

## Brief Communications

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### Phosphoglycerate Mutase Isozyme Marker for Tissue Differentiation in Man

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In the course of population screening for genetically determined variation of the glycolytic enzymes in human brain [1], we have found electrophoretic evidence for tissue-specific isozymes of phosphoglycerate mutase (PGAM; E.C.2.7.5.3). PGAM reversibly converts 3-phosphoglycerate to 2-phosphoglycerate. Skeletal muscle PGAM migrates slowly, while PGAM from brain and other tissues migrates rapidly toward the anode in Tris-citrate (*p*H 7.5) or in Tris-EDTA-borate (*p*H 8.6) horizontal starch gels [2]. Extracts of heart muscle give a three-banded pattern of PGAM activity consistent with a dimer structure with a molecular weight of approximately 60,000 reported for chicken, rabbit, and sheep skeletal muscle and porcine kidney [3-6]. In this paper we report striking developmental and neoplastic transitions in the phenotype of PGAM in human tissues.

#### MATERIALS AND METHODS

Thigh muscle, brain, heart muscle, and other tissues from human fetuses were obtained from the Central Embryology Laboratory at the University of Washington (Dr. T. Shepard). Fetal specimens ranged from approximately 50 to 166 days gestational age, estimated from crown-to-rump length; usually there was good agreement with the estimates from the last menstrual period. Stillbirths and adult tissues were obtained from the autopsy service of the Department of Pathology, University Hospital. Surgical specimens were provided by members of the Neurosurgery Department. Clinical records and histological diagnoses were reviewed carefully.

Tissue extracts were prepared by homogenization with 4 vol of 0.05 M Tris-HCl buffer, *p*H 8.0. Horizontal starch gel electrophoresis was carried out overnight with Tris-EDTA-borate (*p*H 8.6) and Tris-citrate (*p*H 7.5) systems. Specific staining for PGAM activity employed 4 mM 3-phosphoglycerate, 1 mM 2,3-diphosphoglycerate, 20 mM MgCl<sub>2</sub>, 3 mM ADP, 12 mg NADH, and 10 U each of enolase, pyruvate kinase, and lactate dehydrogenase in 10 ml Tris-HCl buffer (*p*H 8.0). The coupled system led to production of NAD (non-fluorescent) from fluorescent NADH. Specificity was confirmed by starting with 2-

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phosphoglycerate as substrate and coupling up the glycolytic pathway with phosphoglycerate kinase and glyceraldehyde-3-phosphate dehydrogenase, as described by Chen et al. [7]. Quantitative assays of PGAM activity utilized the same system, with measurement of the change in  $OD_{340}$  as a function of time in a Gilford model 2000 spectrophotometer. Specific staining for creatine phosphokinase (CPK; E.C.2.7.3.2.) activity employed 2mM creatine phosphate, 3 mM ADP, 0.1 mM NADP, 10 mM glucose, 10 mM  $MgCl_2$ , 10 U each of hexokinase and glucose-6-phosphate dehydrogenase, 5 mg MTT, 0.5 mg PMS, and 0.5% agar in 10 ml Tris-HCl buffer ( $pH$  7.0). All reagents were purchased from Sigma Chemical Company, Saint Louis.

#### RESULTS

As shown in figure 1, there is a striking transition in the PGAM pattern during development of human skeletal muscle. In the earliest specimens (approximately

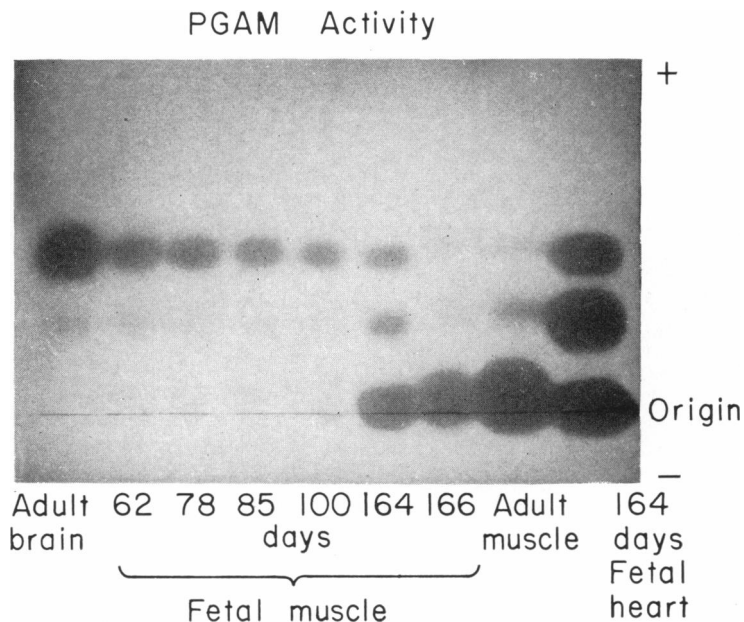


FIG. 1.—Developmental transition of PGAM in human fetal muscle. Starch gel with Tris-EDTA-borate buffer ( $pH$  8.6) stained specifically for PGAM activity in adult brain, adult muscle, fetal muscle, and fetal heart of gestational ages indicated.

