HEXOKINASE ISOENZYMES IN HUMAN ERYTHROCYTES OF ADULTS AND NEWBORNS

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In hemolysates of adult erythrocytes two electrophoretically distinct regions of hexokinase activity have been demonstrated by staining the gels with tetrazolium dyes. Eaton et al.(1966) found a wide region B, which was divided into 6 to 7 subbands and inconstantly a faster migrating band A. In hemolysates of newborn infants, Holmes et al.(1967) saw additionally a third region. Because of their electrophoretic migration these three bands were designated as types I to III in analogy to the rat and human liver types (Grossbard and Schimke,1966; Brown et al., 1967). Types I and III were inconstantly demonstrated in both adults and newborn infants, whereas type II was seen in each blood specimen of newborns (Holmes et al., 1967).

Identification of hexokinase isoenzymes is difficult in the presence of tetrazolium dyes because of the low activity of hexokinase in the red blood cells. Therefore, in this study a new method was used. In erythrocytes of both adults and newborns two electrophoretically distinct bands of hexokinase activity could be demonstrated constantly, which are similar to the type I and III hexokinase isoenzymes from human liver. The higher hexokinase activity of newborn erythrocytes is

located in the type I region. No type II isoenzyme could be demonstrated in newborn infants.

METHODS AND RESULTS

High-voltage starch-gel electrophoresis (Wieland and Pfleiderer, 1955) was used. The hexokinase isoenzymes were identified by a more sensitive method using the fluorescence of the formed NADPH at a wave length of 366 mμ.

In the hemolysates of each blood specimen of 40 adults and 40 newborns, aged 1 to 5 days, we constantly found two hexokinase isoenzymes, which migrated towards the anode. The intensity of the faster migrating band (region A) was the same in both adults and newborns, whereas the slower migrating band (region B) showed a much more marked fluorescence in newborns than in adults, both in intensity and breadth. Region B was wider than region A both in hemolysates of newborns and of adults and partly covered by hemoglobin. When electrophoresis was performed for 4 1/2 hours instead of the usual 3 1/2 hours, region B is divided into two subbands by hemoglobin F so that the uncovered leading part of region B could impress as the type II hexokinase isoenzyme and the end of region B, visible only occasionally, as the type I. No variant of hexokinase isoenzymes could be demonstrated in the 80 blood samples. During the first year of life both the intensity and the breadth of region B diminish and at about 5 months of age had decreased to adult levels (Fig.1). These changes correlated well with the decrease of hemoglobin F (Betke et al., 1959) and the increase of hemoglobin A_1.

Table 1 demonstrates the effect of different concentrations of glucose and fructose on the intensity of the

