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6-Phosphogluconate Dehydrogenase: Hemizygous Manifestation in a Patient with Leukemia

Abstract. *In a study of 41 patients with chronic myelocytic leukemia, two were found to have the 6-phosphogluconate dehydrogenase heterozygous phenotype A-B, and two had the phenotype characteristic of* Pd^B *homozygosity. Since one of the two with* Pd^B *homozygosity was the mother of two children with the A phenotype, it was presumed that she carried a* Pd^A *gene not expressed in her blood cells. This was confirmed by electrophoretic analysis of her fibroblasts, which had the A-B phenotypic pattern. Gene deletion is considered to be the most likely explanation.*

In patients with chronic myelocytic leukemia (CML), the myelogenous cells have the Philadelphia[1] (Ph^1) chromosome, a G-group chromosome with a deletion of the long arm involving about a third of its DNA (*1*). Cytogenetic (*2*) and genetic evidence (*3*) support a clonal origin of red cells and granulocytes in patients with this disease; thus, both of these cell types from CML patients can be used for deletion mapping of the involved G chromosome. During such a study of markers for 15 autosomal loci in 41 patients with CML (*4*) and 29 members of their families, we found one patient with a 6-phosphogluconate dehydrogenase (6PGD) (E.C. 1.1.1.44) anomaly which forms the basis for this report.

The starch-gel electrophoretic pattern (Fig. 1) of 6PGD in granulocytes of normal subjects homozygous for either the common allele, Pd^A (*5–7*) or its less common allele, Pd^B, shows a single band with characteristic mobility (*8*). The electrophoretic pattern of the heterozygous phenotype has both A and B enzyme bands as well as a band with intermediate mobility which probably represents a hybrid of A and B subunits (*8*). Of the 41 patients with CML, 37 had A, two had A-B, and two had B phenotypes indistinguishable from those of normal subjects. Comparison of the phenotype frequencies in this small sample with those in the general population is difficult because the sample was drawn from a very heterogeneous population.

In the family of one of the patients (Mrs. I), the husband and both children had the A phenotype typical of Pd^A homozygosity. Nevertheless, both the red cells and granulocytes of Mrs. I had the B phenotype typical of Pd^B homozygosity. The same results were obtained from two subsequent analyses at 2-month intervals. Mrs. I, in complete remission on busulfan therapy, had never undergone transfusion. The results of other genetic marker tests supported her assertion that she was the mother of her children. Thus, she had to be a carrier of the Pd^A gene, even though this gene was not expressed in her granulocytes or red cells. That she was the carrier was confirmed by analysis of her cultured skin fibroblasts, which revealed an A-B phenotype characteristic of cells from a Pd^A/Pd^B heterozygote (Fig. 1).

No other examples of anomalous inheritance were found in our study. In the one other patient with the blood cell B phenotype, the same type was found in cultured fibroblasts; also, her children had type A-B. She was therefore presumed to be a Pd^B homozygote.

There are several possible explanations for the discordance of 6PGD types between the myelogenous and nonmyelogenous cells of Mrs. I. For example, the electrophoretic migration of the enzyme might be altered as a consequence of CML or its treatment. However, none of the other patients on busulfan therapy had an unexpected isozyme pattern. Thus, it seems more likely that Mrs. I's anomaly is due to a genetic event in a stem cell, occurring early in embryogenesis or later in the single stem cell from which the leukemia arose.

There have been no examples of unusual 6PGD inheritance in studies of several hundred families of nonleukemic subjects (*7–9*). Therefore, since the association of CML and discordant 6PGD phenotype in Mrs. I is apparently not fortuitous, the responsible genetic event probably occurred in the leukemic stem cell. This event could have been somatic mutation or recombination, or genetic inactivation, or—more likely—deletion.

From the observations reported here, the site of the assumed deletion cannot be determined. If it is part of the deleted segment of Mrs. I's Ph^1 chromosome, then one must account for the presence of the blood cell A-B pheno-

