AUTOSOMAL CONTROL OF AN ELECTROPHORETIC VARIANT OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE IN THE MOUSE (*MUS MUSCULUS*)

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CONSIDERABLE interest exists in the enzyme glucose-6-phosphate dehydrogenase (G6PD) because (1) deficiency of the enzyme in man is sex-linked and can result in hemolysis (TARLOV, BREWER, CARSON, and ALVING 1962; BEUTLER 1966); (2) electrophoretically variant forms in man (KIRKMAN, McCURDY, and NAIMAN 1964), equine species (MATHAI, OHNO, and BEUTLER 1966), and Drosophila (YOUNG, PORTER, and CHILDS 1964) are X-linked and have contributed to our understanding of the control of genetic expression of the X-chromosome (LYON 1962; DAVIDSON, NITOWSKY, and CHILDS 1963); (3) the X-linked variants in man can be biochemically characterized (KIRKMAN, McCURDY, and NAIMAN 1964) and used as genetic markers for studies relating to human population genetics (MOTULSKY and CAMPBELL-KRAUT 1961); (4) variant forms of G6PD have served as markers in somatic cell populations of human origin cultivated *in vitro* (GARTLER, GANDINI, and CEPPELLINI 1962); and (5) recently, autosomally determined electrophoretic forms of G6PD have been described for the deer mouse, *Peromyscus maniculatus* (SHAW and BARTO 1965).

In this report we present evidence for a second instance of an autosomal form of G6PD. This was previously reported as an isozymic polymorphism found when comparing inbred strains of mice, *Mus musculus* (RUDDLE 1963).

MATERIALS AND METHODS

Genetic analysis was made on enzymes from kidney homogenates from animals 6 to 8 weeks old. Animals were killed by a blow on the head followed by cervical dislocation. Kidneys were rapidly removed and washed in ice cold isotonic saline. The organs were homogenized in a Potter-Elvehjem tissue grinder in a volume of distilled water equal to the volume of the specimen. The homogenates were frozen and thawed once and then stored at —90°C (Revco Freezer) for 5–10 days prior to analysis. Immediately before electrophoresis the extracts were thawed and centrifuged at 27,000 *g* for one hour at 4°C.

In some cases enzyme phenotypes were studied from erythrocyte lysates and plasma. Plasma was obtained by draining blood from the retro-orbital sinus with heparin treated pipettes. Erythrocytes were removed by centrifugation, washed 3 times in isotonic saline (0.85%) at room temperature, hemolyzed in an equal volume of water, and then extracted with toluene (0.4 ml toluene/ml hemolysate). Particulate material was removed by centrifuging at 27,000 *g* for 1 hour.

Electrophoresis was done in a vertical starch gel (BUCHLER) using a modification of the Tris-EDTA-borate buffer system of BOYER, FAINER, and NAUGHTON (1963). Stock buffer (pH 8.6) was composed of tris (0.9 M), tetrasodium salt of EDTA (0.02 M), and boric acid (0.5 M). The

buffer stock was diluted 1/20 with distilled water for gel buffer, 1/5 for negative electrode buffer, and 1/7 for positive electrode buffer. Hydrolysed starch (CONNAUGHT) was used at a concentration of 13.2%. Electrophoresis was carried out at 3°C for 18 hours at 10 volts/cm across the gel. Gels were stained at 37°C for about two hours in a 100 ml incubation mixture consisting of 0.1 M Tris-HCl buffer at pH 8.0, 35 mg $NADP^+$, 25 mg nitro blue tetrazolium, 3 mg phenazine methosulphate, and 400 mg Na_2Glucose-6-$PO_4 \cdot 3H_2O$. This is a modification of the method of SHOWS, TASHIAN, BREWER, and DERN (1964).

Esterase isozymes were visualized by methods we previously reported (RUDDLE and RODERICK 1965, 1966). Isocitrate and malate dehydrogenase were visualized by modifications of the methods given by HENDERSON (1965, 1966).