50 days), fetal muscle contains almost exclusively the single-banded type-B PGAM pattern, as in adult brain. The appearance of the band of intermediate mobility (80–100 days) signifies type-M PGAM production; at first type-M subunits combine with type-B PGAM subunits to make the hybrid dimer; then the three-banded pattern is seen (115–164 days). In the specimen of 166 days gestational age and on through term, the muscle-type pattern of the adult predominates; when gels are overstained, trace amounts of hybrid and type-B PGAM are detected.

The phenotypes of PGAM in adult brain and muscle are readily distinguished by electrophoresis [2] and by DEAE-cellulose chromatography. Determination of  $K_m$  for 3-phosphoglycerate, the physiological substrate, revealed no differences between the PGAM of adult brain and muscle. At either 37° C or 24° C, the  $K_m$  of brain and muscle PGAM is about 1.4 mM. However, marked differences exist in heat stability. Figure 2 shows the much greater liability to elevated temperature of brain PGAM activity compared to muscle PGAM activity both in the presence and absence of the stabilizing effect of 2,3-diphosphoglycerate.

Creatine phosphokinase is known to undergo a similar transition from a brain-type to a muscle-type pattern [8]. In specimens (62 and 78 days) which still show brain-type PGAM, there is definite expression of hybrid and muscle-type CPK (fig. 3). In fact, comparison of 19 specimens of 50–166 days gestation

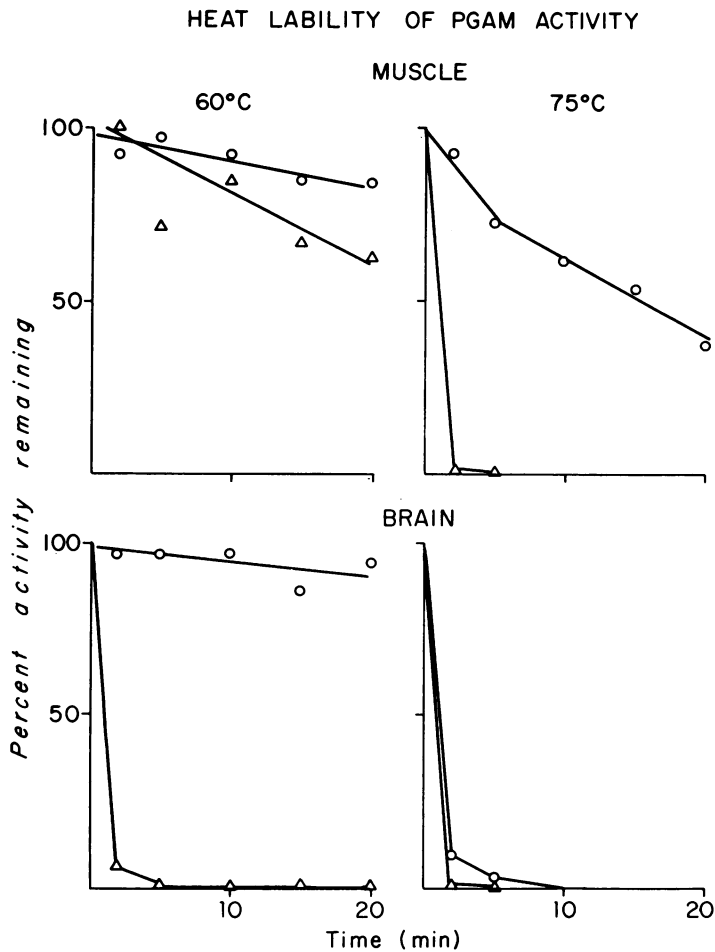


FIG. 2.—Effect of elevated temperature on PGAM activity in adult muscle and adult brain extracts in the presence (circles) and absence (triangles) of 2mM 2,3-diphosphoglycerate.

