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Strain variation in spermatozoal β-glucuronidase in mice

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SUMMARY

Variation in spermatozoal β-glucuronidase levels of inbred strains of mice was found to parallel the known variation usually studied in liver with one outstanding exception: BALB/cJ spermatozoa, but not other tissues, showed an anomalously low level. This difference in spermatozoal β-glucuronidase activity was studied in hybrids and backcrosses but the data did not establish the number of loci affecting the difference. The levels of β-glucuronidase in F_1's between C3H/HeJ and A/J depended markedly on the sex direction of the cross.

1. INTRODUCTION

Studies on the biochemical genetics of β-glucuronidase in mice have been highly fruitful. The original variant is a heat-labile form with about 5% of standard activity in livers of homozygous strains, *Gus*^{h} (Morrow, Greenspan & Carroll, 1949). An 'architectural' mutant causing a deficiency of β-glucuronidase in the microsomal compartment acts by a failure in synthesis or attachment of a polypeptide found on the microsomal components of β-glucuronidase (Swank & Paigen, 1973). Electrophoretic variants exist among the high liver β-glucuronidase activity strains; these change the mobility of both the lysosomal and microsomal components (Lalley & Shows, 1974). Finally, the first well-established cis-acting, regulator-type mutation in eukaryotes is a locus (*Gur*) closely linked to the structural locus (*Gus*) which affects the induction of β-glucuronidase by dihydrotestosterone in kidneys (Swank, Paigen & Ganschow, 1973). The high level or low level (of induction) allele affects only the structural allele to which it is *cis*. An anomalous variation in kidney levels has been described in C57BL and DBA/2 substrains (Lundin & Hakansson, 1973).

Most of the above work has concerned the parenchymal organs, liver or kidney, although developmental curves have been studied in other organs, e.g. brain (Meisler & Paigen, 1972). An interest in the mode of expression of genes in spermatozoa (Erickson, 1974) has led to this study on the genetics of β-glucuronidase in spermatozoa. There have been few observations on the enzyme in spermatozoa although it has been studied histochemically in the testes of amphibia (Varute, 1971) and been reported in the midpiece of mouse spermatozoa (Mathur, 1971).

2. MATERIALS AND METHODS

(i) *Mice*

Inbred mice from the Jackson Laboratory, Bar Harbor, Maine, were kept in a room with controlled lighting (14 h light, 10 h dark). Shavings were used as bedding. The female parent is written first in the symbolization of hybrids.

(ii) *Preparation of tissue extracts*

Spermatozoa from the epididymides and ducti deferentes of 8-week or older animals were harvested by slicing these tissues to about 1 mm pieces, which were left in a solution of 0·2 M glucose, 0·05 M-NaCl, 0·02 M-Na_2HPO_4, and 0·0026 M-KH_2PO_4 for 5–10 min. The majority of tissue fragments were removed by passing the suspension through a no. 16 screen, the washes bringing the final volume to about 7 ml. Ten minutes at 1 g were allowed for smaller tissue fragments to settle out and the supernatant was transferred to a centrifuge tube and centrifuged at 700 *g* for 10 min at 4 °C. The pellet was resuspended in a similar volume and the centrifugation repeated. These sperm were then lysed by freeze-thawing in 0·25 ml distilled water and the solution cleared by centrifugation at 2500 *g* for 20 min. Tests showed that the specific activity of β-glucuronidase in the pellet remained stable after one washing and that Triton X-100 did not release significantly more β-glucuronidase than did the lysis procedure. Homogenates, 10% wt/vol., of other tissues were prepared in distilled water and the extracts cleared by centrifugation as above.

(iii) *β-Glucuronidase assay*

β-Glucuronidase was assayed at 37 °C. using 10^{-3} M *p*-nitrophenol-β-glucuronide (Sigma Chemical Co.), 0·2 M, pH 4·6 Na acetate buffer. Incubations were usually for 1 h, an equal vol. of 0·2 M glycine-NaOH, pH 10·0, was added, and the OD_{420} was read. Preliminary experiments showed that the assay was linear with time and amount of added extract for the ranges used. Protein determinations were performed by the method of Lowry. All statistical calculations utilize one enzyme activity value per mouse (the average of several separate determinations).

