of a freshwater reservoir thermocline was reported in California (*10*).

In all of these cases, solar heating occurs only when the water is segregated into density layers of sufficient contrast. The density of the brine even when hot is greater than the density of cooler freshwater; thus the density profile remains constant. The freshwater layer provides insulation to the hot brine, conserving the heat.

The chemistry of the hot natural brines as distinct from geothermal brines is not as yet well understood. The unusually hot temperatures that are generated can stimulate dissolution, recrystallization, and reconstitution of some bottom sediments. Preliminary examination of the uppermost 1.5 m of bottom sediments cored in Lago Pueblo indicates that secondary changes have occurred in places.

The density-stratified brines in natural or artificial reservoirs should be reconsidered as solar energy collectors. Even in temperate and polar latitudes (namely, the examples in New York State and Lake Vanda, Antarctica), appreciable solar energy can be trapped. The chief advantages of such collectors are their relatively low cost of construction and maintenance and the high efficiency of energy absorption.

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Lysosomal and Microsomal Glucuronidase: Genetic Variant Alters Electrophoretic Mobility of Both Hydrolases

Abstract. *An electrophoretic variant of β-glucuronidase is present in certain inbred mouse lines. The variant simultaneously affects the mobility of the lysosomal and microsomal forms of the enzyme. The difference is inherited as a single gene mapping at the position of the structural gene on chromosome 5. This confirms that β-glucuronidase at both intracellular sites is coded by the same structural gene.*

Acid hydrolases function in cellular catabolism, and deficient activity has been associated with lysosomal storage diseases in man (*1*). Several acid hydrolases, such as β-glucuronidase, β-D-*N*-acetylhexosaminidase, β-galactosidase, α-galactosidase, aryl sulfatase, and acid phosphatase, exist in multiple molecular forms (isozymes) when separated by gel electrophoresis (*2–4*). Biochemical, immunological, and genetic evidence indicate that a genetic and structural relationship may exist between the isozymes of any one acid hydrolase (*5–7*).

Investigation of the relationships of the multiple forms of an acid hydrolase would be facilitated by the existence of electrophoretic variants. Such variants would aid in determining whether the isozymes of a specific acid hydrolase are (i) coded by separate structural genes or (ii) share a common gene product coded by a single structural gene.

In the mouse, β-glucuronidase (E.C. 3.2.1.31) is assocated with the lysosomal and microsomal subcellular fractions (*8*), and both forms can be separated by gel electrophoresis (*9*). The structural gene locus for murine β-glucuronidase has been established on chromosome 5 with the use of a heat labile mutation of the enzyme (*10*). We report an electrophoretic variant of β-glucuronidase in inbred strains of mice which simultaneously alters the mobility of the lysosomal and microsomal glucuronidase activities, confirming earlier biochemical evidence indicating that a single structural gene codes for both forms of the enzyme (*8*). We believe that this is the first genetic electrophoretic variant that alters the mobility of an enzyme associated with two different subcellular structures. Previously, an electrophoretic variant of lysosomal β-glucuronidase was suggested in a wild mouse, but no mention was made of an altered mobility of the microsomal form of the enzyme (*11*).

Vertical starch-gel electrophoresis separates β-glucuronidase into two distinct zones of activity (Fig. 1). The more anodal zone (L) is associated with the lysosomes, while the slower migrating zone (M) is associated with the microsomes (*9, 12*). Although lysosomal β-glucuronidase was expressed in all tissues examined (liver, kidney, spleen, lung, and brain), the expression of microsomal β-glucuronidase varied from tissue to tissue. Liver homogenates were chosen for electrophoretic and genetic studies since liver best expressed the L and M forms of the enzyme. A survey of 28 inbred strains of mice revealed two glucuronidase phenotypes, one of which expressed faster migrating L and M enzymes than the other phenotype. The anodally migrating phenotype (Fig. 1, channels 3 and 7) was observed in six strains: A/J, A/St, BALB/cJ, LG/J, SEA/GnJ, and SM/J. This phenotype has been designated GUS-A. The slower migrating L and M types (Fig. 1, channels 1 and 5), designated GUS-B, were observed in 23 strains: AU/SsJ, BUB/BmJ, CBA/J, C57BL/6J, C57BL/10J, C57BR/cdJ, C57c/Ha, C57L/J, C58/J, C3H/HeJ, CE/J, DBA/2J, LP/J, MA/J, P/J, PL/J, RF/J, SJL/J, ST/bJ, SWR/J, WB/ReCz, and 129/J. Inbred mouse lines were obtained from Jackson Laboratory, Bar Harbor, Maine (designated J), and Roswell Park Memorial Institute.

