GLUCURONIDASE PHENOTYPES OF INBRED MOUSE STRAINS¹

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MAMMALIAN β -glucuronidase has been relatively well characterized with regard to such parameters as substrate specificity, tissue levels, pH dependence, intracellular localization, and protein structure. For a recent review the reader may consult LEVY and CONCHIE (1966). Although this information failed to elucidate the physiological role of glucuronidase, it has provided a convenient tool for the study of biochemical genetics in mammalian systems. In one of the earliest reports of a biochemical variant in higher organisms, MORROW, GREENSPAN, and CARROLL (1949, 1950) described two closely related inbred mouse strains (*Mus musculus*) that showed only one tenth the liver glucuronidase activity of six other lines of inbred mice. Subsequently, from an extensive genetic study, LAW, MORROW, and GREENSPAN (1952) concluded that a single recessive allele was responsible for this difference. The locus involved is presumed to be the structural gene for glucuronidase since mutants produce a physically altered enzyme (PAIGEN 1961a). Genetically it is closely linked to the *retinal degeneration* gene (PAIGEN and NOELL 1961) and recently has been identified as part of linkage group XVII (SIDMAN and GREEN 1965).

The low glucuronidase phenotype is the consequence of both a generalized decrease in enzyme activity and an altered time course of glucuronidase accumulation during post-natal development (PAIGEN 1961b). The formation of a structurally altered enzyme protein also influences the intracellular localization of the enzyme. In the hepatic cells of normal animals the enzyme is present in approximately equal amounts in the lysosomes and the membranes of the ergastoplasm, while in the structural gene mutant the residual activity is preferentially located in lysosomes.

The present study reports an attempt to find additional variants of glucuronidase which might be useful in the analysis of this enzyme in particular and, hopefully, the general problem of gene expression in higher organisms. For this purpose the glucuronidase phenotypes of a large number of inbred strains of the house mouse have been studied.

Inbred mice were chosen as the subjects for several reasons: (1) a large number of inbred strains of independent origins exist, thus providing the necessary genetic diversity among which variants could be expected to occur; (2) if a new variant is discovered, a genetically uniform stock is immediately available; (3) mice are large enough to provide adequate quantities of tissue for biochemical

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studies; and (4) the genetic linkage groups of *Mus musculus* have been well characterized and strains are now available for the rapid determination of the linkage relationships of genes. The phenotypic characters of glucuronidase chosen as most likely to reveal new genetic variants were the tissue concentrations in liver, spleen, and kidney; the intracellular location in liver; and the primary protein structure, as reflected by the heat lability and electrophoretic mobility of enzyme activity. Based upon these characteristics, the results suggest that three, and possibly four, distinct phenotypes are present among inbred strains.

MATERIALS AND METHODS

Strains of inbred mice: Animals used in this study belonged to various inbred strains of the house mouse, *Mus musculus*. Strains are designated according to the Third Listing of the Standardized Nomenclature for Inbred Strains of Mice (STAATS 1964). Table 1 lists the sources of strains used in this study. Each strain is at least ten generations distant from a common ancestor of any other strain.

Preparation of tissue homogenates: Mice were killed by cervical dislocation, and 5% (w/v) homogenates of excised tissue were prepared in cold 0.25 M sucrose, buffered at pH 7.4 with 0.02 M imidazole-HCl, using a Potter-Elvehjem type homogenizer. The homogenates were filtered through four layers of gauze to remove unbroken cells and tissue debris. All necessary dilutions of the homogenates were made with 0.25 M sucrose, buffered at pH 7.4 with 0.02 M imidazole-HCl.

Osmotic shock technique: Homogenates were centrifuged at $105,000 \times g$ for 30 min in a Spinco No. 40 rotor to separate particulate from soluble enzymes. The supernatant was removed and the sediment resuspended in hypotonic buffer (0.02 M imidazole-HCl, pH 7.4) in order to solubilize lysosomal enzyme. The osmotically released enzyme was then separated from osmotically resistant enzyme by centrifuging the resuspended particles at $105,000 \times g$ for 30 min. Enzyme activities of the first supernatant and the final supernatant and sediment were determined.

