buffer, *p*H 7·0, did not restore ACHE activity. No ACHE activity could be detected in the supernatant solution after penicillin treatment. When erythrocytes were treated with penicillin solutions which had been heated for 60 min at 56° C, inactivation of ACHE activity was not affected. Erythrocyte membrane integrity was not necessary for the inactivating effect of penicillin, because soluble bovine erythrocyte ACHE rapidly lost activity when treated with the antibiotic (Fig. 3).

In further experiments, blood group *A* and *B* erythrocytes from normal adults were preincubated with concentrations of penicillin which cause only a slight reduction of ACHE activity. These cells were then washed and exposed to specific immune isoantibodies, and the reduction in ACHE activity was no greater than that caused by the antibiotic alone. Penicillin-treated *A* or *B* cells, however, became more susceptible to haemolysis after incubation with the isoantibodies in contrast to controls consisting of blood group *A* or *B* cells treated with sucrose or blood group O cells preincubated with penicillin. Treatment of erythrocytes with lytic, polyene antibiotics[11] such as filipin and amphotericin *B* or with up to 0·4 molar streptomycin sulphate caused no inactivation of ACHE activity.

This work was supported by a research grant from the National Institutes of Health, U.S. Public Health Service.

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Amino-acid Substitution in Haemoglobin I (Texas Variant)

AN electrophoretically fast genetic variant of adult haemoglobin was described in six members of a Negro family living in Texas by Thompson *et al.* in 1963 (ref. 1). Fingerprints of the purified haemoglobin established the identity of this variant with haemoglobin I previously described by Murayama and Ingram[2], in which the third tryptic peptide from the N-terminus of the α chain contained an amino-acid alteration responsible for the rapid electrophoretic migration of the intact haemoglobin. Lysine, which is the sixteenth residue, had been replaced in haemoglobin I by an acidic amino-acid. The substitution was described in both I variants from Texas[1] and Philadelphia[3] as being lysine to aspartic acid; however, recent elucidation of the genetic code has eliminated the substitution lys→asp from the class of mutations which could result from alteration of a single nucleotide base in the triplet code[4]. A nucleotide triplet coding for lysine (AAA or AAG) could not be altered in a single base so that it would specify aspartic acid (GAU or GAC). This led Beale and Lehmann[5] to re-examine the I variant from Philadelphia. Their findings established the substitution to be lys→glu, which is in agreement with an alteration in a single nucleotide (AAA or AAG to GAA or GAG).

The purpose of the present investigation was to re-examine haemoglobin I from Texas (from individual J. A.[1]), because this was the only structural variant of adult haemoglobin which could not be explained genetically by a single transition or transversion. By using more sensitive analytical techniques now available, the peptide I αTIII, IV was analysed on an automatic amino-acid analyser. The amino-acid composition is presented in Table 1. The lysine residue in the sixty-eighth position of the α chain of haemoglobin A is replaced by glutamic acid in haemoglobin I. This substitution is responsible for one less tryptic peptide in fingerprints and accounts for the higher number of negative charges in the intact haemoglobin. It was evident that the excess of aspartic acid reported previously in αTIII, IV of haemoglobin I (refs. 1 and 3) resulted from contamination of βTV (oxidized) which contains three residues of aspartic acid and tends to smear over αTIII, IV in chromatography and electrophoresis at *p*H 6·4.

Table 1. αIII–IV FROM HAEMOGLOBIN I

	Micromoles × 10^{-2}	Molar ratios	A III + IV composition
Lysine	–	–	1
Histidine	1·2	0·9	1
Arginine	1·3	1·0	1
Aspartic acid	0·2	0·1	–
Glutamic acid	5·3	4·0	3
Proline	–	–	–
Glycine	5·2	4·0	4
Alanine	7·5	5·8	6
Half cystine	–	–	–
Valine	1·5	1·1	1
Methionine	–	–	–
Isoleucine	–	–	–
Leucine	1·5	1·1	1
Tyrosine	1·2	0·9	1
Phenylalanine	–	–	–
Tryptophan	positive Ehrlich stain	(1)	1

The amino-acid substitution in haemoglobin I from Texas is identical with that found in I from Philadelphia by Beale and Lehmann[5]; furthermore, it can now be stated that each amino-acid substitution described in a foetal or adult haemoglobin variant is consistent with a genetic event involving an alteration of a single base in the triplet code.

This work was supported by grants from the U.S. National Institutes of Health.

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CYTOLOGY

Technique for Investigation of Sex Chromatin in Amniotic Membrane of Rat Foetuses

THE letter of Bianchi and de Bianchi[1] prompts us to comment on our own experience in preparing slides for the study of sex chromatin in amniotic membrane cells from rat foetuses. They mentioned in their letter the difficulty of fixing the amniotic membrane of rat foetuses with absolute or 95 per cent alcohol, resulting in coagulation of mucopolysaccharides and shrinkage of the cells.

We have tried fixing the membrane with 95 per cent ethanol, and staining with cresyl violet or with lactic acetic orcein. Both these techniques have been unsatisfactory in our hands. To overcome these difficulties we have used a quick squash technique, which has given satisfactory results. This technique is a modification of that used to stain the sex chromatin of cells from human buccal smears[2].