Nomenclature. The zymogram pattern is divided into a number of broad divisions, called zones, whose numerical designations increase with distance from the origin (RUDDLE 1966). The zones are defined largely on the basis of discontinuities in the electrophoretic pattern, and are subdivided according to their substituent individual enzymes which appear as bands. These are also designated numerically with higher numbers assigned to the enzymes more distantly removed from the origin. The bands are assigned in multiples of ten in order to allow an insertion of additional enzymes. As an example, an enzyme designated II-10 describes a band 10 in Zone II of the pattern. Cathodally migrating enzymes are designated in exactly the same way, except they are termed negative, (—). This system of classification gives consistent results if the conditions of electrophoretic analysis are exactly reproduced.

RESULTS

Zone I G6PD. The electrophoretic pattern for kidney extract G6PD is illustrated in Figures 1 and 2. Zone I appears as a single discrete narrow band. The G6PD polymorphism reported here occurs only in Zone I as a slow migrating form, a; and a fast migrating form, b. The a form of the enzyme is found in inbred strains C57BL/6J, PL/J, C58/J, CE/J, 129/J, C57BR/cdJ, RF/J, and P/J. The b form of the enzyme is found in the inbred strains SJL/J, LP/J, MA/J, ST/bJ, DBA/2J, A/HeJ, AKR/J, C3HeB/FeJ, CBA/J, BALB/cJ SWR/J, and BUB/Bn.

F_1, F_2, BC_1 crosses were made between inbred strains SJL/J and C57BL/6J in order to test the mode of inheritance of the variant enzymes. The results of the crosses are given in Table 1, where without exception both fast and slow bands were expressed codominantly in the F_1 animals. The F_2 progeny showed good statistical agreement with 1:2:1 ratio for a, ab, and b phenotypes, respectively. In the backcross to SJL/J, b and ab animals were found in a ratio not significantly

TABLE 1

G6PD phenotypes in strains SJL/J, C57BL/6J, and crosses between them

	Phenotypes of progeny								
	G6pd-1a			G6pd-1ab			G6pd-1b		
Parental conditions	♂♂	♀♀	Σ	♂♂	♀♀	Σ	♂♂	♀♀	Σ
SJL/J	..	..	..	..	..	..	6	4	10
C57BL/6J	6	4	10	..	..	..	..	..	..
F_1	..	..	..	4	6	10	..	..	..
F_2	9	28	37	37	40	77	12	21	33
F_1 × SJL/J	..	..	..	4	3	7	4	5	9
F_1 × C57BL/6J	6	2	8	3	2	5	..	..	..