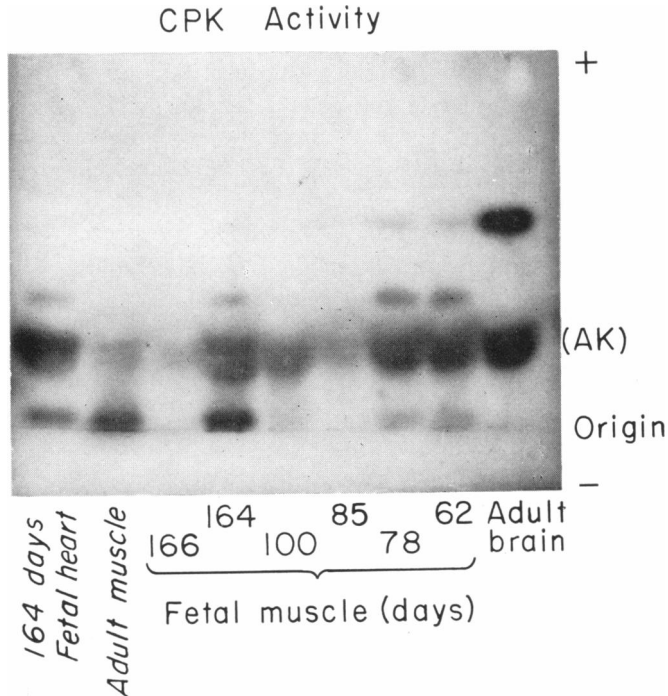


FIG. 3.—Top half of gel shown in fig. 1 stained for CPK (and adenylate kinase) activity. Not all samples have adequate activity.

strongly suggests that the developmental transitions of PGAM and CPK are not synchronous, especially for the first step of turning on the muscle-type genes (fig. 4) in muscle.

A complementary transition in the electrophoretic phenotype and genetic expression of PGAM has been observed in human brain tumors (fig. 5). Relatively benign tumors, such as a meningioma or grade II astrocytomas, gave patterns identical with normal brain. (Overstained gels showed a trace of hybrid band.) However, highly malignant grade III and IV astrocytomas (glioblastoma multiforme) and a recurrent cerebellar hemangioblastoma gave electrophoretic patterns with prominent hybrid and muscle-type bands. This phenomenon cannot be due to unrecognized contamination with blood, since erythrocyte PGAM is type B. No such neoplastic transformation to muscle-type CPK occurred in these tumors.

#### DISCUSSION

The developmental transition in the phenotype of phosphoglycerate mutase in human fetal skeletal muscle represents a two-step process, turning on or turning up the gene for muscle-type (type M) PGAM and turning off the gene for brain-type (type B) PGAM. The disappearance of the type B and hybrid bands on the gels may depend also on the biological half-life of the enzyme. It may be

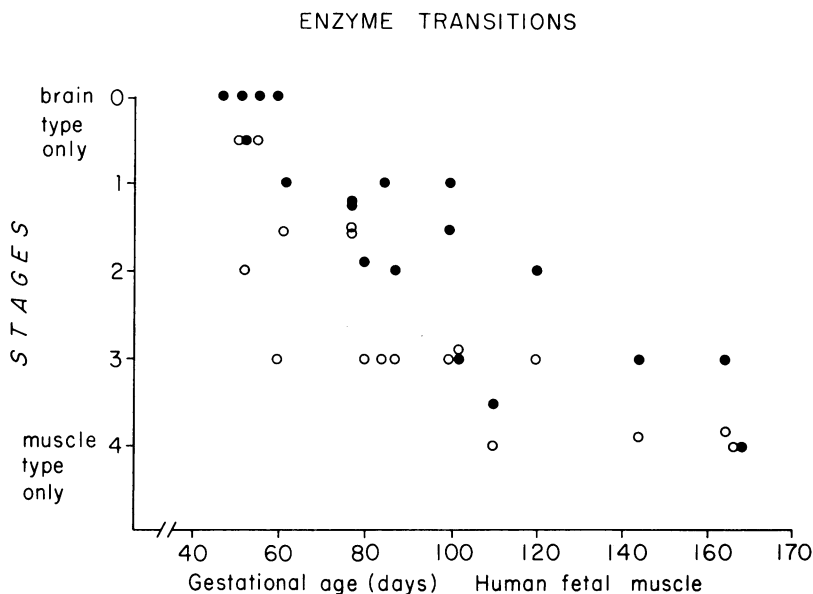


FIG. 4.—Scattergram showing results for PGAM (closed circles) and CPK (open circles) as a function of gestational age. Scores range from zero for brain-type band only, to two for equal amounts of brain and muscle bands with hybrid band, to four for muscle-type band only.

