Genet. Res., Camb. (1978), **32**, *pp.* 183–193
With 2 text-figures
Printed in Great Britain

Strain variation in spermatozoal glycosidases in inbred mice

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(*Received* 15 *May* 1978)

SUMMARY

Ten glycosidases were measured in suspensions of spermatozoa from the vasa deferentia of two inbred mouse strains and their intercrosses. Eight of these glycosidases were associated with the sperm cells and all of these showed genetical variation between the strains except α-L-fucosidase with optimal activity at pH 5·4. In contrast liver enzyme activities showed no significant variation except α-L-fucosidase. Genetic studies indicated that the variation of spermatozoal β-D-hexosaminidase, α-D-mannosidase, α-L-fucosidase and β-D-galactosidase are inherited at autosomal loci and α-D-galactosidase variation shows *X*-linked inheritance. We propose a new provisional gene symbol (*Afuc-2*) for a spermatozoal variant of α-L-fucosidase.

1. INTRODUCTION

The biochemical genetics of several murine glycosidases have been extensively studied in the somatic tissues. Variants have been found for α-D-galactosidase (Lusis & West, 1976), β-D-galactosidase (Seyedyazdani & Lundin, 1973; Felton, Meisler & Paigen, 1974), β-D-glucuronidase (Morrow, Greenspan & Carroll, 1949; Swank & Paigen, 1973; Lalley & Shows, 1974; Swank, Paigen & Ganschow, 1973) and α-D-mannosidase (Dizik & Elliott, 1977). In contrast, little is known about the biochemical genetics of glycosidases associated with spermatozoa. Several glycosidase activities have been detected in murine spermatozoa cytochemically (Mathur, 1971) and strain variation has been shown for hyaluronidase (Erickson & Krzanowska, 1974) and β-D-glucuronidase (Erickson, 1976, 1977).

The mammalian male reproductive tract is a very rich source of glycosidase activities (Conchie & Mann, 1957; Conchie, Findlay & Levvy, 1959). There is considerable interest in the possible role of these enzymes in fertilization (Allison & Hartree, 1970). It has been shown that for some glycosidases distinct isoenzymes are associated with the spermatozoa (Zaneveld, Polakoski & Schumacher, 1973; Majumder & Turkington, 1974; Bullock, 1975), and that hormones affect the induction of these enzymes (Majumder, Lessin & Turkington, 1975).

This paper describes measurements of ten glycosidases in suspensions of

spermatozoa from the vasa deferentia of two inbred strains of mice and their intercrosses. The genetical basis of the observed differences between the strains is discussed and compared with enzymic activities in somatic tissue.

2. MATERIALS AND METHODS

(i) *Mice*

All mice were bred in the Specific Pathogen Free unit at Searle Research Laboratories, High Wycombe, Bucks., U.K. The female parent is written first in the notation of hybrids.

(ii) *Preparations of suspensions of spermatozoa from the vasa deferentia*

Adult male mice were killed by cervical dislocation. Each testis complete with epididymis and vas deferens was freed from the abdominal cavity but left attached to the urethra. It was placed on filter paper which was soaked in Dulbecco's phosphate-buffered saline (PBS). The blood vessel running along the length of the vas deferens was stripped away. The vas deferens was cannulated *in situ* at the distal end with a 15-gauge hypodermic needle attached by fine polythene tubing (Dural Plastics, New South Wales, Australia) to a plastic disposable syringe of 2 cm^3 capacity containing PBS. The needle was held in place by forceps. The cannulated vas deferens was then severed at the proximal end and detached from the urethra. It was held vertically and any adhering blood was rinsed off with PBS. Its contents were flushed out with 1 cm^3 of PBS as a stream of mucus into a 2·5 cm^3 capacity polystyrene tube (Luckhams, Sussex, U.K.). The contents of both vasa deferentia were pooled and left at room temperature for 5–10 min to allow the mucus to dissociate and dissolve, thereby releasing the spermatozoa. The concentration of spermatozoa was measured using a haemocytometer. Suspensions of spermatozoa were cooled on ice for 10–15 min before sonication for 15 sec in an MSE 100 Watt Ultrasonic Disintegrator with an amplitude of 8 μm peak to peak and at a frequency of approximately 20 kHz.

(iii) *Preparation of liver homogenate*

Homogenates (10 %, w/v) of liver were prepared in 10 mM-sodium phosphate buffer, pH 6·0, at 4 °C using a glass homogenizer. The crude homogenate was filtered through two layers of muslin to remove any unhomogenized tissue fragments.

