1100°C (*3*). They are rhombohedral with $a \simeq 6.5$ Å and $\alpha > 90°$ for the small cations such as Cu, Mg, Zn, Cd, and Ag; for the larger cations $\alpha < 90°$. Since the rhombohedral angles are very close to 90°, the metric symmetry of these compounds is almost cubic. Although the structural arrangement is not yet known, it is reasonable to assume that these compounds are not of an intercalation nature as the ratio of cation to sulfur is unity.

We anticipated superconductivity in most of these compounds, and our expectations were verified as shown in Table 1. Because of their high transition temperatures, one could speculate that their arrangement should also be pseudocubic (*4*). In any event this new system of superconductors illustrates once again the incompatibility between pseudo two-dimensional metal networks, as in the layer compounds, and high transition temperatures. A detailed report together with the crystal structure of these compounds will be published elsewhere (*5*).

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References and Notes

1. R. B. Somoano and A. Rembaum, *Phys. Rev. Lett.* **27**, 402 (1971).
2. H. E. Barz, A. S. Cooper, E. Corenzwit, M. Marezio, B. T. Matthias, P. H. Schmidt, *Science* **175**, 884 (1972).
3. R. Chevrel, M. Sergent, J. Prigent, *J. Solid State Chem.* **3**, 515 (1971).
4. B. T. Matthias, E. Corenzwit, A. S. Cooper, L. D. Longinotti, *Proc. Nat. Acad. Sci. U.S.* **68**, 56 (1971).
5. B. T. Matthias, M. Marezio, E. Corenzwit, A. S. Cooper, H. E. Barz, in preparation.

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Genetic Variation in Human Erythrocyte Acetylcholinesterase

Abstract. *A method for solubilization of human erythrocyte membranes was developed and used to survey 70 unselected human blood samples for isozymic variation of stromal acetylcholinesterase. Three variants were observed. Pedigrees of families studied by this method indicated that this variation represented the phenotypic expression of two codominant alleles at a single locus.*

Familial reduction in the activity of human erythrocyte acetylcholinesterase (AChE) (E.C. 3.1.1.7) has been reported (*1*); reduction in enzyme activity had no associated symptoms. This report prompted us to examine AChE for normal, genetically determined isozymic variation. This enzyme is a firmly bound component of the human erythrocyte membrane (*2*). The membrane, or stroma, is a complex framework of proteins and lipids. It has been characterized by electrophoresis and found to consist of several classes of polypeptides (*3*, *4*), which can be distinguished by their molecular weights (*4*, *5*). In most studies of this kind, however, strong protein-denaturing agents have been used, such as urea (*6*) and sodium dodecyl sulfate (*4*, *7*), which destroy the catalytic activity of enzymes. To examine the possibility of isozymic variation by gel electrophoresis, we devised a rapid method for the solubilization of human erythrocyte membranes without loss of AChE activity.

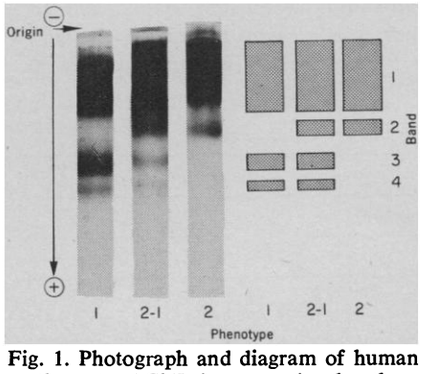

Fig. 1. Photograph and diagram of human erythrocyte AChE isozymes in the three AChE phenotypes: 1, 2-1, and 2. Band 3 is not always heavier in phenotype AChE 1 than in phenotype AChE 2-1, as in the photograph.

Venous blood was collected in tubes containing heparin and centrifuged, and the plasma was removed. The cells were washed three times in isotonic saline and hemolyzed in 1.4 volumes of distilled water and 0.4 volume of toluene (*8*). After vigorous shaking, the mixture was centrifuged at 6000*g* for 20 minutes. The stromata could be seen between a top layer of toluene, which contained dissolved lipids, and the bottom layer, which contained the water-soluble hemolyzate. Both toluene and hemolyzate were removed by aspiration. The extraction of stromata was repeated once, after which the stromata were pink or white. They were suspended in a solution of 5 percent Triton X-100 in 0.01*M* phosphate buffer, *p*H 7.4; the volume of the suspension was made equal to the original volume of packed cells. The suspension was stored at −10°C.