The structural gene for β-glucuronidase, formerly designated *G*, is now designated *Gus* (*12*). The alleles for the fast and slow phenotypes, respectively, are designated Gus^a and Gus^b, and give rise to three possible phenotypes, GUS-A, GUS-B, and GUS-AB, in mating experiments. The respective genotypes are Gus^a/Gus^a, Gus^b/Gus^b, and Gus^a/Gus^b.

In a mating between SM/J mice (GUS-A, Fig. 1, channels 3 and 7) and SWR/J mice (GUS-B, Fig. 1, channels 1 and 5), all F_1 progeny expressed a heterozygous phenotype (GUS-AB) consisting of a broad band of activity intermediate between the two parental types in both the lysosomal and microsomal forms of the enzyme (Fig. 1, channels 2 and 6). Such a phenotype

for the heterozygote indicates that unresolved heteropolymers are present in hybrid tissues. An artificial mixture of the homogenates of the two types, A and B, exhibited a composite of the two parental phenotypes (Fig. 1, channels 4 and 8) which was not identical to the F_1 phenotype.

Since β-glucuronidase is a tetramer (*12, 13*), five bands of activity would be expected in the heterozygote, because of the random association of the parental subunits. The five bands would correspond to the two parental forms and three heteropolymeric forms (*14*). Our system could not resolve the five bands, but it was sufficient to distinguish accurately between the three possible phenotypes.

In reciprocal backcross matings (Table 1), segregation of both parental nonrecombinant phenotypes was in essentially 1 : 1 ratios for both sexes. These results are consistent with autosomal segregation of two codominant allelic forms. Seven markers were available for gene linkage analysis in this cross. Linkage was not observed between *Gus* and *Gpi* [linkage group (LG) 1, chromosome 7] (*15*); *Pgm*-2 (LG VIII, chromosome 4) (*16*); *Id*-1 (LG XIII, chromosome 1) (*17*); *Got*-2 (LG XVIII, chromosome 8) (*18*); or agouti locus *a* (LG V, chromosome 2). Positive linkage was observed between *Gus* and *Pgm*-1 (LG XVII, chromosome 5) (*19*) (Table 1). The recombination frequency ± the standard error between *Gus* and *Pgm*-1 was 36.8 ± 4.3 percent, observed in progeny from reciprocal backcross matings. This would place the locus determining *Gus* electrophoretic mobility on chromosome 5 at a position expected for the structural gene as determined previously for a thermolabile β-glucuronidase variant (Gus^h) (*10, 19*).

The electrophoretic variant described here confirms earlier genetic and biochemical results which indicated that the enzyme at both cellular sites was coded by the same structural gene (*8*). Recent biochemical evidence demonstrated that the glucuronidase structural gene product undergoes secondary modification to give rise to microsomal glucuronidase (*12*). In microsomes the enzyme exists in noncovalent association with a peptide which stabilizes the enzyme in the microsomal membranes (*12*). This peptide (molecular weight, 50,000 to 55,000) is coded by the *Eg* gene (*20*), which is located on chromosome 8 (*21*), while the structural gene that codes for identical subunits (molecular weight, 60,000 to 70,000) common to both the lysosomal and microsomal glucuronidase is located on chromosome 5.

This unique genetic and molecular model, which involves the secondary modification of a structural gene product, may be applicable to other hydrolases associated with subcellular structures. Evidence has been presented indicating that similar structural and genetic relationships exist between the isozymes of human hexosaminidase, deficient activity of which is associated with Tay-Sachs disease (*22*). With the use of man-mouse somatic cell hybrids (*23*), it was demonstrated that the expression of human hexosaminidase A was dependent on the presence of hexosaminidase B. The data suggest that a structural relationship exists between hexosaminidases A and B, and that the gene responsible for the expression of hexosaminidase A functions by modifying hexosaminidase B or the hexosaminidase B gene product. Biochemical and immunological evidence further support the existence of a structural relationship between the two hexosaminidase isozymes (*3, 7*). Knowledge of the genetic control and structural relationship of the multiple forms of a specific acid hydrolase is important for understanding the basic genetic defect associated with such lysosomal storage diseases as Tay-Sachs disease (*22*).