3. RESULTS

(i) *Strain variation in β-glucuronidase activity*

The specific activity of spermatozoal β-glucuronidase was measured in a number of inbred strains (Table 1). Spermatozoal glucuronidase activity was greater than 0·1 nmol/h/μg in all strains of mice but two (BALB/c and C3H), and variations among these 'high' sperm β-glucuronidase activity strains was not significant by Student's *t* test and did not correlate with *Gus* and *Gur* genotypes. The low activity of the Gus^h/Gus^h strain, C3H/HeJ, was reflected in spermatozoa. BALB/cJ stood out among the other inbred strains for having the lowest spermatozoal level while the liver level was comparable to that of other Gus^a/Gus^a or Gus^b/Gus^b strains. The difference was statistically significant by Student's *t* test ($P < 0·01$) for BALB/cJ against A/J; but BALB/cJ was not statistically different from C57*L*/J or AU/SsJ.

(ii) *Genetics of BALB/cJ spermatozoal β-glucuronidase*

We sought to elucidate the factor(s) which determined this lower level of spermatozoal β-glucuronidase in the BALB/cJ strain, using the A/J strain as a convenient high activity control. Of the tissues examined, the low specific activity of β-glucuronidase was limited to spermatozoa. As seen in Table 2, liver, kidney, testes and coagulating gland of BALB/cJ gave higher specific activities than did the same tissues in the A/J strains, while BALB/cJ spleen showed a lower, but not significantly lower, activity than did A/J spleen.

Table 1. *Strain specific variation in spermatozoal, compared to liver, β-glucuronidase*

Strain	*Gus** genotype	*Gur*† genotype	β-Glucuronidase: Spermatozoa,‡ 37 °C (nmol/h/μg prot.)	β-Glucuronidase: Liver§ 56 °C (nmol/h/μg wet wt)
C57BL/6J	*b/b*	*b/b*	(5) 0·205 ± 0·020	0·087
C57L/J	*b/b*	—	(7) 0·101 ± 0·024	—
DBA/2J	*b/b*	*b/b*	(5) 0·143 ± 0·023	0·077; S.D. 0·016
SWR/J	*b/b*	*b/b*	(5) 0·276 ± 0·067	0·051
AU/SsJ	*b/b*	*a/a*	(3) 0·104 ± 0·027	—
A/J	*a/a*	*a/a*	(5) 0·173 ± 0·021	0·049
SM/J	*a/a*	*a/a*	(3) 0·255 ± 0·077	0·070
BALB/cJ	*a/a*	*a/a*	(7) 0·055 ± 0·014	0·063
C3H/HeJ	*h/h*	—	(5) 0·011 ± 0·006	0·005; S.D. 0·001

* Lalley & Shows (1974).

† Swank, Paigen & Ganschow (1973).

‡ (Number of mice) mean ± standard error.

§ Mean of three mice, standard deviation (S.D.) given in two cases, from Ganschow & Paigen (1968).

Intercrosses and backcrosses were performed between BALB/cJ and A/J mice to investigate the genetic basis of the difference. The F_1's showed the expected intermediate values (Table 3). The two crosses by parental sex are given for reasons that will become apparent below. There was no significant difference between the two sex directions for the cross, and the backcross data (Table 4) are presented with data pooled from the eight possible sex direction backcrosses (based on the two direction intercrosses), since visual inspection of the data plotted separately for each backcross by the multiple sex directions did not suggest any trend of differences. As seen in Fig. 1, there is a suggestion of a trimodal distribution in both backcrosses. However, as tabulated in Table 4, the mean of the backcross to BALB/cJ is coincident with the F_1 mean, while the mean for the backcross to A/J falls between the F_1 and the parental values (and is significantly different from the other backcross, $t = 2{\cdot}41$, $P < 0{\cdot}02$). More than one locus and non-additive effects, as also suggested by the shift of the F_1 mean towards the A/J parental strain value, might be postulated but the data do not allow definitive conclusions.