Disc electrophoresis in polyacrylamide gels was done essentially by the method of Clarke (*9*), with 7 percent gels in glass tubing 90 mm long with an inner diameter of 5 mm. Stromal samples diluted 1 : 5 were applied to the top of the gel columns in Sephadex G-200. Electrophoresis was conducted at 2 ma per tube for 45 to 60 minutes. Activity of AChE on these gels was detected histochemically by a copper-thiocholine technique (*10*).

In a survey of blood samples from 70 randomly selected adults, all samples showed a slowly migrating major zone of activity (Fig. 1, band 1). In addition there were up to three minor components, which migrated faster than the major zone. Bands 3 and 4 appeared in samples from 53 individuals (76 percent); band 2 alone occurred in 5 samples (7 percent); and all 3 components occurred in 12 samples (17 percent). Isozymic variation was not associated with significant differences in total AChE activity. These patterns were reproducible when several determinations were made on the same sample and when several samples were taken at different times from the same individual.

When benzoylcholine was used as substrate, according to the method of

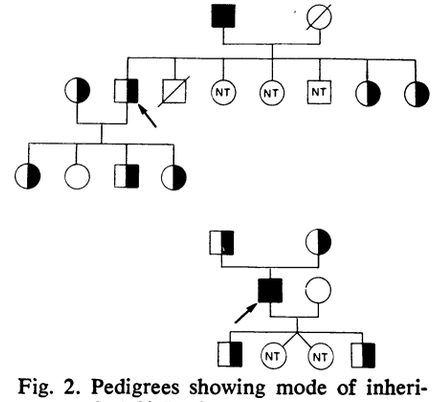

Fig. 2. Pedigrees showing mode of inheritance of AChE phenotypes. Squares are males; and circles, females; open symbols are AChE 1; solid, AChE 2; and half-solid, AChE 2-1; NT means not tested; and the diagonal line indicates that individual was deceased. Arrows refer to probands.

Kalow and Lindsay (*11*), the enzyme activity was very slight—only 5 to 10 units. Moreover, this activity was not inhibited by $2 \times 10^{-5}M$ quinidine sulfate (*12*). Both results indicate that the hydrolysis was not due to plasma cholinesterase.

When families were studied by this method (Fig. 2), the variation in bands 2, 3, and 4 of the zymograms was shown to represent the phenotypic expression of two alleles—*AChE*^{1} and *AChE*^{2}—at a single gene locus. The homozygous phenotypes are arbitrarily designated AChE 1 and AChE 2, and the heterozygous type is called AChE 2-1. The two-allele hypothesis was consistent with data from three additional families.

Human erythrocyte AChE activity can be resolved into two components by cellulose-acetate electrophoresis and by anion-exchange chromatography; the two components differ in molecular charge, but not in molecular weight (*13*). The major zone of activity reported here (Fig. 1, band 1) appeared as two discrete bands when a sample diluted 1 : 25 was applied to the gel. This dilution was not used routinely, however, because at this dilution bands 2, 3, and 4 could not always be seen.

We believe that this is the first report of polymorphic variation in a stromal enzyme. With the use of this technique, it may be possible to examine other membrane-bound enzymes for genetic variation.

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References and Notes

1. R. J. Johns, *N. Engl. J. Med.* **267**, 1344 (1962).
2. D. Vincent, G. Segonzac, G. Sesque, *C. R. Soc. Biol.* **155**, 662 (1961).
3. S. L. Marchesi, E. Steers, V. T. Marchesi, T. W. Tillack, *Biochemistry* **9**, 50 (1970); J. Lenard, *ibid.*, p. 1129.
4. G. Fairbanks, T. L. Steck, D. F. H. Wallach, *ibid.* **10**, 2606 (1971); H. R. Trayer, Y. Nozaki, J. A. Reynolds, C. Tanford, *J. Biol. Chem.* **246**, 4485 (1971).
5. J. T. Gwynne and C. Tanford, *J. Biol. Chem.* **245**, 3269 (1970).
6. E. A. Azen, S. Orr, O. Smithies, *J. Lab. Clin. Med.* **65**, 440 (1965).
7. S. A. Rosenberg and G. Guidotti, *J. Biol. Chem.* **243**, 1985 (1968).
8. R. E. Tashian, *Proc. Soc. Exp. Biol. Med.* **108**, 364 (1961).
9. J. T. Clarke, *Ann. N.Y. Acad. Sci.* **121**, 428 (1964).
10. G. A. Davis and B. W. Agranoff, *Nature* **220**, 277 (1968).
11. W. Kalow and H. A. Lindsay, *Can. J. Biochem. Physiol.* **33**, 568 (1955).
12. G. L. Ellman, K. D. Courtney, V. Andres, R. M. Featherstone, *Biochem. Pharmacol.* **7**, 88 (1961).
13. T. Shafai and J. A. Cortner, *Biochim. Biophys. Acta* **236**, 612 (1971).
14. Supported by the Medical Research Council of Canada.