Assay of β -glucuronidase activity: Glucuronidase (I.U.B. 3.2.1.31) was assayed by a modification of the procedure of NIMMO-SMITH (1961) employing the substrate *p*-nitrophenyl glucuronide (Cyclo Chemical Co.). Equal volumes of enzyme extract and substrate solution were mixed. The substrate solution contained 0.002 M *p*-nitrophenyl glucuronide, 0.2 M acetate buffer, pH 4.6, and 0.2% of the surface active agent Triton X-100 (ROHM and HAAS). The latter was added to insure complete activation of latent lysosomal enzymes (WATTIAUX and DE DUVE, 1956). The enzyme-substrate mixture was then incubated at 56°C for appropriate times and the reaction stopped by placing the tubes in a boiling water bath for 3 min. They were then cooled and the protein precipitate was centrifuged down. The absorbance of the supernatant was determined at a wavelength of 415 m μ before and after the addition of approximately 30 μ l of a 7 M solution of the base, 2-amino-2-methyl-1:3 propane diol, which ionized the liberated *p*-nitrophenol. The liberation of *p*-nitrophenol was a linear function of both enzyme concentration and time up to four hours, the limit tested. Results are expressed as μ M of *p*-nitrophenol liberated per hour at 56°C.

Heat inactivation of glucuronidase: Aliquots of 0.5 ml of the enzyme sample were added to 0.25 ml of 0.3 M acetate buffer (pH 4.6) in separate tubes and warmed at 56°C. The tubes were then held in a water bath at 71°C for periods up to 60 min after which they were chilled and the surviving enzyme activity measured.

Electrophoresis of glucuronidase: Five percent liver homogenates prepared in 0.1 M acetate buffer (pH 4.6) were incubated for 2.5 hours at 56°C, a procedure which solubilizes 90% of the glucuronidase activity. Suspended solids were removed by highspeed centrifugation ($105,000 \times g$ for 30 minutes). The sediment was discarded and the supernatant was dialyzed twice against 20 volumes of distilled water in order to reduce the ionic strength. 150 μ l of the dialyzed extract were subjected to electrophoresis in a polyacrylamide-gel column according to

the method of DAVIS (1964). This utilizes a glycine buffer at pH 9.5 and a constant current of 5 ma per 0.5 cm diameter gel during a 30 min run. The gels were then stained for glucuronidase by the simultaneous-coupling azo dye method of HAYASHI, NAKAJIMA and FISHMAN (1964), which results in an intense, nondiffusing red band in regions of glucuronidase activity after incubation of the gels in a solution containing 2.5×10^{-4} M naphthol AS-BI glucuronide and 1.8×10^{-3} M diazotized pararosaniline. Stained gels could be stored indefinitely in 7% acetic acid. Among other electrophoretic systems tested were the polyacrylamide gel systems of ALLEN and GOCKERMAN (1964), which uses a histidine-HCl buffer at pH 7.5; CLARKE (1964), which utilizes a TRIS-glycine buffer at pH 8.1; and WILLIAMS and REISFIELD (1964), which uses a TRIS-phosphate buffer at pH 7.5 in the gels and a TRIS-barbital electrode buffer at pH 7.0.

Presentation of data: The results for each strain are presented in Table 1. Mean values for quantitative characters are presented, and since only three animals of each strain were tested, the variability is expressed as one-half of the range. In the case of DBA/2 and C3H/HeHa, where larger numbers of animals were examined, the variability is expressed as the standard deviation from the mean.

RESULTS

Liver enzyme levels: Glucuronidase specific activity of livers from three males of each strain was determined, using DBA/2 and C3H/HeHa males as standards in each set of determinations. The results are presented in Table 1.

Of the strains examined, 55 out of 63, including DBA/2, are considered to have high enzyme activities. Their mean specific activities ranged from 38 to 102 units per gram. As shown on the abscissa of Figure 1, this group is clearly distinguished from six other strains which exhibit mean levels between 3 and 6 activity units (AU). The low activity group includes several C3H sublines, CBA/J, and AKR. One strain, YBR, had average liver enzyme levels of 24 AU, and is intermediate between the high and low activity groups. As shall be shown, this strain is unique in other respects as well.