(iv) *Glycosidase assays*

Glycosidase activities were measured using the appropriate 4-methylumbelliferyl glycoside (Koch–Light Laboratories Ltd., Colnbrook, Bucks., U.K.) as a fluorogenic substrate. The incubation mixture consisted of 200 μl of suitably diluted sonicated sperm suspension or liver homogenate and 200 μl of a 1 mM-solution of the appropriate substrate in 0·2 M-Na_2HPO_4 adjusted to the required pH with 0·1 M-citric acid. After incubation for 1–2 h at 37 °C the reaction was stopped by the addition of 2 cm^3 0·5 M-glycine adjusted to pH 10·4 with NaOH.

The fluorescence of the liberated 4-methylumbelliferone was measured in the flow-cell of a Locarte Fluorimeter Mk 2 with excitation at 340–380 nm (filter LF2) and emission above 450 nm (filter LF5). Quinine sulphate (4 μg cm^{-3}) in 0·05 M-H_2SO_4 was used as the primary fluorescence standard. Enzymic activity was calculated by reference to a standard curve for 4-methylumbelliferone. One unit of activity is that amount of enzyme that transforms 1 μmole of substrate per minute under the specified conditions. The optimum pH for each substrate was determined in the McIlvaine (1921) buffer system using sonicated suspensions of spermatozoa from BALB/c mice. Each assay procedure was validated for enzyme concentration and time. Assays were performed in triplicate.

(v) *Protein determination*

Protein was determined by the Folin method (Lowry *et al.* 1951) using bovine serum albumin as a standard.

(vi) *Histocompatibility typing*

BALB/c has haplotype *H-2^d* and B10.M is congenic with C57BL/10 ScSn with haplotype *H-2^f*. Antisera used to test lymph node cells of appropriate backcross mice for *H-2^d* or H-2^f by the chromium release cytotoxicity test of Wigzell (1965) and Sanderson (1965) were made by immunizing respectively B10.M mice with spleen cells from BALB/c and B10.D2 (congenic with C57BL/10 with haplotype *H-2^d*) with cells from B10.M. Positive controls from (B10.M × BALB/c) F_1 hybrids were tested on each occasion.

3. RESULTS

(i) *Correlation between the number of spermatozoa and the concentration of protein in the suspensions of spermatozoa*

Spermatozoa accounted for the great majority (always > 96 %) of the cells present in the material flushed out of the vasa deferentia. Other cells included epithelial cells, polymorphs and macrophages. The correlation between the number of spermatozoa and the protein concentration in suspensions of spermatozoa from BALB/c, (BALB/c × B10.M) F_1, (B10.M × BALB/c) F_1 and B10.M mice was computed (Table 1). This showed that the variation in protein concentration was related to the number of spermatozoa present. However, as the linear regression lines did not pass through zero some of the protein is not directly associated with the spermatozoa.

(ii) *pH-dependence of spermatozoal glycosidases in BALB/c mice*

The pH-dependence of the enzyme activity in sonicated suspensions of spermatozoa from the vasa deferentia of BALB/c mice towards eight 4-methylumbelliferyl substrates was investigated. A single pH optimum was observed for all the substrates except the α-L-fucoside, which had a major peak of activity optimal at pH 5·4 and a minor one at pH 3·5. All subsequent measurements of glycosidase

activities were made at these pH-optima with α-L-fucosidase being assayed at both pH 5·4 and pH 3·5. α-D-Mannosidase was also assayed at pH 6·4 because activity in the somatic tissues has pH-optima at pH 4·6 and pH 6·4. Although α-D-mannosidase and β-D-galactosidase activity with a neutral pH optimum are also usually present in mammalian somatic and reproductive tissues they were not detected in suspensions of spermatozoa.

Table 1. *Correlation between number of spermatozoa and protein concentration in suspensions of spermatozoa*

Strain	Correlation*
BALB/c	0·840 (17), $P < 0·001$
(BALB/c × B10.M)F_1	0·927 (10), $P < 0·001$
(B10.M × BALB/c)F_1	0·914 (12), $P < 0·001$
B10.M	0·669 (10), $P < 0·05$

* Correlation coefficient r (number of mice).

(iii) *Strain variation in glycosidase activities*

The specific glycosidase activities of suspensions of spermatozoa and liver homogenates from BALB/c and B10.M mice are given in Table 2. Significant differences ($P < 0·01$ or $P < 0·001$) between the strains were found for all the spermatozoal glycosidases except for α-L-fucosidase assayed at pH 5·4 ($P > 0·1$) and β-D-fucosidase ($0·02 > P > 0·01$). In contrast no differences were found between the strains for the liver enzymes except for α-L-fucosidase assayed at both pH 3·5 and pH 5·4.

