Nb_3Al (*4*), and recently Nb_3Ge (*5*), are the only known superconductors with transition temperatures in the vicinity of 18°K. In itself, Nb_3Ge is a rather ambiguous compound. Under normal conditions of formation it crystallizes in the nonstoichiometric ratio of about 3.3 Nb to 1 Ge, thus being very rich in niobium compared to the ideal ratio of 3 to 1. The $Nb_{3.3}Ge$ has a superconducting transition temperature from 6° to 7°K. When more stoichiometric ratios are enforced by extremely rapid cooling methods, the transition temperature can be raised to 18°K. It has not yet been possible to decide with certainty how close these compounds now are to the 3 to 1 ratio. In the Nb-Al system there is a temperature-dependent homogeneity range (*6*) which makes the 3 to 1 ratio difficult to obtain.

We, therefore, decided to investigate the Nb_3Al–Nb_3Ge system. It is interesting to note that recently reported similar efforts have failed to reach even 18°K (*7*). The maximum temperature of 20°K reached at present for more or less ordered crystals is near a composition of 4 Nb_3Al to 1 Nb_3Ge. Alloys prepared by arc melting were annealed over a period of days at temperatures below 1000°C.

The superconducting transition was measured magnetically by observing superconducting shielding currents and subsequently was measured calorically. The latter method verified that the superconductivity of our samples was a bulk effect. The specific heat versus temperature is given in Fig. 1 and shows a transition into the superconducting state at 20.05°K. Superconducting shielding currents were observed with a ballistic galvanometer on a similarly prepared sample when immersed in liquid hydrogen at 20.05°K. The electronic specific heat, γ, obtained from the measured entropy in the superconducting state and assuming the validity of the third law of thermodynamics, shows a large decrease of more than a factor of 2 from the values measured for Nb_3Sn or V_3Si (*8*). This variation of T_c with γ, and consequently with the density of states, is opposite to the prediction given by microscopic theories. This is not too surprising since it has become quite evident during the last few years (*9*) that any correlation between γ and T_c is tenuous at least and usually of the wrong sign. In fact, when the measured transition temperature and γ are used in the microscopic theory to define empirically some attractive electron-electron interaction (which is neither measured nor calculated) it is found that variations in density of states must then be almost completely compensated by variations in the interaction parameter for all the high-temperature superconductors (*10*).

We have at present no real explanation for the high transition temperatures apart from the hypothesis that the stoichiometry of Nb_3Al or perhaps even of the intermediate composition has been improved considerably over that of the normal Nb_3Al or Nb_3Ge. We do not think that the variation of the average number of valence electrons per atom is decisive (*11*).

The only conclusion, by analogy, that can be drawn at present with certainty is that V_3Al, should we ever manage to crystallize it in the β-W structure, would be a very high superconductor. Since there is no theory for the high transition temperatures of a superconductor, it is impossible to say how much this value would be raised above 18°K.

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12. We wish to thank A. M. Clogston and G. S. Indig for many helpful discussions. The work of B.T.M. at La Jolla was sponsored by the Air Force Office of Scientific Research, Office of Aerospace Research, U.S. Air Force, under grant AF-AFOSR-631-67.

22 April 1967

Hexokinase Isoenzymes in Human Erythrocytes: Association of Type II with Fetal Hemoglobin

Abstract. *Hexokinase activity in human erythrocytes is associated with three electrophoretically distinct bands. Normal adult erythrocytes contain hexokinases Types I and III. Type II hexokinase is present in the erythrocytes of newborn infants and absent from those of normal adults; it is, however, present in erythrocytes of adults with hereditary persistence of fetal hemoglobin. Type II hexokinase and fetal hemoglobin appear to be associated.*

Heparinized samples of venous blood were spun at 600*g* for 10 minutes at 4°C, and the buffy coat and plasma were removed. The red cells were suspended in 0.9 percent saline containing glucose (0.01*M*), and the suspension was centrifuged at 2000*g* for 20 minutes. This process was repeated three times. The washed red cells were resuspended in an amount, equal to their volume, of 0.01*M* phosphate buffer, *p*H 7.0, containing 2-mercaptoethanol (5 mmole/liter), sodium ethylenediaminetetraacetate (EDTA, 5 mmole/liter), glucose (0.01 mole/liter). They were lysed by freezing and thawing three times in a bath of dry ice and acetone. The hemolyzate was spun at 20,000*g* for 20 minutes, the hemoglobin content of the clear supernatant was determined and adjusted to 15 g/100 ml by the addition of phosphate buffer. Thirty-microliter samples were applied to the gel.