Several families of ancestrally related strains are included in our sample. In the C3H family, 6 of the 13 strains display mean liver glucuronidase levels between 50 and 75 AU, and six others have uniformly low average levels (3 to 5 AU). However, the C3H/Bi strain includes representatives of both groups. The three animals examined showed values of 3, 31 and 34 AU. The A family of strains shows remarkably similar average enzyme levels, although one of the strains (A/HeJ) does exhibit marked differences among individual animals.

PAIGEN and NOELL (1961) have performed liver activity determinations on 19 of the 63 strains herein examined, and the review by FISHMAN (1955) includes data on 16 of the strains tested in the present study. A comparison of values from these studies with those of Table 2 reveals no major differences. However, within that group of animals in Table 1 which have high liver glucuronidase levels (38 to 102 AU), the relative enzyme levels do not always correspond to the relative levels of the same strains reported in earlier studies. This is not too surprising in view of the small numbers of animals used in both this and the previous studies.

Kidney enzyme levels: Specific activity values for kidney glucuronidase from males of each inbred mouse strain are presented in Table 1. Extremes are represented by the high activity of DBA/2 (mean = 392; S.D. = 124), which is more

TABLE 1

Type, tissue levels, and intracellular localization of glucuronidase in 63 strains of inbred mice

Strain*	Source†	ENZYME TYPE		TISSUE LEVELS OF GLUCURONIDASE						DISTRIBUTION INDEX‡	
		Heat inactivation‡	Electrophoresis§	LIVER		KIDNEY		SPLEEN		Mean	d/2
A/Ha	Ha	A	SF	49	4	114	71	45	18	0.05	0.04
A/HeJ	JAX	A	SF	46	14	78	40	60	12	0.00	0.04
A/J	JAX	A	SF	49	7	55	12	66	8	0.06	0.04
A/St	Mann	A	SF	46	2	65	12	70	10	-0.01	0.07
DBA/2 (11)	Ha	D	SF	77	SD=16	392	SD=124	58	SD=18	0.17	SD=0.06
BALB/cJ	JAX	D	SF	63	9	117	49	54	12	0.19	0.05
C	St	D	SF	59	6	27	8	91	36	0.34	0.17
CBA/St	Mann	D	SF	58	6	74	63	55	3	0.14	0.04
C3HB(2)	St	D	SF	51	4	162	3	57	6	0.07	0.04
C3H/Bi(2)	Kp	D	SF	33	2	25	2	43	3	0.19	0.01
C3H/St	Mann	D	SF	63	4	153	51	47	10	0.12	0.02
CHI	Mann	D	SF	54	8	105	14	60	22	0.09	0.02
C3H/ HeHa(11)	Ha	C	S	5	SD=1	15	SD=5	24	SD=3	0.43	SD=0.06
CBA/J(2)	JAX	C	S	4	0	13	6	21	1	0.45	0.02
C3H/Bi(1)	Kp	C	S	3	.	9	.	20	.	0.47	.
C3H/HeJ	JAX	C	S	5	0	15	7	20	0	0.40	0.06
C3Hp/Ha	Ha	C	S	5	1	35	2	37	16	0.41	0.12
C3H/StHa	Ha	C	S	3	0	20	10	20	10	0.66	0.08
Poly 1	St	D	SF	74	4	76	12	58	7	0.26	0.04
Poly 2	St	D	SF	66	2	29	4	54	9	0.18	0.04
Poly 4	St	D	SF	82	7	53	22	71	10	0.22	0.06
Poly 5	St	D	SF	56	3	45	15	45	10	0.32	0.04
Poly 6	St	D	SF	65	16	24	6	77	14	0.12	0.10
Poly 101	St	A	SF	67	16	27	3	108	14	0.21	0.06
Poly 201	St	D	SF	66	9	56	20	74	20	0.32	0.06
Poly 501(1)	St	A	SF	64	.	84	.	102	.	0.15	.
Al	Mann	D	SF	51	6	63	28	47	2	0.29	0.18
C57BL	Ha	D	SF	87	10	24	4	58	4	0.26	0.02
C58	Mann	D	SF	85	20	30	8	100	13	0.20	0.08
Na	Mann	D	SF	62	6	47	10	56	6	0.18	0.07
Op	Mann	D	SF	77	14	36	23	82	8	0.08	0.10
Ra	Mann	D	SF	82	7	28	6	50	8	0.20	0.14
PRUNT	St	D	SF	64	8	110	32	63	24	0.14	0.03
5-PRUNT(2)	St	A	SF	74	15	130	16	46	8	0.07	0.04
AKR	Ha	C	S	4	0	70	46	25	3	0.58	0.14
DA	St	D	SF	60	6	53	15	69	26	0.16	0.06
L	St	A	SF	53	8	72	40	66	10	0.04	0.08
LaA	St	D	SF	64	11	54	38	76	6	0.12	0.02
LOW(2)	St	D	SF	83	7	34	10	109	22	0.20	0.04
LP/J	JAX	D	SF	39	6	68	8	99	5	0.14	0.03
BRS(2)	Mann	D	SF	88	9	113	75	52	3	0.02	0.03
BRSUNT	St	D	SF	70	1	62	28	60	10	0.22	0.09
FZ	Mann	D	SF	102	16	88	58	75	22	0.37	0.06
H	St	D	SF	65	6	33	10	64	10	0.10	0.06