(iv) *Association of glycosidase activities in suspensions of spermatozoa with number of sperm and non-sperm cells*

The correlation between the glycosidase activity and the number of sperm or non-sperm cells present in suspensions of spermatozoa was calculated. All the glycosidase activities measured correlate ($P < 0·001$) with the number of sperm cells except β-D-fucosidase and α-L-arabinosidase ($P > 0·1$) which appear to correlate with the non-sperm cells ($P < 0·001$).

(v) *Genetics of spermatozoal glycosidases*

To investigate whether the differences of sperm-associated glycosidase levels between the BALB/c and B10.M strains were genetical, activities were measured in the progeny from intercrosses and backcrosses between the strains. Because of the large spread of glycosidase activities of individual mice within a parental strain or intercross we were unable to distinguish progeny from backcrosses unequivocally into parental activity groups for most of the glycosidases assayed. Backcross data has been presented below for only those glycosidase activities for which separations can be clearly made, namely β-D-*N*-acetylglucosaminidase, β-D-*N*-acetylgalactosaminidase and α-L-fucosidase assayed at pH 3·5. The variation of all these glycosidases in the backcrosses did not appear to be linked to their known genotypes for the marker loci *H-2*, brown *b* or albino *c*.

Table 2. *Strain variation in spermatozoal and liver glycosidases*

Glycosidase	Assay pH	Specific activity (mU/mg protein)							
		Spermatozoa				Liver			
		BALB/c		B10.M		BALB/c		B10.M	
β-D-*N*-Acetylglucosaminidase	4·2	7·40	1·54 (7)*	1·19	0·089 (10)	0·715	0·038 (4)	0·972	0·069 (4)
β-D-*N*-Acetylgalactosaminidase	3·7	9·08	0·685 (17)	1·37	0·135 (10)	0·755	0·036 (4)	1·22	0·062 (4)
α-D-Mannosidase	4·6	16·5	1·54 (12)	8·27	0·405 (10)	0·649	0·045 (9)	0·915	0·059 (4)
α-D-Mannosidase	6·4	2·89	0·237 (10)	1·76	0·089 (7)	1·37	0·071 (4)	1·61	0·089 (4)
α-D-Galactosidase	4·0	0·530	0·034 (12)	0·212	0·012 (10)	0·210	0·027 (9)	0·174	0·012 (4)
β-D-Galactosidase	3·5	7·57	0·497 (12)	2·17	0·105 (10)	1·28	0·107 (9)	1·70	0·073 (4)
α-L-Fucosidase	3·5	0·184	0·040 (12)	1·58	0·080 (10)	0·028	0·0034 (4)	0·470	0·017 (4)
α-L-Fucosidase	5·4	6·25	0·567 (12)	6·22	0·277 (10)	0·118	0·007 (4)	0·534	0·010 (4)
β-D-Fucosidase	5·6	1·01	0·132 (12)	1·51	0·123 (10)	0·020	0·0005 (4)	0·028	0·0015 (4)
α-L-Arabinosidase	6·0	0·317	0·038 (12)	0·512	0·039 (10)	0·017	0·0011 (4)	0·028	0·0015 (4)

* Mean, standard error of mean (number of mice).

The data for α-L-fucosidase assayed at pH 3·5 are presented in Table 3. The specific activity of (B10.M × BALB/c) F_1 was similar to that of the maternal parental strain B10.M ($P > 0{\cdot}1$) suggesting dominance or an X-linked inheritance. However (BALB/c × B10.M) F_1 mice had an intermediate activity between the parental strains (Table 3). The (B10.M × BALB/c) F_1 backcrossed to BALB/c produced progeny which segregated into equal groups (Fig. 1). One group had the

Table 3. *Spermatozoal α-L-fucosidase in BALB/c, B10 . M and F_1 progeny*

Strain	Specific activity (mU/mg) protein			
	Assayed at pH 3·5		Assayed at pH 5·4	
BALB/c	0·184	0·040 (12)*	6·25	0·567 (12)
(BALB/c × B10.M) F_1	0·779	0·062 (10)	5·97	0·688 (10)
(B10.M × BALB/c) F_1	1·33	0·131 (12)	6·08	0·374 (12)
B10.M	1·58	0·080 (10)	6·22	0·277 (10)

* Mean, standard error of mean (number of mice).

same activity as the F_1 parent and the other had intermediate activity between the parents. The results of the backcross to B10.M are most readily interpreted as showing two groups. Their closeness may explain the similarities between the B10.M and (B10.M × BALB/c) F_1 specific activities (Table 3). These data can be explained by the effects of a single autosomal gene controlling α-L-fucosidase activity at pH 3·5 in sperm and we propose for it the provisional gene symbol *Afuc-2*, spermatozoal α-L-fucosidase. We suggest that *Afuc-1* is reserved for the somatic α-L-fucosidase locus.