Table 2. *β-Glucuronidase activity in various tissues of BALB/cJ and A/J mice*

Organ	BALB/cJ	nmol/h/μg protein probability of difference by Student's *t* test	A/J
Liver	(3) 0·141 ± 0·003[1]	< 0·05	(3) 0·096 ± 0·016
Spleen	(3) 0·078 ± 0·010	n.s.	(3) 0·134 ± 0·035
Kidney	(3) 0·102 ± 0·039	n.s.	(3) 0·033 ± 0·002
Testes	(3) 0·026 ± 0·009	n.s.	(3) 0·013 ± 0·002
Coagulating gland	(3) 0·147 ± 0·006	< 0·02	(3) 0·048 ± 0·023

* (Number of mice) mean ± standard error.

Table 3. *Spermatozoal β-glucuronidase in F_1's*

	nmol/h/μg protein	Student's *t* test
BALB/cJ × A/J		
(1) BALB/cJ (♀) × A/J (♂)	(8) 0·096 ± 0·012*	$t = 1{\cdot}80$, $0{\cdot}1 > P > 0{\cdot}05$
(2) A/J (♀) × BALB/CJ (♂)	(17) 0·138 ± 0·015	
C3H/HeJ × A/J		
(1) C3H/HeJ (♀) × A/J (♂)	(9) 0·149 ± 0·026	$t = 3{\cdot}19$, $P < 0{\cdot}01$
(2) A/J (♀) × C3H/HeJ (♂)	(7) 0·040 ± 0·010	

* (Number of animals) mean ± standard error.

Table 4. *Mean spermatozoal β-glucuronidase in parental F_1, and backcross mice for A/J, BALB/c*

	Mean ± standard error spermatozoal β-glucuronidase specific activities (nmol/h/μg protein)		
Parental ...	BALB/cJ 0·055 ± 0·014	—	AJ 0·173 ± 0·021
F_1 (both directions by sex of cross)	—	0·125 ± 0·012	—
Backcrosses (both directions by sex of cross)	To BALB/cJ 0·124 ± 0·007		To A/J 0·151 ± 0·008

(ii) *Genetics of C3H/HeJ spermatozoal β-glucuronidase*

The *Gus*^{h}/*Gus*^{a} hybrid of C3H/HeJ with A/J showed intermediate spermatozoal specific activities with a marked effect of the sex direction of the cross (Table 3). The spermatozoal β-glucuronidase activity was much greater when the high strain (A/J) was the father than when it was the mother. The sperm β-glucuronidase levels from small numbers of individuals from four of the eight possible sex-direction backcrosses were plotted and a clear distinction between maternal inheritance, *X*-linked, or *Y*-linked hypotheses was not seen. The pooled data for each of the two kinds of backcrosses supported the expected segregation of *Gus*^{a} and *Gus*^{h} alleles (Fig. 2). The backcross to A/J resulted in apparently equal groups above and below 0·16 nmol/h/μg protein (presumed *Gus*^{a}/*Gus*^{a} and *Gus*^{a}/*Gus*^{h}) while the backcross

to C3H/HeJ resulted in about equal groups below 0·08 nmol/h/μg protein (presumed *Gus*^{h}/*Gus*^{h} and in the 0·08–0·16 nmol/h/μg protein (presumed *Gus*^{a}/*Gus*^{h}) classes.

