that administration of disulfiram to rats in vivo decreases aldehyde dehydrogenase activity in the liver and that recovery of activity is dependent on synthesis of new enzyme. (Activity was not regained when cycloheximide, an inhibitor of protein synthesis, was also administered.) This observation appears to be inconsistent with our proposed mechanism (Fig. 1B), especially on consideration of the fact that glutathione, the physiological reducing agent, is present in the liver at concentrations ranging from 2 to 12 m*M*, depending on the species (*12*). However, when we incubated the unlabeled, inhibited enzyme with 9 m*M* glutathione (*p*H 7) for up to 18 hours, we found that only about 5 percent of the catalytic activity was restored, compared to 100 percent when 2-mercaptoethanol was used. These results could explain why synthesis of new enzyme is required for return of aldehyde dehydrogenase activity in vivo, even though the modification is reversible in vitro in the presence of the nonphysiological reducing agent 2-mercaptoethanol. The reason why glutathione is inefficient in returning oxidized aldehyde dehydrogenase to its reduced form is not clear, but will probably be found in the primary structure of the enzyme surrounding the points of disulfiram interaction.

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15 December 1981; revised 25 January 1982

Phosphoglucomutase: Evidence for a New Locus Expressed in Human Milk

Abstract. *Electrophoretic study of phosphoglucomutase (PGM) in human milk revealed different patterns that can be explained by the existence of a locus distinct from the common PGM1, PGM2, and PGM3. One hundred and forty samples were tested and the results showed four different alleles of PGM4 whose frequencies were under Hardy-Weinberg equilibrium.*

Phosphoglucomutase (PGM), α-D-glucose 1,6-bisphosphate: α-D-glucose-1-phosphate phosphotransferase (E.C. 2.7.5.1), is known to be the product of three loci: PGM1 (*1*), PGM2, and PGM3, each determining a characteristic set of two or three isozymes. Electrophoretic variants attributable to alleles have been identified for the three genes (*2*). In most tissues, PGM1 isozymes account for 85 to 95 percent of the total PGM activity, PGM2 for 2 to 5 percent, and PGM3 for 1 to 2 percent. In erythrocytes, however, PGM1 and PGM2 isozymes are found in almost equal amounts, with practically no PGM3 activity (*3*).

We used electrophoresis to determine, by the method of Spencer *et al.* (*4*), the PGM phenotypes in 140 samples of human milk from Mexican *mestizas* who had given birth 3 to 10 days previously. We also examined samples of erythrocytes from the same women. Milk samples were treated with CCl_4 to remove fat, which produces an unspecific stained background in this electrophoretic system. The PGM patterns in human milk were different and independent from those observed in the erythrocytes (Figs. 1 and 2). Eight phenotypes were easily distinguishable, and were explained on the basis of a distinct PGM4 locus with four alleles conventionally designated here as PGM4*1, PGM4*2, PGM4*3, and PGM4*4. The homozygotes PGM4*1/PGM4*1, PGM4*2/PGM4*2, and PGM4*3/PGM4*3 showed three bands with activities decreasing from the central region to the anode. The phenotypes in the heterozygotes PGM4*1/PGM4*2, PGM4*1/PGM4*3, and PGM4*2/PGM4*3 showed the combination of the expected bands. PGM4*4 could not be found in homozygosis but only combined with alleles *1 and *2; it migrates as a single intense band with mobility similar to the slowest band produced by PGM4*1/PGM4*1 homozygotes. The distribution of the PGM phenotypes in the 140 samples is presented in Table 1. The gene frequencies of each of these alleles were: 0.346 for PGM4*1, 0.475 for PGM4*2, 0.114 for PGM4*3, and 0.065 for PGM4*4. The statistical analysis of these frequencies under Hardy-Weinberg equilibrium showed good agreement between the observed and the expected [χ^2 (9) = 10.5].

Tests for phosphopentomutase (PPM) activity, present in PGM2 (*5*), were carried out in ten milk samples, giving nega-

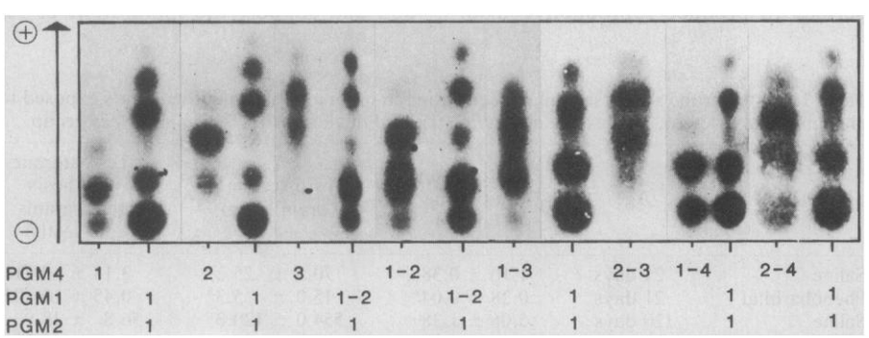

Fig. 1. Photograph showing the PGM4 phenotype in human milk compared with the phenotypes of PGM1 and PGM2 in erythrocytes.