Spermatozoal α-L-fucosidase assayed at pH 5·4 did not parallel the observed variation in the liver. No significant differences ($P > 0{\cdot}1$) in specific activity between parental and reciprocal intercrosses were observed (Table 3).

β-D-*N*-acetylglucosaminidase and β-D-*N*-acetylgalactosaminidase activities segregate together in the backcross progeny ($r = 0{\cdot}998$ and $r = 0{\cdot}993$ for (B10.M × BALB/c) F_1 backcrossed to BALB/c and B10.M respectively), suggesting that loci affecting each enzyme activity are closely linked or that the same enzyme is responsible for hydrolysing both substrates.

Suspensions of spermatozoa from BALB/c and F_1's have the same specific β-D-*N*-acetylglucosaminidase activity and β-D-*N*-acetylgalactosaminidase activity (Table 4). The simplest explanation of the mode of inheritance of these activity variations is that of a dominant allele from BALB/c at a single autosomal locus. The backcross data from β-D-*N*-acetylglucosaminidase are presented in Fig. 2. The backcross to B10.M resulted in an apparently trimodal distribution of activity, two groups having the same activity as the (B10.M × BALB/c) F_1 and B10.M parents respectively and a third group having activity significant lower than the B10.M parent. The results of backcross to BALB/c are most readily interpreted as belonging to two groups. These data suggest that β-D-*N*-acetylglucosaminidase and β-D-*N*-acetylgalactosaminidase activities are controlled by more than one locus.

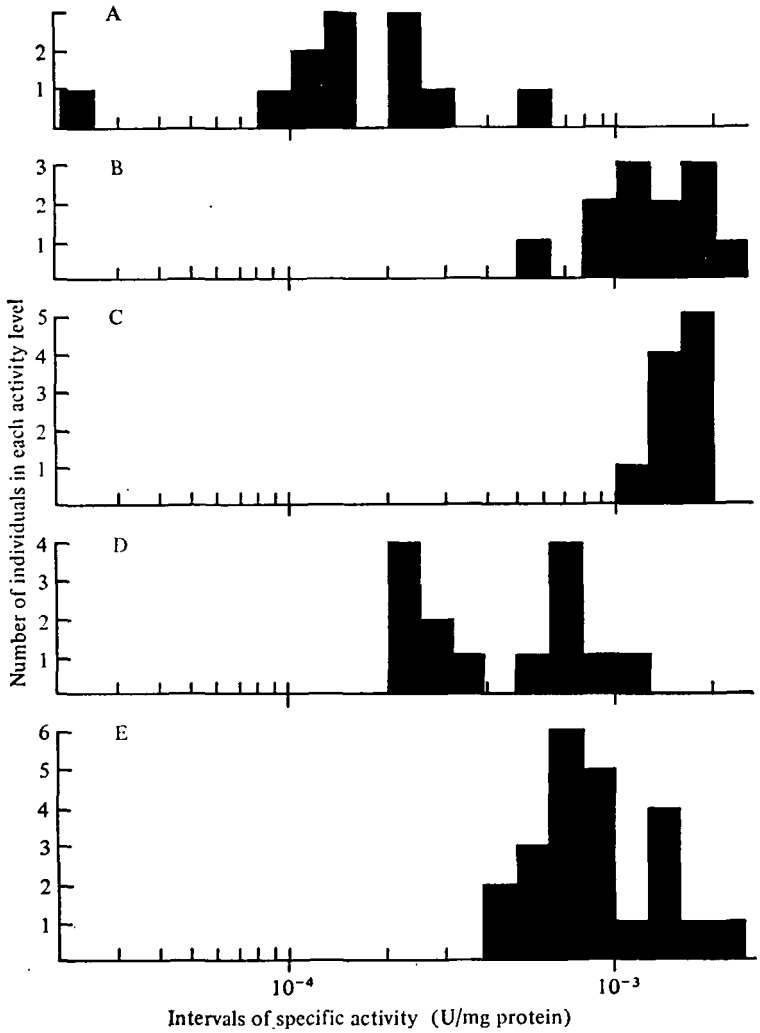

Fig. 1. Distribution of spermatozoal α-L-fucosidase assayed at pH 3·5 in individual mice. (A) BALB/c, (B) (B10.M × BALB/c) F_1, (C) B10.M, (D) (B10.M × BALB/c) F_1 backcrossed to BALB/c and (E) (B10.M × BALB/c) F_1 backcrossed to B10.M.