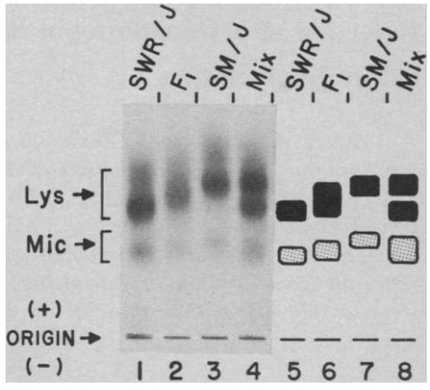

Fig. 1. Liver phenotypes and genetic variants of murine β-glucuronidase are demonstrated by electrophoretic patterns (channels 1 to 4) and a diagram (channels 5 to 8). The more anodal band is the lysosomal (*Lys*) form in each extract, and the zone closer to the cathode is the microsomal (*Mic*) form in each extract. In the fast migrating variant, SM/J (channels 3 and 7), both lysosomal and microsomal glucuronidase migrate faster than lysosomal and microsomal glucuronidase, respectively, in the slow variant, SWR/J (channels 1 and 5). The heterozygous phenotype (channels 2 and 6) consists of a broad band of activity intermediate in mobility between the fast and slow variants in both lysosomal and miscrosomal forms. Tissues were extracted and prepared for electrophoresis as described (*26*). Vertical starch-gel electrophoresis (63 g/500 ml; Electrostarch) was perfoimed at 4°C for 18 hours at 200 volts in a tris-citrate buffer system, *p*H 7.0 (*26*). After electrophoresis, gels were incubated for 30 minutes at room temperature in 0.2*M* acetate buffer, *p*H 5.0, to adjust the gel to the optimum *p*H for β-glucuronidase. Gels were specifically stained for glucuronidase activity by the simultaneous diazo coupling method of Hayashi *et al.* (*27*) with naphthol AS-BI, β-D-glucuronidase (Sigma) as substrate. The method was modified slightly by reducing the concentration of *p*-rosaniline to 0.3 m*M* to decrease background staining.

Table 1. Recombination of β-glucuronidase (*Gus*) and phosphoglucomutase (*Pgm-1*).

Mating		Nonrecombinants		Recombinants*		Totals
(SWR/J × SM/J) F_1 × SM/J						
$\frac{Gus^a\ Pgm\text{-}1^a}{Gus^b\ Pgm\text{-}1^b}$ × $\frac{Gus^a\ Pgm\text{-}1^a}{Gus^a\ Pgm\text{-}1^a}$		$\frac{Gus^a\ Pgm\text{-}1^a}{Gus^a\ Pgm\text{-}1^a}$	$\frac{Gus^a\ Pgm\text{-}1^a}{Gus^b\ Pgm\text{-}1^b}$	$\frac{Gus^a\ Pgm\text{-}1^b}{Gus^a\ Pgm\text{-}1^b}$	$\frac{Gus^b\ Pgm\text{-}1^a}{Gus^a\ Pgm\text{-}1^a}$	
		25	31	22	13	91
(SWR/J × SM/J) F_1 × SWR/J						
$\frac{Gus^a\ Pgm\text{-}1^a}{Gus^b\ Pgm\text{-}1^b}$ × $\frac{Gus^b\ Pgm\text{-}1^b}{Gus^b\ Pgm\text{-}1^b}$		$\frac{Gus^b\ Pgm\text{-}1^b}{Gus^b\ Pgm\text{-}1^b}$	$\frac{Gus^a\ Pgm\text{-}1^a}{Gus^b\ Pgm\text{-}1^b}$	$\frac{Gus^a\ Pgm\text{-}1^b}{Gus^b\ Pgm\text{-}1^b}$	$\frac{Gus^b\ Pgm\text{-}1^a}{Gus^b\ Pgm\text{-}1^b}$	
		13	10	7	4	34
	Total progeny	38	41	29	17	125

* Frequency of recombination ± S.E. = 36.8 ± 4.3 percent; $\chi^2 = 8.712$.

Sandhoff-Jatzkewitz disease (*6*), generalized gangliosidosis (*24*), Fabry's disease (*25*), and metachromatic leukodystrophy (*4*).

The significance of our experiments is (i) the demonstration of an inherited single mutation of murine β-glucuronidase which alters the electrophoretic mobility of the enzyme localized at two different subcellular sites and (ii) the support that this variant lends to a model in which the multiple molecular forms of an acid hydrolase are coded by a single structural gene whose gene product undergoes secondary modifications.