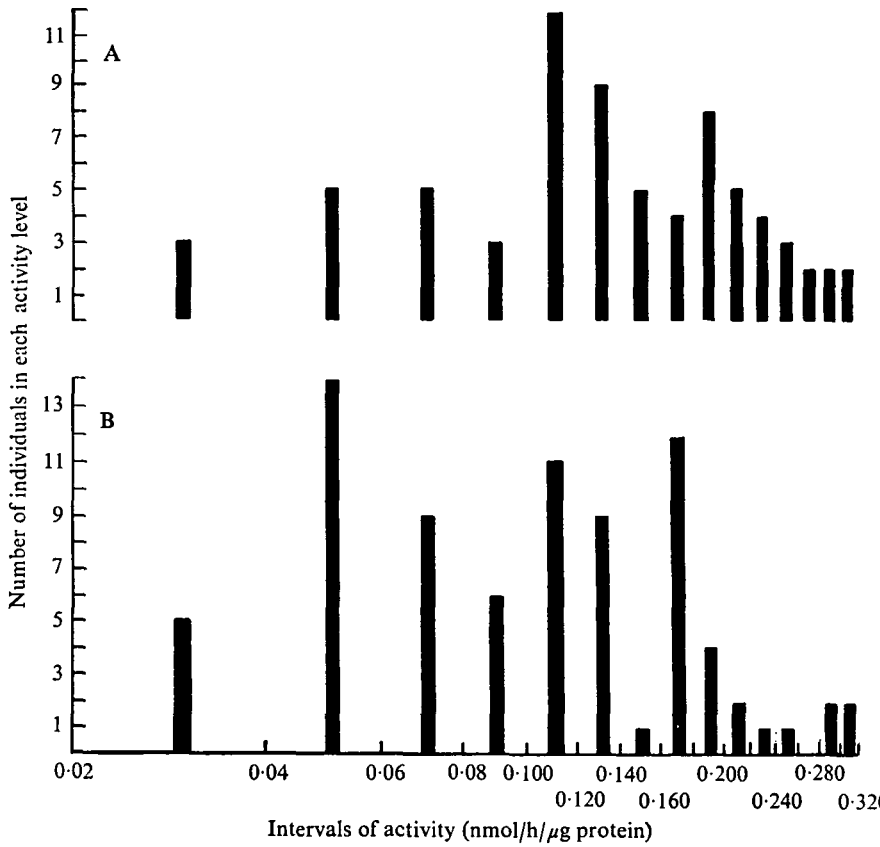

Fig. 1. Distribution of spermatozoal β-glucuronidase specific activities in individual mice from BALB/cJ × A/J F_1's backcrossed to A/J (panel A, 72 mice) or to BALB/cJ (panel B, 79 mice). Data from the various crosses by direction of sex are pooled.

4. DISCUSSION

As mentioned in the introduction, most of the biochemical genetics of β-glucuronidase in mice has been studied in a single tissue, liver, in which the variations are explicable by the effects of alleles at two unlinked loci, *Gus* and *Eg*. The first is the structural gene for the enzyme, while the second affects the distribution of the enzyme between subcellular organelles. When other tissues were examined, the *Gur* locus (closely linked to the *Gus* locus) affecting testosterone induction in kidney was found (Swank *et al.* 1973). Lundin & Hakansson (1973) found a Mendelian difference for kidney β-glucuronidase between the C57BL and DBA/2 strains which are thought to share identical *Gus* and *Gur* alleles (but substrain variation could explain the result). We have found further strain variations by examining another tissue, spermatozoa. In the survey of inbred strains for spermatozoal β-glucuronidase

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levels (Table 1) no correlation of the variable specific activities with *Gus* or *Gur* alleles was found. The strain with the lowest specific activity for a non-Gus^h strain, BALB/cJ was studied in detail but the fact that BALB/cJ shows unusual kinetics of induction of kidney β-glucuronidase by dihydrotestosterone, even though it is classified as Gur^a/Gur^a (Swank *et al.* 1973) might suggest the presence of a *Gur* allele differing from that in other strains, e.g. A/J. Thus it is possible that unique aspects of testosterone induction of β-glucuronidase in spermatozoa may explain the low spermatozoal β-glucuronidase in the BALB/cJ strain. Arguing against this interpretation is the finding that electrophoretograms of Gur^bGus^b/Gur^aGus^a spermatozoal β-glucuronidase did not show a shift of the mobility of the F_1 spermatozoal β-glucuronidase toward the Gur^a cis allele (Gus^a) as is observed in testosterone-induced kidney (Erickson, in preparation).