Table 4. *Spermatozoal β-D-N-acetylglucosaminidase and β-D-N-acetylgalactosaminidase in BALB/c, B10.M and F_1 progeny*

Strain	Specific activity (mU/mg protein)			
	β-D-*N*-Acetyl-glucosaminidase		β-D-*N*-Acetyl-galactosaminidase	
BALB/c	7·40	1·54 (7)*	9·08	0·685 (17)
(BALB/c × B10.M) F_1	4·58	0·476 (10)	8·37	1·21 (10)
(B10.M × BALB/c) F_1	7·24	0·744 (12)	8·08	0·811 (12)
B10.M	1·19	0·089 (10)	1·37	0·135 (10)

* Mean, standard error of mean (number of mice).

https://doi.org/10.1017/S0016672300018668 Published online by Cambridge University Press

The α-D-mannosidase activities in the F_1's were intermediate between the two parental strains and there was no significant difference between the two sex directions for the intercross (Table 5). This indicates that the α-D-mannosidase variation is inherited as a single autosomal locus. A similar conclusion can be made about the variation of spermatozoal β-D-galactosidase (Table 5).

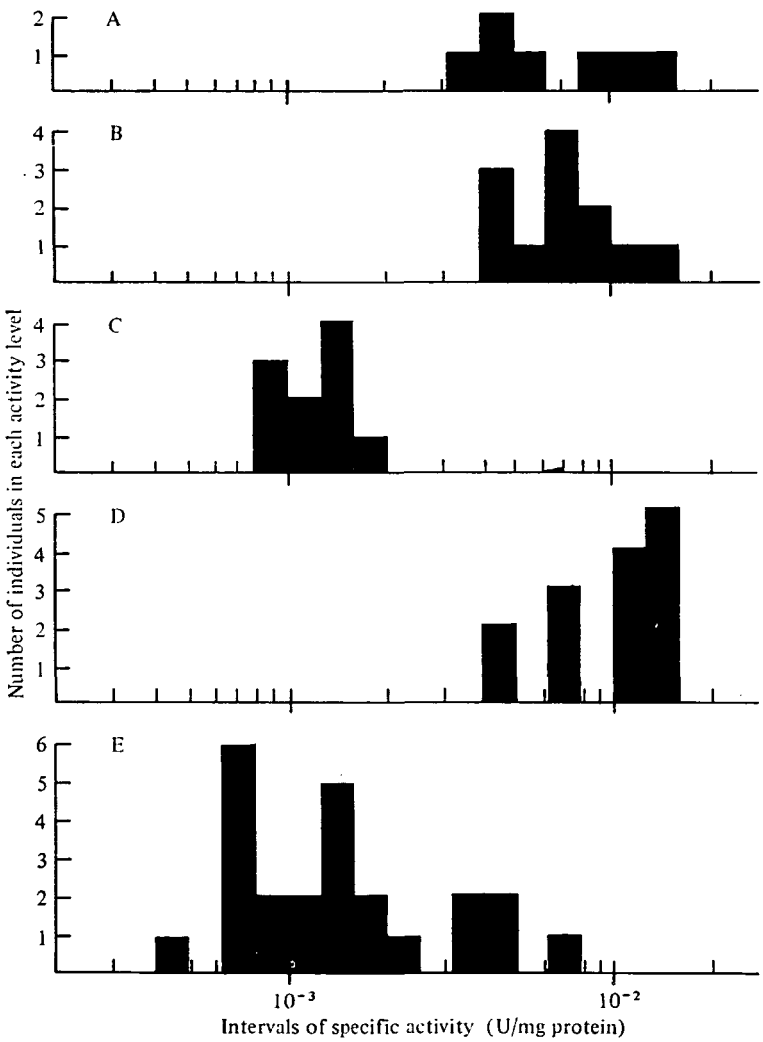

Fig. 2. Distribution of spermatozoal β-D-*N*-acetylglucosaminidase in individual mice. (A) BALB/c, (B) (B10.M × BALB/c) F_1, (C) B10.M, (D) (B10.M × BALB/c) F_1 backcrossed to BALB/c and (E) (B10.M × BALB/c) F_1 backcrossed to B10.M.