Strain*	Source†	ENZYME TYPE		TISSUE LEVELS OF GLUCURONIDASE‡						DISTRIBUTION INDEX¶	
		Heat inactivation‡	Electrophoresis§	LIVER		KIDNEY		SPLEEN		Mean	d/2
I(2)	Mann	D	SF	80	6	56	.	93	6	0.11	0.06
IPBR	Mann	D	SF	50	8	52	4	76	14	0.07	0.12
JK	St	D	SF	63	9	93	68	84	11	0.11	0.10
PBR	Mann	D	SF	58	4	101	52	58	10	0.10	0.08
BDP/J	JAX	D	SF	50	4	46	14	95	16	0.23	0.06
P/J	JAX	D	SF	51	4	113	8	96	21	0.26	0.10
CE/J	JAX	D	SF	69	10	36	4	43	11	0.14	0.07
DE/J	JAX	D	SF	42	5	78	20	52	2	0.12	0.10
129/J	JAX	D	SF	72	10	27	5	102	6	0.31	0.04
129/RrJ	Ha	D	SF	61	6	33	10	110	46	0.32	0.02
MA/J	JAX	D	SF	55	6	164	58	66	11	0.15	0.10
PIN	Mann	D	SF	52	2	125	22	64	19	0.14	0.04
PL/J	JAX	D	SF	62	12	47	1	95	16	0.17	0.16
RF/J	JAX	D	SF	64	6	178	45	85	3	0.27	0.04
SJL/J(2)	JAX	D	SF	48	4	33	6	57	28	0.14	0.01
SM/J	JAX	D	SF	70	4	51	16	80	9	0.36	0.03
ST/J	JAX	D	SF	40	6	21	2	60	2	0.16	0.10
SWR/J	JAX	A	SF	51	2	48	15	69	2	0.25	0.04
Y/Wi	Mann	D	SF	58	6	62	27	72	5	0.25	0.10
YBR(4)	Ha	A	F	24	8	106	48	45	12	0.82	0.02

*Strains are arranged in ancestrally related groups. Those strains with no known relationship to any other tested strain are arranged alphabetically in the last group. All animals tested were between 90 and 120 days of age.

†Key to symbols: Ha—Dr. THEODORE HAUSCHKA, Roswell Park Mem. Inst., Buffalo, N.Y.; JAX—Jackson Mem. Lab., Bar Harbor, Me.; Kp—Dr. HILARY KOPROWSKI, The Wistar Inst., Philadelphia, Pa.; Mann—Dr. STANLEY MANN, Biological Station, Roswell Park Mem. Inst., Springville, N.Y.; St—Dr. LEONELL STRONG, Biological Station, Roswell Park Mem. Inst., Springville, N.Y.

‡Symbols specify which one of the three inactivation profiles of Figure 4 most closely resembles that of the indicated strain. Strains resembling A/J profiles are designated by the symbol A, those like C3H/HeHa by C, and those like DBA/2 by D.