Vertical starch-gel electrophoresis (*1*) of hemolyzates was performed with a 0.02*M* sodium barbital gel buffer, *p*H 8.6, which contained Na_2EDTA (1.0 mmole/liter) and 2-mercaptoethanol (5 mmole/liter), at 6 volt/cm for 19 hours at 4°C. The electrode buffer was 0.06*M* sodium barbital, *p*H 8.6, containing Na_2EDTA (1.7 mmole/liter) and 2-mercaptoethanol (5 mmole/liter). Thin slices of the gel were stained for hexokinase activity for 90 minutes at 37°C by immersion in 0.1*M* tris(hydrozymethyl)aminomethane (tris), *p*H 7.4, containing nicotinamide-adenine dinucleotide phosphate (5×10^{-3} mole/liter), $MgCl_2$ (5×10^{-3} mole/liter), adenosine triphosphate (5×10^{-3} mole/liter), KCN (2×10^{-3} mole/liter), glucose-6-phosphate dehydrogenase (0.4 international units/ml), phenazine methosulphate (1.3×10^{-4} mole/liter),

nitro blue tetrazolium (4.7×10^{-4} mole/liter), and glucose (either 5×10^{-4} or 0.1 mole/liter).

Four forms of hexokinase have been separated from mammalian tissues by vertical starch-gel electrophoresis and chromatography on diethylaminoethyl (DEAE)-cellulose (*2*). In the rat, these include a glucokinase with a high K_m found only in liver and designated Type IV on the basis of its characteristic electrophoretic migration on starch gel (Fig. 1). In addition, three hexokinases with low K_m's designated Types I, II, and III (Fig. 1) have been purified approximately 150- to 400-fold from one or more rat tissues (*2*). Each purified enzyme type, regardless of tissue source, has characteristic electrophoretic, chromatographic, heat stability, and kinetic properties that distinguish it from the other hexokinase types with low K_m's (*2*). The available evidence indicates that these hexokinase types represent different molecular forms and that they are not artifacts of preparation (*2*). A striking similarity between the various mammalian species studied has been noted with respect to both the number and tissue distribution of the multiple forms of hexokinase (*2*). A recent report by Brown *et al.* (*3*) indicates that human liver and adipose tissue have, on electrophoresis, patterns of hexokinase isoenzymes similar to those observed in other mammalian species.

Our studies indicate that hexokinase activity in hemolyzates of erythrocytes from adult humans who have no evidence of hematologic disease is associated with two electrophoretically distinct proteins here designated as Types I and III to comply with the nomenclature employed for other tissues (Fig. 1). Type III hexokinase was consistently demonstrated in hemolyzates from 21 normal adults; it stains more intensely at $5 \times 10^{-4}M$ glucose than at $0.1M$ glucose, thus resembling in staining characteristics the Type III hexokinase of rat liver, which is inhibited by $0.1M$ glucose (*2*). An additional band of hexokinase activity migrating similarly to the Type I isoenzyme of rat liver was observed in 17 of the 21 hemolyzates prepared from the blood of normal adults. In no instance was hexokinase activity with an electrophoretic mobility similar to that of the Type II hexokinase of rat liver observed in normal adult erythrocytes.

Type II hexokinase was demonstrated in the hemolyzates of 17 of 17 newborn infants who were studied within the first 4 days of life. In five instances, Type II was the only hexokinase demonstrated. Twelve of the 17 newborns had Type III in addition to Type II, and 4 of 17 had both Types I and III in addition to Type II. In hemolyzates from four infants studied at 1 month of age, Types II and III were demonstrated in every instance, and in one infant Types I, II, and III were present.