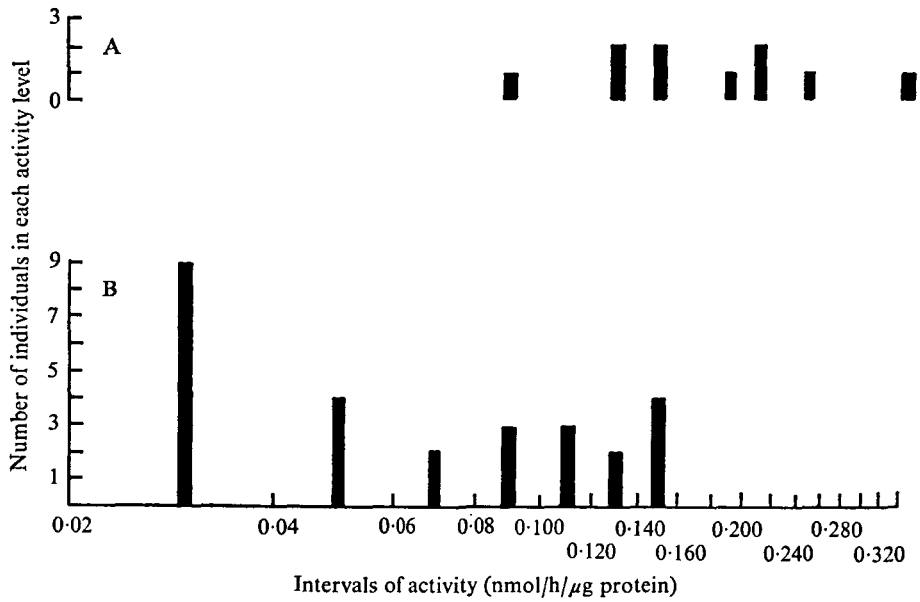

Fig. 2. Distribution of spermatozoal β-glucuronidase specific activities in individual mice from C3H/HeJ × A/J F_1's backcrossed to A/J (panel A) or C3H/HeJ (panel B). Data from the various crosses by direction of sex are pooled.

The BALB/cJ difference in β-glucuronidase levels, compared to other Gus^a/Gus^a strains was limited to spermatozoa among those tissues studied, but a complete survey was not made. The data from the F_1 and backcrosses between the BALB/cJ and A/J strains suggested the effects of more than one locus and non-additive effects, but no conclusions could be drawn. Since the Gus^a and Gus^b alleles did not correlate with the specific activity of spermatozoal β-glucuronidase, a segregating factor should be something other than *Gus*. Linkage studies were not performed so it is not possible to say whether a part of the difference might be an expression of BALB/cJ's postulated unusual Gur^a allele.

In a preliminary study of the mode of expression of the Gus^h allele in spermatozoa, a large difference was found in the specific activities of β-glucuronidase depending on the sex direction of the A/J, C3H/HeJ cross. This could be explained by *X*- or *Y*-linked factors or by a maternal effect – presumably on the developing male gonad during its fetal development. *H*-2-linked factors are known to affect androgen function (Ivanyi *et al.* 1973) while an autosomal locus of unknown linkage affects the kinetics of spermatogenesis (Bruce *et al.* 1973). Examination of spermatozoal β-glucuronidase specific activities for small numbers of individual mice in four of the eight possible sex direction backcrosses did not distinguish between the three possibilities. The expected segregation of the Gus^a and Gus^h alleles was seen in these backcrossed mice, further confirming that *Gus* is the structural gene for spermatozoal β-glucuronidase.

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