GLUCOSE-6-PHOSPHATE DEHYDROGENASE

F_2 progeny from single sibship

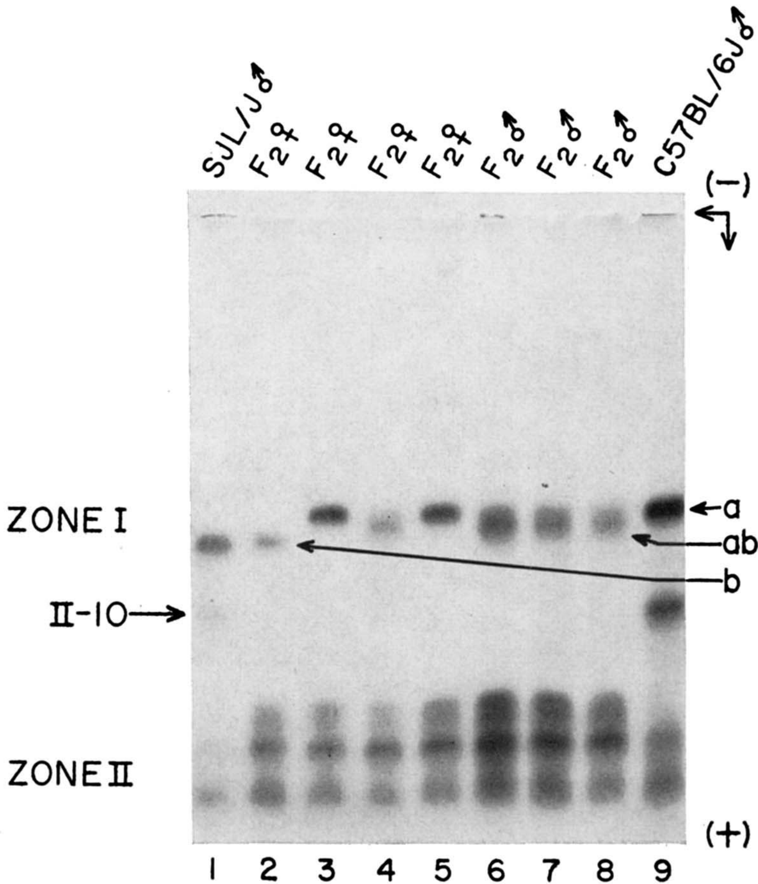

FIGURE 1.—Starch gel electrophoretic patterns of G6PD from kidney extract, without TPN. All F_2 animals are from a single sibship. Samples in channels 1 and 9 have been frozen and thawed several times. This causes band II-10 to migrate in a more cathodal position.

different from 1:1. In the backcross to C57BL/6J, a and ab animals were observed also in a ratio not significantly different from 1:1. The progeny data, therefore, describe a phenotypic difference controlled at one locus by two codominant alleles. The gene is not sex-linked, because heterozygous male individuals occurred in the F_1, F_2, and BC_1 progeny and all three phenotypes occurred in both sexes in one of the F_2 sibships.

The heterozygous phenotype for Zone I is distinctly different from either of

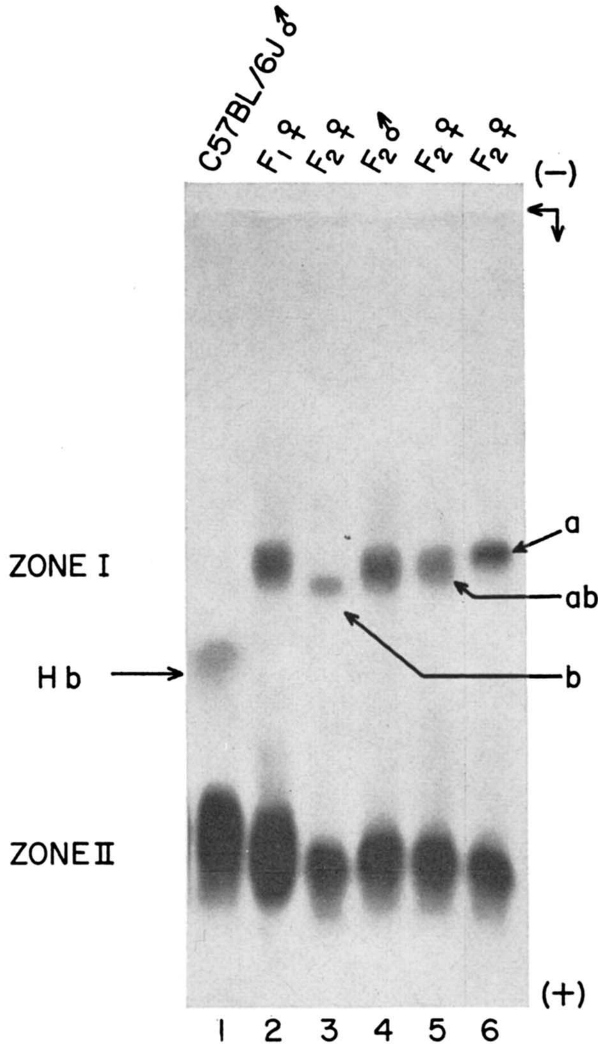

FIGURE 2.—Starch gel electrophoretic patterns of G6PD from kidney extracts with TPN. Erythrocyte lysate has been run in channel 1. Hb marks the position of hemoglobin.

the homozygous phenotypes (Figure 1). In the heterozygote, three bands occur. Two of these correspond to the a and b parental forms, whereas the third band (ab) has an intermediate mobility, and possesses approximately double the histochemical staining activity of either a or b in the heterozygote. The genetic evidence indicates that two alleles exist which code for enzyme subunits which

randomly hybridize as dimers. This would give in a heterozygous individual the observed phenotype 1a:2ab:1b. These results are in agreement with SHAW and BARTO (1965) who reported that an autosomally linked form of G6PD in *Peromyscus maniculatus* was a dimer.