§The staining pattern of liver glucuronidase activity after polyacrylamide gel electrophoresis (pH 9.5) is indicated by the symbols S and F. SF refers to the presence of both slow and fast moving activity zones shown in Figure 5, S to the presence of only the slow zone, and F to the presence of only the fast zone.

||Mean levels of glucuronidase activity for the indicated tissues of a strain are presented in terms of μM of *p*-nitrophenol liberated per g wet weight of tissue per hour at 56°C. Variability of individual animals within a strain is indicated by d/2, which is one-half the difference between the highest and lowest values obtained. If no value is given for d/2, only one animal was examined. Variability of C3H/HeHa and DBA/2 animals is expressed in terms of the standard deviation (SD) from the mean since a larger number of animals were examined. Unless indicated in parenthesis next to the strain designation, values were obtained for 3 animals of each strain.

¶The distribution index is used to portray the relative amounts of glucuronidase in lysosomes and ergastoplasm of liver cells. This index is defined as the total lysosomal activity minus total microsomal activity divided by the total particulate activity. Mean values for three animals are presented as well as one-half the difference between the highest and lowest values obtained (d/2).

than twice the average value of any other strain, and by the relatively low activity of animals of several sublines within the C3H family. However, in contrast to the discontinuous distribution of mean liver glucuronidase levels for the 63 strains, the distribution of kidney values, as represented on the ordinate of Figure 1, is continuous. Thus, the strains are not readily divided into high and low activity groupings for kidney.

FIGURE 1.—Comparison between liver and kidney glucuronidase levels. Values are plotted on a logarithmic scale in order to emphasize relative values.

However, Figure 1 also illustrates the fact that strains with high levels of liver activity (38 to 102 AU) generally have kidney glucuronidase levels in the high range, and that the opposite holds true for strains with low liver enzyme levels. The lowest levels of kidney activities, as in the case of liver, are found among several strains of the C3H family. Furthermore, two C3H/Bi animals with high liver activity (34 and 31 AU) also have high kidney activity (23 and 26 AU) while the third animal, previously characterized as having a low level of liver enzyme (3 AU), is also low in kidney activity (9 AU). Beyond this generalization, there is no obvious correlation between liver and kidney activity, and even this generalization has several notable exceptions. The AKR strain, with a low liver specific activity (4 AU), has a relatively high average value for kidney (70 AU), and the YBR strain, which is distinctly intermediate with respect to liver activity, has a relatively high level of kidney activity (106 AU). As illustrated in Table 1, very large differences in kidney activity are commonly observed between males of a given strain as well as between strains. These variations between and within strains are probably due to hormonal effects since it has been demonstrated that androgens dramatically influence the level of kidney

FIGURE 2.—Comparison between liver and spleen glucuronidase levels. Values, obtained from Table 1, are plotted on a logarithmic scale.

glucuronidase activity (FISHMAN and FARMELANT 1953; PETTENGILL and FISHMAN 1962; PLAUT and FISHMAN 1963).

Spleen enzyme levels: Specific activity values for spleen glucuronidase of each inbred mouse strain are presented in Table 1. As shown in Figure 2, the distribution of these values is similar to that of liver. Strains with high liver glucuronidase levels have high levels of spleen enzyme, while strains with the lowest liver levels have the lowest spleen levels. The range of spleen specific activity values for high activity strains is very similar to the range for liver values. However, the low activity strains have a 5- to 10-fold greater concentration of glucuronidase activity in spleen than in liver. This difference is due to a failure of liver glucuronidase to accumulate after the twelfth postnatal day in animals with low adult liver levels, whereas spleen activity accumulates throughout the entire postnatal period of spleen growth (PAIGEN 1961b).

