown (*1*). While examining what effect metabolic inhibitors might have on this action of CO_2, we obtained the following result using 2,4-dinitrophenol (DNP). The indicated values are the percentages of germination of subterranean clover seed on the 8th day after the named treatments: water, 16; 2.5 percent CO_2, 99; 2.5 percent CO_2 plus 0.1 m*M* DNP, 97; 0.1 m*M* DNP, 54. Not