We propose to designate this locus *Gpd-1* in accordance with nomenclature recommended by the Committee on Standardized Genetic Nomenclature for Mice (STAATS 1966). The alleles coding for the slow and fast forms of the enzyme will be designated *Gpd-1^a* and *Gpd-1^b*, respectively. The phenotypes will be designated G6pd-1a for the slow and G6pd-1b for the fast and G6pd-1ab for the heterozygote. Although the polymorphic enzyme reported here is probably homologous with that termed hexose-6-phosphate dehydrogenase (H6PD) in man by OHNO, PAYNE, MORRISON and BEUTLER (1966) and in Peromyscus by SHAW (1966), we prefer the G6PD terminology. Our reasons are that (1) G6PD refers to an actual enzyme whereas H6PD does not, and (2) the established mouse terminology can readily accommodate different loci which code for different G6PD enzymes (*viz.*, *Gpd-1*, *Gpd-2* . . .).

The Zone I form of G6PD is broadly distributed in the tissues of the mouse. Highest enzyme activity was found in kidney extracts; lower activity was observed in extracts of brain, liver, tongue, skeletal and cardiac muscle. It is *not* detectable in erythrocyte lysates (Figure 2, channel 1). G6PD zymograms of *Peromyscus maniculatus* liver and kidney homogenates and erythrocyte hemolysates were similar to those of *Mus musculus* in the position and general appearance of Zone I and II isozymes. The Zone I enzyme is also expressed in fibroblasts derived from mouse fetuses which have been maintained in continuous cultivation *in vitro* for more than one year. Cultures originating from C57BL/6J, (C57BL/6J × SJL/J) F_1, and SJL/J fetuses express G6pd-1a, G6pd-1ab, and G6pd-1b phenotypes, respectively. Details relating to the *in vitro* expression of allelic forms of enzyme variants will be published elsewhere as a separate report.

Zone II G6PD. Zone II is broadly diffuse, migrates anodally with respect to Zone I, and possesses considerably more G6PD activity than the Zone I form of the enzyme. Zone II is probably composed of more than one molecular form. Three bands can be seen in Zone II (Figure 1) in gels lacking TPN. These we designated II-10, 20, and 30 in order of increasing mobility. These distinct subcomponents are lost if TPN (5×10^{-5} M) is added to the gel (Figure 2). Another observation indicating that molecular differences exist in Zone II is the difference in electrophoretic behavior in old and fresh extracts when electrophoresed on gels containing TPN. In fresh extracts, the subcomponents of Zone II migrate as a group. In older extracts, or in extracts which have been frozen and thawed several times, band II-10 migrates in a more cathodal position (Figure 1, channel 9). This effect can be reversed by adding TPN (10^{-3} M) to the old extract. These subtle transient differences in Zone II enzyme might relate to the reversible association of a single primary structured form of the enzyme with certain ligands. Differences in ligand binding may also be related to the association of the enzyme with different subcellular organelles.

There was no indication of a genetic association between Zone II and Zone I

enzyme, because in no instance did Zone II vary between individuals of the parental strains or their progeny in the crosses reported here. Additional evidence for a lack of association is the fact that Zone II enzyme is found in hemolysates (Figure 2, channel 1) as well as in kidney, brain, liver, and tongue extracts. Zone II is also strongly expressed in fibroblast cell populations *in vitro*.