Intracellular distribution: As a measure of the relative amounts of glucuronidase localized in the lysosomes and in the ergastoplasm of liver cells, cytoplasmic particle fractions of liver homogenates were osmotically shocked in order to solubilize the lysosomal glucuronidase. Subsequently, the solubilized and residual

particulate glucuronidase activities were separated centrifugally and determined. The distribution index, which is defined as the total lysosomal activity minus total microsomal activity divided by the total particulate activity, was calculated for animals of each strain. An equal distribution of enzyme between lysosomes and ergastoplasm results in an index of zero. An index above zero reflects an excess of lysosomal enzyme. Strains with high liver glucuronidase levels have low distribution indices in the range -0.01 to 0.37 (Table 1, Figure 3). Among the high strains, the A family is characterized by uniformly low distribution indices (-0.01 to 0.06). Conversely, all of the strains which exhibited low liver enzyme levels showed high distribution indices (0.41 to 0.66). This extends the earlier observation in C3H/HeHa that animals with low liver activity have a greater proportion of enzyme localized in the lysosomes than do high activity strains. In obvious contrast to all others was the YBR strain with an approximate distribution index of 0.82 , an indication that YBR liver activity resides primarily in the lysosomes with very little, if any, ergastoplasmic enzyme present.

Heat inactivation kinetics: C3H/He enzyme is inactivated at 71°C several times as rapidly as is DBA/2 enzyme (PAIGEN 1961a), suggesting a difference in primary structure. The heat inactivation kinetics of liver glucuronidase activity from each of the inbred strains studied were compared with those of DBA/2 and C3H/HeHa. Most strains showed inactivation curves indistinguishable from that given by one of these two strains. However, several mouse strains, including all members of the A family, YBR, L, SWR/J, 5-PRUNT, Poly 101, and Poly 501, gave a slightly different result. Typical data illustrating each of the three inacti-

FIGURE 3.—Comparison between concentration and intracellular distribution of liver glucuronidase. Values for liver activities are plotted semi-logarithmically against the distribution indices.

FIGURE 4.—Heat-inactivation of liver glucuronidase. The survival of enzyme from DBA/2 (○—○), C3H/HeHa (●—●), and A/J (▲—▲) is plotted as a function of the duration of exposure to heating at 71°C. Activities are expressed as percent of initial activity present in each case.

vation curves are shown in Figure 4. Experimentally, there was no ambiguity in assigning each strain to one of these classes. These results are listed in Column 3 of Table 1.

The results with DBA/2 contrast with the unimolecular inactivation kinetics found previously (PAIGEN 1961a). It has not been possible to demonstrate the basis for this difference. The present results suggest that more than one enzyme component may be present in strains exhibiting inactivation kinetics similar to DBA/2.

Electrophoresis: The search for variants of the primary structure of glucuronidase also included a comparison of the electrophoretic mobilities of liver glucuronidase. Rat liver enzyme was found to be a suitable distance standard. Preliminary experiments showed that both mouse and rat enzyme separated into two zones after electrophoresis in the polyacrylamide gel system (TRIS-glycine buffer, pH 9.5) of DAVIS (1964). However, enzyme from rat liver migrates toward the anode at a slower rate than does mouse enzyme. When run together, the "fast" zone of rat enzyme is continuous with the "slow" zone of mouse enzyme, as shown in Figure 5. Samples of enzyme from each strain were subjected to electrophoresis in the presence and in the absence of rat enzyme, and the gels were subsequently stained for glucuronidase activity with naphthol-AS-BI-glucuronide. Each set of enzyme samples also included a DBA/2 representative

FIGURE 5.—Disc electrophoresis (pH 9.5) of liver glucuronidase.

as a standard against which the mobility of enzyme from all other mouse strains was compared.

Other electrophoretic systems tested (see MATERIALS AND METHODS) did not prove useful and gave a single, diffuse enzyme band. However, on two occasions the ALLEN and GOCKERMAN method succeeded in resolving the broad smear of DBA/2 liver enzyme activity into five distinct components.

The electrophoretic mobility of enzyme from high activity strains was indistinguishable from that of DBA/2. Strains with low liver activity showed only the slower migrating zone (Figure 5) and the YBR strain gave only the fast zone. Mixtures of C3H/HeHa and YBR enzyme, as well as liver enzyme from F_1 animals obtained by crossing C3H and YBR, gave both zones of activity (Figure 6).

The correlation between heat-inactivation classes and electrophoretic mobility was not complete. All strains exhibiting heat-inactivation kinetics similar to those of C3H/HeHa showed only the S component, and strains resembling DBA/2 exhibited both the S and F components. However, most strains with heat-inactivation kinetics like those of A/J also showed both S and F components, with the exception of YBR which contained only the F enzyme component.