The α-D-galactosidase activities in the F_1's depend on the sex direction of the intercross (Table 6). The specific activity of each F_1 cross was idential to that of the maternal parental strain. This is consistent with an *X*-linked mode of inheritance of spermatozoal α-D-galactosidase variation.

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Table 5. *Spermatozoal α-D-mannosidase (assayed at pH 4·6) and β-D-galactosidase in BALB/c, B10.M and F_1 progeny*

Strain	Specific activity (mU/mg protein)			
	α-D-Mannosidase		β-D-Galactosidase	
BALB/c	16·2	1·54 (12)*	7·57	0·497 (12)
(BALB/c × B10.M)F_1	10·4	1·29 (10)	3·21	0·267 (10)
(B10.M × BALB/c)F_1	10·1	0·422 (12)	3·00	0·136 (12)
B10.M	8·27	0·405 (10)	2·17	0·105 (10)
Student's *t*-test difference between F_1's	$t = 0·21$, $P > 0·1$		$t = 0·68$, $P > 0·1$	

* Mean, standard error of mean (number of mice).

Table 6. *Spermatozoal α-D-galactosidase in BALB/c, B10.M and F_1 progeny*

Strain	Specific activity (mU/mg protein)		Student's *t* test
BALB/c	0·530	0·034 (12)*	$t = 2·09$, $P = 0·05$
(BALB/c × B10.M)F_1	0·443	0·015 (10)	
(B10.M × BALB/c)F_1	0·226	0·012 (12)	$t = 0·83$, $P > 0·5$
B10.M	0·212	0·012 (10)	

* Mean, standard error of mean (number of mice).

4. DISCUSSION

BALB/c and B10.M mouse strains show significant differences in levels of the spermatozoal glycosidases measured except α-L-fucosidase assayed at pH 5·4. In contrast no significant differences were found between the strains for the liver enzymes except for α-L-fucosidase activity. Preliminary genetical analysis of the spermatozoal glycosidase variation indicated that for some of these enzymes more than one locus was implicated in the variation.

The variation of spermatozoal β-D-galactosidase appears to be affected by at least one autosomal locus. The gene *Bgs* controls the levels of β-D-galactosidase in all somatic tissues (Felton *et al.* 1974). B10.M (which is congenic with C57BL/10 ScSn) and BALB/c both have the Bgs^h/Bgs^h genotype and as expected have the same liver β-D-galactosidase activity (Table 2). The variation of spermatozoal β-D-galactosidase between the strains therefore suggests that another gene controls the spermatozoal levels.

The variation of spermatozoal α-D-galactosidase showed an *X*-linked mode of inheritance (Table 6). Although a structural gene *Ags* coding for α-D-galactosidase in all tissues has been shown to be *X*-linked (Lusis & West, 1976) we conclude that spermatozoal α-D-galactosidase levels are affected by another *X*-linked gene because all inbred lines tested including BALB/c and C57BL/10 have the genotype Ags^h/Ags^h.

Spermatozoal α-L-fucosidase did not parallel the observed significant variation

https://doi.org/10.1017/S0016672300018668 Published online by Cambridge University Press

in the liver (Table 2). We suggest that the genes involved in this variation are not expressed in the spermatozoa. Their variations are attributable to the gene which we have provisionally named *Afuc-2*.

α-L-Arabinosidase, β-D-fucosidase and α-D-mannosidase with activity optimal at pH 6·4 were not associated with spermatozoa although they are found in liver homogenates. These observations indicate that genes affecting these glycosidases may not be expressed in sperm cells.

Erickson's (1976, 1977) data on murine spermatozoal β-D-glucuronidase show similarities with our data on eight other glycosidases. He found in a survey of inbred strains of mice that the variation in spermatozoal β-D-glucuronidase levels did not correlate with the known *Gus* and *Gur* genotypes, although the low activity of Gus^h/Gus^h mice was reflected in spermatozoal suggesting that *Gus* is also the structural gene for spermatozoal β-D-glucuronidase. Our observations suggest that the expression of genes associated with somatic tissue glycosidases may be repressed in the spermatids while other genes are uniquely expressed for the control or formation of spermatozoal glycosidases. The results also indicate that there is a higher degree of polymorphism associated with these spermatozoal glycosidases.

S.J.S. was the recipient of a S.R.C. studentship.

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