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Detection of a Gonadotropin in Rabbit Blastocyst before Implantation

Abstract. *A gonadotropin similar to human chorionic gonadotropin or luteinizing hormone has been demonstrated in rabbit blastocyst prior to implantation. The gonadotropin has been detected by a radioreceptor assay for human chorionic gonadotropin with the use of the plasma membranes of bovine corpora lutea obtained during the first trimester of pregnancy. The concentrations of the human chorionic gonadotropin or luteinizing hormone per milliliter of blastocyst fluid were tenfold higher than those in the blood of pregnant rabbits on days 5 and 6 after mating.*

The detection of pregnancy as early as days 6 to 8 after conception (*1*) suggested to us that we investigate the possible presence of a substance similar to human chorionic gonadotropin (hCG) or luteinizing hormone (LH) in the blastocyst prior to implantation. The existence of pregnancy was determined by the measurement of hCG-like or LH-like material in the blood by a radioreceptor assay for hCG, on the basis of competitive protein binding (*2, 3*).

Ten albino virgin rabbits were mated. On day 6 after mating, the animals were killed. The uterine horns were dissected on a petri dish and flushed with physiological saline by the aid of a syringe. The blastocysts, approximately three to ten from each rabbit, were transferred to a test tube with a Pasteur pipet. The fluid adhering to the blastocysts was drawn off. The blastocysts were disrupted by a needle and centrifuged. The volume of the supernatant was approximately 15 μl per blastocyst. The supernatant was tested in the radioreceptor assay of hCG-LH concentrations of 10, 20, 50, and 100 μl. Samples of plasma from nonpregnant and pregnant rabbits were also tested in the radioreceptor assay as follows. Samples of 100 μl of standard solution of hCG (12,000 international units per milligram) in doubling dilution [for example, 3.0, 6.3, 12.5, 25, 50, and 100 ng per milliliter of 10 m*M* tris-HCl buffer (*p*H 7.2) containing 1 m*M* $CaCl_2$, 1 m*M* $MgCl_2$, 0.1 percent bovine serum albumin], 10 international units of Trasylol in 20 μl of tris-HCl buffer, plasma membranes (*3*) (25 mg of protein in 100 μl incubation buffer), and ^{125}I (approximately 50,000 count/min in 100 μl of incubation buffer; specific activity, 40 to 50 μc/μg) were added to disposable plastic vials (75 × 100 mm) to both standard and unknown samples. The reaction mixture was then incubated at 37°C for 20 minutes. The tubes were placed in an ice bath, and 1 ml of chilled tris-HCl buffer was added to each tube. The contents of the tube were mixed on a Vortex mixer and centrifuged for 10 minutes at 5000*g*. The supernatants were aspirated, and the radioactivity bound to the plasma membranes was counted in an autogamma counter (Packard Instruments) with a 51 percent efficiency for ^{125}I.

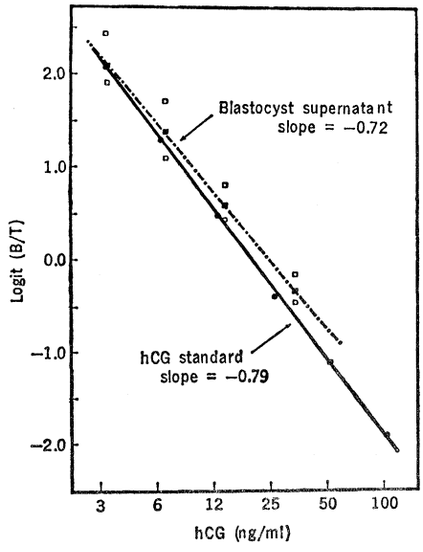

Fig. 1. Dose-response curve for human chorionic gonadotropin (hCG) and the supernatant of the ruptured and centrifuged blastocysts expressed as logit-log transformation. The ratio B/T is the ratio of ^{125}I-labeled hCG bound in the membrane receptor to the total ^{125}I-labeled hCG. Open squares represent duplicate experiments.

The dose-response curve for hCG and blastocyst supernatant are presented as logit-log transformations (*2*) in Fig. 1. Increasing quantities of the blastocyst supernatant progressively inhibited the binding of ^{125}I-labeled hCG, yielding a slope of −0.72, a value which is close to that of the hCG standard of −0.79, suggesting the presence of a material resembling hCG or LH in the blastocyst fluid. Ten assays performed on 50 blastocysts yielded, per