DISCUSSION

Phenotypic classes: The inbred mouse strains we have surveyed are naturally divisible into several types (Table 2). *Type I* includes most of the strains examined; it is characterized by (a) multicomponent inactivation kinetics of liver glucuronidase activity at 71°C; (b) liver glucuronidase which resolves into two zones after polyacrylamide gel electrophoresis at pH 9.5; (c) relatively high

FIGURE 6.—Disc electrophoresis (pH 9.5) of mouse liver glucuronidase.

levels of liver, kidney, and spleen glucuronidase activity; and (d) relatively small differences in glucuronidase activity between lysosomes and ergastoplasm of liver (i.e., a distribution index generally between 0.2 and 0.4). *Type II* includes most sublines of the C3H strain and the CBA/J and AKR strains, all of which display (a) rapid inactivation of glucuronidase at 71°C; (b) a single slowly migrating zone of glucuronidase activity in electrophoresis; (c) low liver specific activity; and (d) a relative excess of lysosomal over ergastoplasmic enzyme (distribution indices above 0.40). It is possible to distinguish a third phenotype exemplified by those strains with liver, spleen, and kidney enzyme levels similar to *Type I*, but with distinctly lower mean distribution indices (below 0.1) and enzyme which decays at a slow, constant rate at 71°C. However, it is difficult to say whether this is really a distinct type or only a subpopulation of *Type I*.

TABLE 2

Glucuronidase phenotypes among inbred mouse strains

Liver glucuronidase characteristics	Type I strains*	Type II strains	YBR strain
Heat inactivation kinetics at 71°C	Multicomponent	Rapid, single component	Single component
Pattern after disc electrophoresis at pH 9.5	Two zones	One zone corresponding to slow zone of Type I	One zone corresponding to fast zone of Type I
Enzyme level	High	Low	Intermediate
Mean intracellular distribution indices†	0.2 to 0.4	0.4 to 0.6	~0.8

* It is possible to distinguish an additional phenotype exemplified by those strains with liver, kidney and spleen enzyme levels similar to *Type I*, but with single component heat-inactivation kinetics at 71°C and distinctly lower mean distribution indices (below 0.1). It is not possible at the present time to state whether this is a distinct type or only a subpopulation of *Type I*.

† The distribution index is defined as the total lysosomal activity minus total microsomal activity divided by the total particulate activity.

One of the strains examined is distinctly different from all of the others. The YBR strain exhibits (a) a level of liver glucuronidase intermediate between *Types I* and *II*; (b) a very high distribution index suggesting that little, if any, enzyme is contained in the ergastoplasm; and (c) a single fast zone of glucuronidase after polyacrylamide gel electrophoresis. Genetic and biochemical analyses of this variant have been performed and the results show that a mutation, unlinked to the glucuronidase structural gene, produces this phenotype (GANSCHOW and PAIGEN 1967). The mutant gene is responsible for integration of glucuronidase into the ergastoplasm of liver cells and does not appear to affect other ergastoplasmic proteins or the integration of glucuronidase into lysosomes. The results suggest that the intracellular localization of enzymes may be controlled by factors other than the primary structure of the enzyme.

These results demonstrate the feasibility of surveying for new biochemical variants by searching among inbred mouse strains. It is clear that three, and possibly four, distinct glucuronidase phenotypes are present among inbred mice. Two have been previously described and are characterized by DBA/2 (*Type I*) and C3H/HeHa (*Type II*). The sublines of the A strain may represent a third phenotype, and at least one new glucuronidase phenotype, that of the YBR strain, has been uncovered.

Enzyme components: There is no suggestion that the existence of two electrophoretic bands is related to the presence of enzyme at two intracellular sites. Both the C3H/HeHa and YBR strains contain predominantly lysosomal enzyme, but C3H exhibits only the slowly migrating band whereas YBR shows only the more rapidly migrating band.

The results of electrophoresis of glucuronidase at pH 9.5 suggest that either two electrophoretically distinct forms of glucuronidase exist in mouse liver or that mouse liver glucuronidase activity is the result of an association between two subunits which are dissociated and resolved under the alkaline conditions of electrophoresis. For the second interpretation to be correct, it is necessary that subunits reassociate into active enzyme during the subsequent staining for enzyme activity in the gel at acid pH.