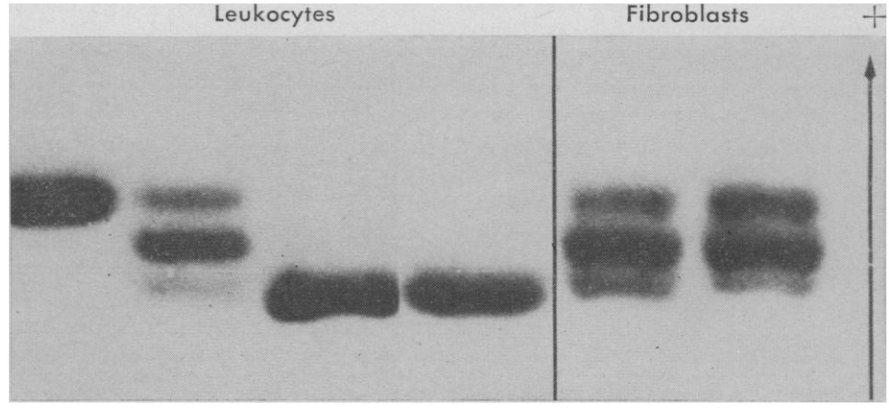

Fig. 1. Starch-gel electrophoresis of 6-phosphogluconate dehydrogenase. From left: slot 1, granulocytes from a normal subject homozygous for the Pd^A gene; slot 2, granulocytes from a normal subject heterozygous for the Pd^A and Pd^B genes; slot 3, granulocytes from a normal subject homozygous for the Pd^B gene; slot 4, granulocytes from Mrs. I; slot 5, cultured skin fibroblasts from Mrs. I; slot 6, cultured skin fibroblasts from a normal subject heterozygous for the Pd^A and Pd^B genes.

type in two other patients with the Ph^1 chromosome. It may be that the Ph^1 deletion varies in size or location from one CML patient to another, or that Mrs. I has an inversion on this chromosome so that the 6PGD locus is deleted. Alternatively, the deletion of the 6PGD locus might affect another chromosome, but the deletion is too small to be detected with currently available cytogenetic techniques.

Other less likely genetic mechanisms include gene inactivation or generalized hypermutability. Inactivation, such as that occurring at X-linked loci, does not occur normally at the 6PGD locus (*10*). Furthermore, the two CML patients with A-B types show that inactivation is not a general phenomenon of this disease, since the intermediate electrophoretic band represents a hybrid of A and B subunits produced within individual cells. Hypermutability was not observed at any other loci, and there were no apparent changes in ABH antigens, such as are observed in some cases of acute leukemia.

If similar results should be obtained in other patients with CML who have the Ph^1 chromosome, the argument for localization of the 6PGD locus on the G-group chromosome would be strengthened. However, only one-half of the A-B heterozygotes with the postulated deletion of the 6PGD locus would be hemizygous for Pd^B. The other half would be hemizygous for Pd^A and would only be detected if there were an anomalous inheritance pattern in their parents or children. Study of 6PGD in patients with trisomy-21 who have A-B phenotypes has potential value in resolving the problem, provided the Ph^1 chromosome is the "21st" chromosome.

The observed aberration appears to involve all (or a great majority) of Mrs. I's red blood cells and granulocytes. Since the most likely explanation for this aberration lies in a genetic mechanism, the observation provides additional support for the earlier conclusion (*3*) that CML has a clonal origin and that granulocytes and red blood cells have a common precursor cell which is the site of the malignant transformation.

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Pars Intermedia: Unitary Electrical Activity Regulated by Light

Abstract. *In the pars intermedia of frogs in the dark two types of spontaneously firing neuronal units have been found; one can be inhibited by and the other is indifferent to increases in illumination. The receptor for the light-inhibited units appears to be the pineal organ. Transection experiments indicate that the axons to the two kinds of units in the pars intermedia are separately grouped in the infundibular floor.*

Pigmentary adaptation in vertebrates requires a system that couples changes in values of environmental light with eventual responses of pigment cells in the skin. The best explored phase of this system involves the release of intermedin or melanocyte stimulating hormone (MSH) from the pars intermedia of the pituitary gland. This hormone directly evokes adaptive changes (darkening) in integumentary pigment cells (*1*), and its action has been well explored. However, the coupling of MSH secretion and release to photoreceptive and other nervous mechanisms is not so well understood. Current research indicates that release of MSH may be under direct inhibitory control of monoaminergic axons that innervate the pars intermedia and synapse with secretory cells (*2*). Stimulatory as well as inhibitory relations of innervation to secretion of MSH have been claimed (*3*), but most experimental evidence in Amphibia, at least, favors a tonic type of inhibitory control.

Since current hypotheses concerning control of MSH release require that neurons link photoreceptive function and the pars intermedia, and since electron microscopy reveals numerous synapses between neurons and secretory cells (*4*), we elected to examine the gland for unitary neuronal electrical activity in frogs.

We recorded such unitary activity from the pars intermedia through microelectrodes (Fig. 1, A and B) and established a lack of this activity in the other large epithelial lobe of the pituitary gland, the pars distalis (Fig. 1C). Furthermore, some of the pars intermedia electrical potentials were shown early in this analysis to be responsive to changes in background illumination (Figs. 1B and 2A).

Seventy-eight adult frogs (*Rana pipiens*) of both sexes, weighing 25 to 60 g, were used. Each frog was lightly anesthetized with ether, immobilized by intramuscular *d*-tubocurarine chloride (1.0 mg per 100 g of body weight), and placed on its back in a transparent plastic frame. Bone and connective tissue were quickly removed from the ventral hypothalamic region under a dissecting microscope, and with minimal bleeding.

A stainless steel microelectrode [electrolytically sharpened, insulated except at the tip (*5*)] led electrical impulses