countered that the transition represents not the maturation of muscle cells but a shift in cell population from predominantly fibroblastic connective tissue to myoblastic cells. Figures 3 and 4 serve to rule out this explanation, since the developmental transitions of PGAM and CPK appear to be nonsynchronous. PGAM, like CPK, can be detected in cultured muscle cells (G. S. Omenn and S. Hauschka, unpublished observation) and provides a good marker for investigation of differentiation *in vitro*. The transition in electrophoretic phenotype reported here accounts for the developmental increase in sensitivity to mercuric ions found for human and chicken skeletal muscle PGAM [9]. Muscle PGAM differed from PGAM of brain, liver, kidney, and erythrocytes in that  $Hg^{++}$  induced a reversible inhibition of the mutase activity and activation of the 2,3-diphosphoglycerate phosphatase activity of PGAM [6].

Although the developmental data, heat lability, and mercuric ion inhibition all suggest significant differences between type-B and type-M PGAM, definitive assignment of these isozymes to different genes requires analysis of electrophoretic variants of PGAM. No variants of PGAM were found in human, monkey, or mouse brain [1]. Two variants of PGAM have been found recently after surveys of 3,104 samples of human erythrocytes [7], but muscle samples were not available for comparison.

The transition in electrophoretic phenotype of PGAM in neoplastic brain tissue appears to be correlated with the degree of malignancy of the tumors. A much larger series with various histological types of brain tumors will be neces-

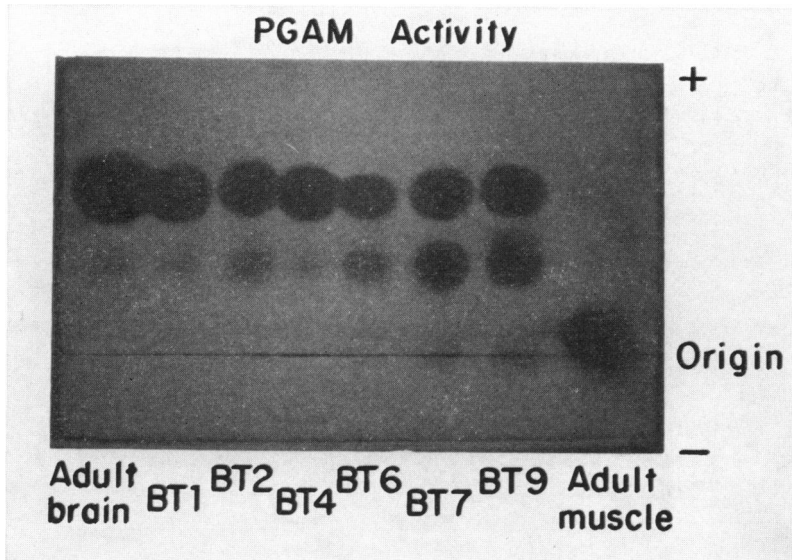


FIG. 5.—Electrophoretic phenotype of PGAM in brain tumors (BT). *BT 1 and BT 4*, Grade II astrocytomas; only minimal hybrid PGAM with an overstained brain band, as in normal brain control. *BT 2, BT 6, and BT 7*, Astrocytomas of more malignant appearance histologically; excess hybrid band and some muscle band. *BT 9*, Recurrent hemangioblastoma; excess hybrid band and some muscle band. Starch gel with Tris-EDTA-borate buffer, pH 8.6. PGAM bands appear dark in fluorescent field photographed under ultraviolet illumination. Top half of gel was stained for CPK activity; all samples had only the brain-type phenotype.

sary to confirm this suggestion. It is likely that the neoplastic transformation somehow activates greater expression of the type-M PGAM gene in brain cells; analogous “ectopic” production of polypeptides with distinctive hormonal, antigenic, and enzymatic activities has become a well recognized clinical and biochemical phenomenon [10–12]. Since fetal brain does not express the type-M PGAM, this transition resembles production of placental-type alkaline phosphatase by colon carcinomas [13] rather than the reexpression of fetal patterns of aldolase or phosphorylase in neoplastic brain or liver [14, 15]. These gene products should be useful markers for studies of tumors and transformed cultured cells.

#### SUMMARY

Electrophoretically distinguishable isozymes of phosphoglycerate mutase in brain and muscle provide biochemical markers for the differentiation of fetal muscle and for neoplastic transformation in man.

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