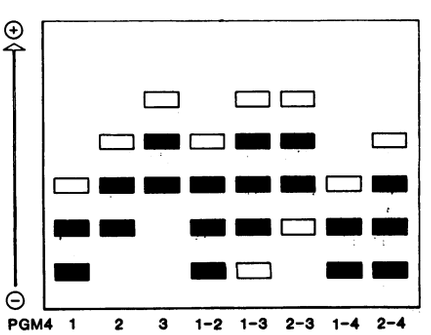

Fig. 2. Diagram of the eight different observed phenotypes of PGM4.

0036-8075/82/0507-0639$01.00/0

Table 1. Distribution of erythrocyte PGM1 and milk PGM4 phenotypes in 140 Mexican women. The phenotype PGM2 1 was found in all samples.

Phenotype PGM1	Phenotype PGM4								Number of samples
	1	2	3	1–2	1–3	2–3	1–4	2–4	
1	14	18	1	26	5	8	10	7	89
1–2	3	11	1	11	4	11	1		41
2		1		1		2			4
1–4				2					2
1–7		1		2					3
2–5				1					1
Number of samples	17	31	1	43	9	21	11	7	Total 140

tive results. Thermostability tests with whole hemolyzate and milk were performed, with the samples being incubated for 15 minutes at 40°, 45°, 50°, 55°, 60°, 65°, and 70°C before electrophoresis. The PGM4 activity disappeared at 60°C, PGM1 at 65°C, and PGM2 at 70°C. When glucose 1-phosphate, glucose 1,6-diphosphate, nicotinamide adenine dinucleotide phosphate, methyl thiazolyl blue, or phenazine methosulfate were omitted in the staining mixture no PGM1, PGM2, or PGM4 isozymes were observed. The omission of G6PD gave faint bands in the position of the endogenous enzyme.

These results show the presence of a different set of PGM isozymes in human milk, coded by a specific gene with at least four alleles probably located in an autosome. Each product of the alleles PGM4*1, PGM4*2, and PGM4*3 gives three anodal bands, the most intense (probably the primary isozyme) being intermediate. This pattern is quite different from the patterns given by PGM1, PGM2, and PGM3, whose secondary isozymes appear toward the anode; PGM1 and PGM3 show one primary and one secondary isozyme, and PGM2 shows one primary and two secondary isozymes (*6*). In the human nonlactating mammary gland, PGM activity is due to PGM1 and PGM2 (*7*). However, these isozymes could not be found in any of the milk samples studied, indicating a switch-off of the PGM1 and PGM2 genes and a simultaneous switch-on of the PGM4. This switching is reversible and probably dependent on hormonal activation of lactation. Since PGM is indispensable for the production of glucose 1-phosphate, which is the first intermediate on the pathway to synthesis of the galactose moiety of lactose (*8*), and since lactose is the main carbohydrate of milk, the need of an efficient PGM has probably been an evolutionary selective force for an independent locus (*9*). Although there are several instances of polymorphism among milk-specific proteins (*10*), to our knowledge this is the first report of a distinct gene for a widely distributed protein being functionally restricted to the lactating mammary gland, since no evidence of its activity has been found in tissues or fibroblasts previously studied (*3*).

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11. We thank E. Franco-Gamboa, A. Alcaraz, and R. Troyo for skillful technical assistance and D. A. Hopkinson for his valuable comments when this work was presented at the 6th International Congress on Human Genetics, 13 to 18 September 1981, Jerusalem, Israel (Book of Abstracts, p. 35).

26 January 1982

Prenatal Exposure to Phenobarbital Permanently Decreases Testosterone and Causes Reproductive Dysfunction

Abstract. *Exposure of rats to phenobarbital during late prenatal development decreased the concentration of testosterone in plasma and the brain during the late fetal, early postnatal, pubertal, and adult periods. By decreasing the production of testosterone in the brain during the period of sexual differentiation, phenobarbital may lead to sexual dysfunction in later life.*

Phenobarbital (PB), when administered during pregnancy to a variety of species including humans, produces both anatomic and neurologic malformations in the offspring (*1, 2*). Recently, we demonstrated that prenatally administered PB is also capable of modifying the development of reproductive function in male and female offspring (*3, 4*). Phenobarbital acts during neuroendocrine development in the late fetal and neonatal periods to produce reproductive disorders in both male and female offspring (*5*). In male offspring there is a delay in testicular descent, with subsequent infertility and decreased production of testosterone and gonadotropin throughout adulthood (*3*).

The mechanism underlying these effects of PB in early life has not been determined. During the fetal and early neonatal periods, testicular hormones masculinize anatomic sexual differentiation and support the maturation of re-

Table 1. Testosterone synthesis and concentration in brain and plasma of male rats exposed to phenobarbital in utero. Values are means ± standard deviations for six animals per group.

Treatment	Age	Testosterone in plasma (ng/ml)	Testosterone in brain (pg/g)	Testosterone synthesis (nanograms per testis)
Saline	21 days	1.10 ± 0.38	70.5 ± 25.2	3.14 ± 0.71
Phenobarbital	21 days	0.28 ± 0.04*	15.0 ± 5.3*	0.45 ± 0.10*
Saline	120 days	5.06 ± 1.28	554.0 ± 120.0	56.8 ± 18.9
Phenobarbital	120 days	2.66 ± 0.05*	375.2 ± 65.7*	32.1 ± 14.2*

*Significantly different from corresponding control value at $P < .01$ (Student's t-test).

0036-8075/82/0507-0640$01.00/0