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Expression of the Mammalian X Chromosome before and after Fertilization

Abstract. *The activity of hypoxanthine-guanine phosphoribosyltransferase in unfertilized mouse ova and in mouse embryos at the two-cell stage is proportional to the number of X chromosomes present during oogenesis. This indicates that the enzyme is X-linked in the mouse and that inactivation of the X chromosome does not occur during oogenesis. However, the genetic dosage effect of the X chromosomes is not present after the increase in hypoxanthine-guanine phosphoribosyltransferase activity in the late morula and the blastocyst stages. These results indicate that the X-linked enzyme locus is expressed sometime after fertilization but before the morula stage.*

We have reported (*1*) that glucose-6-phosphate dehydrogenase (G6PD) is synthesized in a manner that is dependent on the genetic dosage of the X chromosome during oogenesis in the mouse and that inactivation of the X chromosome does not occur in the developing oocyte. These conclusions were based on experimental results showing that the activity of G6PD in ova of XX mice is twice that in ova of XO mice; no difference was observed for autosomally controlled lactate dehydrogenase. Investigations on another enzyme known to be X-linked in man, hypoxanthine-guanine phosphoribosyltransferase (HGPRT) (E.C. 2.4.2.8.), indicate the same genetic dosage effect of the X chromosome on the enzyme's activity in mouse ova. Furthermore, measurements of the activity of HGPRT in the later stages of development of the embryo, but before its implantation in the uterus, indicate that the increase in the enzyme activity (*2*) is determined by events that occur after fertilization.

Mouse embryos at ages of 1 day (two-cell) and 3 days (morula and blastocyst) were obtained from mothers with XO (genotypes *Ta*/*O* and +/*O*) and XX (genotype *Ta*/+) chromosomes (*3*) (Jackson Laboratory). The mice were injected with pregnant mare's serum and human chorionic gonadotropin to induce ovulation and were mated with albino Swiss males (*4*). Groups of embryos were assayed simultaneously for HGPRT and guanine deaminase (E.C. 3.5.4.3) activity (*2*, *5*); the results are shown in Table 1. Guanine deaminase, not known to be X-linked in any species, was used as a control enzyme. The activity of guanine deaminase was nearly the same in both the two-cell embryos from XO mothers and those from XX mothers (XO/XX = 0.90). In contrast, the activity of HGPRT in the two-cell embryos from XO mothers was almost half of that in the embryos of XX mothers (XO/XX = 0.48). There was almost no difference, however, in activity of HGPRT in erythrocytes of XO and those of XX adult mice (XO/XX = 1.08). Thus, the activity of HGPRT at the two-cell stage of development is proportional to the number of X chromosomes present during oogenesis; synthesis of HGPRT in the oocyte appears to be controlled in the same manner as that of G6PD.

Experience indicated that the HGPRT assay was more reliable with two-cell embryos than with fertilized or unfertilized ova. The foregoing experiments were therefore carried out with fertilized two-cell embryos. However, there is a chance that the YO and some of the XO embryos, from XO mothers, destined to die before reaching the blastocyst stage (*6*), might be metabolically abnormal and, as a result, have lower HGPRT activity. Although the likelihood of this is not great [embryos of XO mothers appear normal until after the two-cell stage (*7*)], we nevertheless studied the dosage effect in unfertilized eggs. Reliable results were obtained if the ova were washed six times in medium (*8*) to remove follicular cells and other contaminants before assay. Unfertilized ova (from unmated mice primed with hormones) were treated with hyaluronidase (bovine testicular, Sigma type I, 75 units per milliliter of medium) to remove cumulus cells, were washed six times, and were assayed for activities of HGPRT and guanine deaminase. As in the two-cell embryos, the activity of HGPRT in the unfertilized eggs from XO