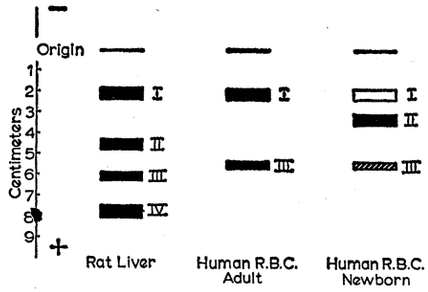

Fig. 1. Diagram of bands of hexokinase activity seen upon starch-gel electrophoresis of hemolyzates of erythrocytes from adults and newborn infants. For comparison, liver from a 100-g Wister rat was homogenized in $0.1M$ tris buffer, *p*H 7.4, containing EDTA and 2-mercaptoethanol (both $5 \times 10^{-3}M$), and the supernatant obtained by centrifugation at 104,000*g* for 1 hour was run in the same system.

To exclude the possibility that the absence of Type II hexokinase from adult erythrocytes and its consistent demonstration in newborns' red cells is due to the greater concentration of young cells in the blood of infants (*4*), we studied the hexokinase activities of "young" and "old" cells prepared from adult blood samples by ultracentrifugation (*5*). Washed erythrocytes from four normal adults were suspended in phosphate buffer $0.01M$, *p*H 7.0, and spun at 106,000*g* for 1 hour at 4°C. The supernatant was removed, and the red cells were frozen *in situ* and sliced to provide samples of the upper and lower thirds representing "young" and "old" cells respectively, from each blood sample (*5*). Types I and III hexokinase were present in both the "young" and "old" cells from each of the four normal subjects, and no Type II hexokinase was observed. It would appear that the absence of Type II hexokinase in the erythrocytes of adults is not related to cell age. Moreover, the shift in hexokinase isoenzyme pattern that occurs in human erythrocytes as the individual ages has a parallel in rat liver (*2*). Katzen and Schimke (*2*) found that Type II hexokinase is readily demonstrable in the liver of newborn rats but is barely perceptible in the liver of adult animals.

Eaton *et al.* (*6*) have recently reported the separation of seven and possibly eight distinct bands of hexokinase activity upon starch-gel electrophoresis of hemolyzates of human erythrocytes; the number of bands of hexokinase activity and their electrophoretic mobilities are dissimilar to those reported by other workers for several mammalian and human tissues (*2, 3*). The methodology employed in the studies of Eaton *et al.* (*6*) differs in many respects from that previously used in the studies of mammalian hexokinases. Major differences include a preliminary extraction of the hemolyzate with toluene, the absence of 2-mercaptoethanol from the hemolyzate and gel buffers, the use of a tris-boric acid gel buffer, and the use of an electric field of 8 volt/cm. Type II hexokinase from rats exists in two electrophoretically distinct bands when the procedure is carried out in the absence of mercaptoethanol (*7*); incubation of rat Type I hexokinase with trypsin results in the formation of a second form of Type I activity that migrates slightly more rapidly than

Table 1. Pattern of hexokinase isoenzymes observed upon starch-gel electrophoresis of hemolyzates from a family containing subject homozygous for hereditary persistence of fetal hemoglobin. Other data provided by the Hematology Division of the Johns Hopkins Medical Institutions which has previously reported this family (*9*). FA indicates that the subject is heterozygous for persistent fetal hemoglobin trait; F-Thalassemia, that he is heterozygous for both persistent fetal hemoglobin trait and thalassemia; FF, homozygous for persistent fetal hemoglobin trait; and A-Thalassemia, heterozygous for thalassemia.

Subject	Age (yr)	Phenotype	Fetal hemoglobin (%)	Erythrocyte hexokinase isoenzymes present
Father	30	FA	26	I, II, III
Mother	27	F-Thalassemia	50	I, II, III
Propositus ♂	7	FF	100	II, III
Sibling ♂	6	A-Thalassemia	2	I, III
Sibling ♀	$2^1/_3$	FA	50	I, II, III

the untreated enzyme does (*2*). In the presence of trypsin and glucose, Type II hexokinase splits into three separate active enzyme forms, none of which migrates with a mobility equal to that of untreated Type II (*2*). The significance of the seven bands observed by Eaton *et al.* thus remains to be established. The possibility that these multiple bands are produced in the course of preparing the hemolyzates and during electrophoresis remains to be excluded (*5*).

A possible association between Type II hexokinase and fetal hemoglobin was suggested by the chance observation that the red cells of a dysmature (*8*) infant studied on the first day of life demonstrated only hemoglobin A and Types I and III hexokinase on starch-gel electrophoresis. The erythrocytes of this newborn dysmature infant thus lacked both the high concentration of fetal hemoglobin and the Type II hexokinase normally present.