Linkage. The F_2 and BC_1 progeny of SJL/J × C57BL/6J crosses segregate for a number of markers, six of which we could conveniently observe in addition to G6PD. To look for linkage relationships, we observed 13 progeny from the backcross to C57BL/6J and 19 from the backcross to SJL/J. They were typed for phenotypes of *Es-1*, *Es-3* (RUDDLE and RODERICK 1967 and earlier), *Id-1*, *Mdh-1* (HENDERSON 1965, 1966), *a*, *c*, and *rd* (GREEN 1966). HENDERSON (1966) thought her data suggested a linkage between *Mdh-1* and dilute (*d*). Linkages are known for *Es-1*, *a*, *c*, and *rd*, (GREEN 1966) but not for *Id-1*, *Es-3*, and *Gpd-1*. POPP (1966) showed *Es-3* is not tightly linked to *d*, *Ca*, *mi*, *ln*, and *Os*. POPP (1967) has recently demonstrated linkage between *Es-1* and *Es-2* (PETRAS 1963). In all combinations of two genes in our backcross progeny there was no suggestion of a predominance of parental types. Thus there is no evidence from our data for close linkages among the 6 loci, *c*, *rd*, *Gpd-1*, *Es-1*, *Es-3*, *Id-1* and *Mdh-1*.

DISCUSSION

The only other reports of an autosomally linked form of G6PD are those of SHAW and BARTO (1965), and SHAW (1966) in *P. maniculatus*. They recognized two electrophoretically distinct enzymatic forms of G6PD which were subsequently called H and G (SHAW and KOEN 1967). This terminology was chosen because the G form oxidizes only the substrate glucose-6-phosphate, whereas the H (hexose) form oxidizes both glucose-6-phosphate and hexose-6-phosphate. The H and G enzymes have also been identified in equine and human liver (SHAW 1966; OHNO, PAYNE, MORRISON, and BEUTLER 1966). The H form migrates more cathodally on Tris-EDTA-borate gels than does the G form. The H form of the enzyme is polymorphic and autosomally linked in Peromyscus and Mus. Since no genetic variant of the G form has been found in mice of these genera, it is still possible that the locus for the G enzyme is sex-linked in mice as in man (KIRKMAN, MCCURDY, and NAIMAN 1964).

The enzymes which we have designated as G6PD Zone I and II most probably relate respectively to SHAW's H and G enzymes, because (1) there exists a general similarity between H in comparison to Zone I and G in comparison to Zone II in terms of their electrophoretic migration and resolution (Figure 2); (2) H and Zone I enzymes oxidize both glucose- and galactose-6-phosphate, whereas G and Zone II enzymes oxidize only glucose-6-phosphate; and (3) the H and Zone I enzymes are both autosomally linked. It is interesting that the Zone I (H) form of the enzyme is polymorphic in both genera, suggesting that polymorphism is in some way adaptive at this locus.

Increasingly, the possibilities of systems which will permit the genetic analysis of somatic cells *in vitro* are beginning to emerge (HARRIS 1966; KLEIN 1963;

GARTLER and PIOUS 1966). The laboratory mouse because of the existence of inbred lines, a highly developed formal genetics, and the cultivability of murine cells *in vitro* is ideally suited to somatic genetic studies. We have demonstrated, in addition, that fibroblasts derived from fetuses of the inbred lines SJL/J, C57BL/6J, and their F_1 hybrids continue to express their characteristic isozyme phenotypes for the enzymes malate dehydrogenase, isocitrate dehydrogenase, and G6PD for at least a year under continuous cultivation (RUDDLE, in preparation). The expression of these marker enzyme phenotypes can be employed to identify and type lines of tissue culture cell populations (GARTLER and PIOUS 1966). Potentially more exciting is the use of heterozygous combinations of co-dominately expressed enzyme variant alleles to test for somatic recombination in tissue culture cell populations.

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SUMMARY

A comparison of the electrophoretic patterns of F_1, F_2, and backcross progeny of two inbred strains of mice has revealed a new autosomal variant of glucose-6-phosphate-dehydrogenase. We have designated this locus *Gpd-1* and its two alleles *Gpd-1*^{a} and *Gpd-1*^{b} which represent respectively the slow and fast migrating electrophoretic forms.

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