Genetic relationships: The present study provides some additional information on glucuronidase polymorphism among the C family of inbred strains. Figure 7 illustrates the derivation and relationships of the strains within this family. C3H/St (PAIGEN and NOELL 1961), several sublines of C3H/Bi (HAMER 1958), and CBA/An (MORROW *et al.* 1950) have been found to exhibit high levels of liver glucuronidase, corresponding to strains of *Type I* in the present study, whereas C3H/An (MORROW *et al.* 1950), C3H/Bi (MORROW *et al.* 1950), and several other sublines of C3H/Bi (HAMER 1958) display lower levels of liver activity, similar to strains in *Type II*. We find that all strains of the C family maintained in the laboratory of Dr. L. C. STRONG, who originated the C family, belong to *Type I*. With the exception of C3H/Bi, all other strains directly or indirectly derived from Dr. Strong's stock belong to *Type II*. The suggestion by HAMER (1958) that segregation for the low glucuronidase allele still occurs among

FIGURE 7.—Genealogy of the C family of inbred mouse strains.

animals of the C3H/Bi strain is supported by the observation that one of three C3H/Bi animals in the present study had low liver activity (as well as a high distribution index and a heat-labile enzyme), whereas the activity of the other two animals was approximately 10-fold greater.

Considering these facts, the most likely explanation for the polymorphism of glucuronidase expression among strains of the C family is that the mutation for low liver activity has been maintained in the heterozygous condition in C3H/St, C3H/Bi, and CBA/St strains, and that the mutant allele has been fixed in C3H/An, C3H/StHa, and CBA/J, which are derived directly from Dr. STRONG's stocks. It seems unlikely that independent mutations to low glucuronidase have occurred within several of the strains in the C family since no differences in glucuronidase structure are apparent among the low activity strains, as judged by the equality of heat-sensitivity and electrophoretic mobility. Nevertheless, independent mutation cannot be discounted completely since the AKR strain, with no apparent ancestral relationship to the C family, is a low glucuronidase strain exhibiting enzyme characteristics identical to the low activity strains of the C family.

Tissue modifiers: Among the strains belonging to each type, there is no obvious correlation of glucuronidase levels between liver, kidney, and spleen (Figures 1 and 2). For example, DBA/2, which has more than twice the average kidney enzyme value of any other strain, has only moderately high liver and spleen specific activities while AKR displays very low liver and spleen activities but a relatively high kidney level.

Furthermore, it is apparent that large interstrain differences exist with respect to activity levels of various tissues, even among strains where a glucuronidase of the same structure is formed as shown by heat-inactivation and electrophoresis. Consequently, genetic factors other than those determining enzyme structure have a significant effect upon glucuronidase activity.

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SUMMARY

Sixty-three inbred strains of mice (*Mus musculus*) were examined for genetic variants of β -glucuronidase using the following enzyme characters: (a) liver, kidney, and spleen concentration; (b) intracellular distribution in liver cells; (c) heat stability at 71°C; and (d) electrophoretic mobility of liver enzyme at pH 9.5. Strains divide into several distinct groups. The vast majority of strains (*Type I*) are characterized by relatively high levels of liver, kidney, and spleen glucuronidase; slightly greater glucuronidase activity in lysosomes than in ergastoplasm; and the presence of a heat stable enzyme which separates into two bands after electrophoresis. *Type II* strains exhibit low liver glucuronidase levels with approximately two-thirds of the liver enzyme localized in lysosomes; and the presence of a thermo-labile enzyme with a single zone of activity after electrophoresis which corresponds to the slow component of *Type I*. One strain (YBR) is clearly distinguished from *Types I* and *II*. It exhibits an intermediate level of liver glucuronidase with little, if any, ergastoplasmic enzyme, and a single zone after electrophoresis which corresponds to the fast component of *Type I*. The screening of inbred mouse strains has proved to be a feasible method for discovering genetically determined biochemical variants. The present results indicate that genetic factors other than those specifying enzyme structure have a significant role in determining the enzyme phenotype.

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