We were fortunate in obtaining blood samples from a well-studied family that includes one subject known to be homozygous for the hereditary persistence of fetal hemoglobin (*9*). Results of starch-gel electrophoresis indicate that Type II hexokinase is present in the red cells of the members of this family who have the trait for persistent fetal hemoglobin (Table 1). Three unrelated adult patients with the persistent fetal hemoglobin trait were also studied and found to have Type II hexokinase in their erythrocytes. This trait thus appears to be associated with the presence of Type II hexokinase in adult erythrocytes. Heretofore it has been thought that, apart from the hemoglobin components, the red cells of the patient homozygous for persistent fetal hemoglobin are comparable to those of the normal adult and are, with respect to enzyme composition, unlike the erythrocytes of the newborn (*9*). These data suggest that hexokinase activity, which is a probable rate-limiting step in red-cell glycolysis (*10*), may be mediated by distinct proteins in the erythrocytes of the fetus and the adult. Furthermore, they strongly indicate that the regulation of synthesis of Type II hexokinase is in some way related to that of the γ-chain of hemoglobin.

The K_m for glucose of Type I hexokinase from human erythrocytes when separated by DEAE-cellulose chromatography is of the order of $5.3 \times 10^{-5}M$, and the K_m for glucose of Type II is $1.4 \times 10^{-4}M$. The substrate specificity and kinetic characteristics of the isoenzymes of red-cell hexokinase may prove to be significant with regard to glucose homeostasis, for, as noted by Krebs (*11*), the obligatory glucose utilization of the erythron is of great quantitative significance in the fasting state.

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12. We thank Drs. S. Charache and A. M. Mondzac of Johns Hopkins University for permitting us to study their patients with hereditary persistent fetal hemoglobin, and Miss M. A. Holzinger for technical assistance. Work supported by research career development award GM 6405-02 (A.I.W.) and PHS grants AM 04722-06 and HD 01919.

13 February 1967

Amino Acids and the Spikes from the Retinal Ganglion Cells

Abstract. *The effects of amino acids or mixtures of amino acids on the spike discharges from the optic ganglion cells were studied. Mixtures of amino acids have different effects than single amino acids. Proper composition of excitatory and inhibitory amino acids can enhance only the light-induced spikes without giving rise to any spontaneous discharges. It is implied that amino acids may play an important role in the regulation of the general sensitivity of cells in the central nervous system.*

The iontophoretic injection of amino acids has given us valuable information on the action of amino acids in the central nervous system. These results have shown that the amino acids could be classified into three types: (i) excitatory amino acids such as glutamic acid which gave rise to spike discharges when they were iontophoretically injected; (ii) depressing amino acids such as γ-aminobutyric acid (GABA) which inhibited (spontaneous) spike discharges and antagonized the action of excitatory amino acids; and (iii) those amino acids such as glutamine which exhibited no apparent effect (*1, 2*).

This report describes preliminary results of experiments in which amino acids or mixtures of amino acids were applied to a vertebrate retina. In the vertebrate retina, the discharge pattern of the ganglion cell is the result of the mutual antagonism between the central and peripheral portion of the receptive field (*3*). The "on" mechanism is thought to be related to an excitatory process and the "off" mechanism to an inhibitory process. Intracellular recording from the ganglion cell of the bullfrog retina also showed that there was a complex interaction of excitatory and inhibitory postsynaptic potentials (*4, 5*). Study of the effects of amino acids on the retinal ganglion cell may help to clarify the role of the amino acids in the central nervous system.

The eye of the bullfrog, *Rana catesbiana*, was dissected and the frontal half removed together with the lens. Test solutions were applied to the vitreous side of the posterior half of the eye by means of a pipette positioned close to the recording site. In the bullfrog the vitreous humor could easily be removed and the solutions could be introduced directly onto the retinal surface. Test solutions were applied in the order of 0.01 ml per trial.

Commercially obtained amino acids were dissolved in the frog Ringer solution, the *p*H of which was adjusted to 7 by HCl or by NaOH. Spikes were recorded extracellularly by glass pipettes filled with saturated NaCl solution. In the vertebrate retina only the ganglion cells are known to produce spikes (*5, 6*) and, therefore, it