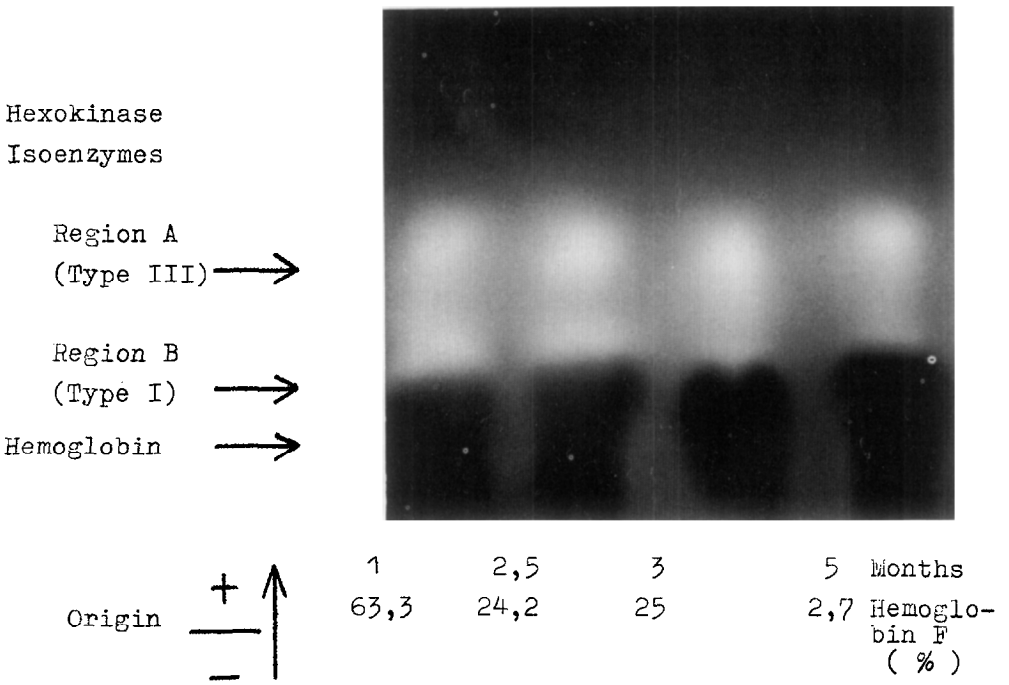

Figure 1. Starch-gel electrophoresis of hexokinase isoenzymes in hemolysates of erythrocytes from infants aged one to five months. Horizontal electrophoresis (23,5 v/cm) was carried out for 3 1/2 hours; the cooling fluid had a temperature of -4 to -6°C. The 0,02 M sodium barbital gel buffer, pH 8,6, contained 0,001 M EDTA and 0,005 M 2-mercaptoethanol. The electrode buffer was 0,06 M sodium barbital, pH 8,6, containing 0,0017 M EDTA and 0,005 M 2-mercaptoethanol. Before lysing, the cells from heparinized venous blood were centrifuged at 600 g for 10 minutes at 4°C, and plasma and the buffy coat were removed. The cells were suspended in 0,9 per cent saline containing 0,02 M glucose, and the suspension was centrifuged at 2000 g for 10 minutes at 4°C. This procedure was repeated twice. Hemolysates were prepared at 4°C by lysing washed red cells for 6 hours with one volume of a solution containing 0,005 M EDTA, 0,005 M 2-mercaptoethanol and 0,02 M glucose, pH 7,0. A clear supernatant was prepared by centrifugation at 20 000 g for 20 minutes. After electrophoresis, the sliced gel was incubated for 2 hours at 37°C with 20 ml of a reaction mixture containing 0,1 M tris-HCl, pH 7,4, 0,0025 M NADP, 0,0025 M ATP, 0,005 M $MgCl_2$, 0,002 M KCN, 8 I.U. glucose-6-phosphate dehydrogenase and 0,0005 M glucose. When either ATP, $MgCl_2$, or glucose were omitted, no bands appeared. The hexokinase isoenzymes were identified as fluorescence of the formed NADPH at 366 mμ.

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hexokinase bands. The activity of region A is inhibited by high concentrations of glucose, a fact which also characterizes hexokinase type III from rat and human liver. Both regions

appear with fructose as substrate. As type III from human liver region A is not inhibited by high concentrations of fructose.

Table 1. Effect of different concentrations of glucose and fructose on the activity of hexokinase isoenzymes.

Molarity of substrate in the incubation mixtures		Newborns		Adults	
		Region B (Type I)	Region A (Type III)	Region B (Type I)	Region A (Type III)
Glucose	10^{-1}	+++	(+)	++	(+)
	10^{-2}	+++	++	++	++
	10^{-3}	+++	++	++	++
	10^{-4}	+++	++	++	++
	5×10^{-4}	+++	++	++	++
	10^{-5}	+	+	+	+
Fructose	10^{-1}	+++	++	++	++
	10^{-2}	+++	++	++	++
	10^{-3}	++	+	+	+
	10^{-4}	-	-	-	-

In the experiments with fructose 10 I.U. phosphohexose isomerase were added to the incubation mixtures. Each concentration of both glucose and fructose was studied in two runs of electrophoresis with each three to four different blood specimens. The intensity of the bands is expressed as following: - no visible band, (+) extremely faint band, + faint band, ++ intermediate band, +++ strong band.

DISCUSSION

The table clearly demonstrates that the higher hexokinase activity of newborns is located in region B. The isoenzyme of this region exhibits a high stability. It could be demonstrated constantly in hemolysates of adults and newborns when the

heparinized blood was stored for 48 to 72 hours at 24°C before lysing. The high stability and the relative widespread band are properties of the type I hexokinase from liver, whereas liver type II is a more instable enzyme. Region A showed the same electrophoretic mobility as the type III hexokinase from liver homogenates of adult rats, which were prepared as described by Holmes et al.(1967).

These results lead us to the suggestion that the two hexokinase bands in the hemolysates of erythrocytes from adults and newborns are identical. According to their electrophoretic migration, their affinity to the substrates glucose and fructose and their stability, we assume that they are similar to the types III (region A) and I (region B) of rat and human liver. The type II isoenzyme seen by Holmes et al. (1967) in the blood of newborns might be an artefact, which is produced by the interruption of region B by hemoglobin F.

When we used the same method as Eaton et al.(1966), we also found the division of region B into 6 to 7 subbands, but in the absence of tetrazolium blue region B was an uniform band in the UV-light.

Our data demonstrate that the higher hexokinase activity of newborn erythrocytes is located in the hexokinase isoenzyme region B (probably type I). This indicates that the regulation of synthesis of type I hexokinase is different from that of type III during development.

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