Genet. Res., Camb. (1981), **38**, *pp.* 225–236
With 1 plate and 5 text-figures
Printed in Great Britain

Galactosyltransferase activities on mouse sperm bearing multiple t^{lethal} and t^{viable} haplotypes of the T/t-complex

BY BARRY D. SHUR

Department of Anatomy, University of Connecticut Health Center, Farmington, Connecticut 06032, U.S.A.

(*Received* 26 *February* 1981 *and in revised form* 30 *March* 1981)

SUMMARY

Segregation-distorting *t*-sperm show a specific increase in *N*-acetylglucosamine:galactosyltransferase activity over wild-type (+/+) due to a deficiency of a wild-type galactosyltransferase inhibitor (Shur & Bennett, 1979). Eight other enzymic activities are indistinguishable between +- and *t*-sperm suspensions. In this study, three additional points are analysed. First, galactosyltransferases are assayed on sperm homozygous for a semilethal haplotype (t^{w2}/t^{w2}), relative to heterozygous ($+/t^{w2}$) and wild-type (+/+) controls. t^{w2}/t^{w2} assays circumvent the +-sperm inhibition of *t*-sperm galactosyltransferases that occurs in heterozygous $+/t$-assays and show that *t*-sperm are actually four times as active as wild-type. Second, sperm which are compound heterozygotes for two complementing lethal *t*-haplotypes (t^{1x}/t^{1y}), have nearly twice the theoretical enzyme level of t^{1x}/t^{1y} sperm. Thus, in either homozygous (t^{w2}/t^{w2}) or double heterozygous (t^{1x}/t^{1y}) form *t*-haplotypes act synergistically on sperm galactosyltransferase activity.

Third, and most interesting, sperm bearing either recombinant, viable *t*-haplotypes ($+/t^{v}$, t^{vx}/t^{vy}), or one of three dominant T/t-complex mutations, were assayed to determine which portions of the T/t-complex are responsible for elevated galactosyltransferase activity. Results show that sperm bearing recombinant, non-segregation-distorting, viable t^{v}-haplotypes no longer show elevated transferase activity. Therefore, the elevated t^{1}-sperm galactosyltransferase activity strictly correlates with the increased transmission frequency of t^{1}-sperm. These studies strengthen further the hypothesis that sperm surface galactosyltransferases are involved in egg binding during fertilization, and that t^{1}-sperm segregation-distortion results, at least in part, from increased galactosyltransferase activity.

1. INTRODUCTION

Cell-surface components are believed to play crucial roles in cell-contact-dependent processes as diverse as fertilization, embryonic morphogenesis, metastasis and immune recognition. Investigators have characterized a variety of

molecules which may be involved in cell social behaviour. The occurrence of lectin-like carbohydrate-binding proteins (Frazier & Glaser, 1979), and of specific aggregation-promoting factors (Rutz & Lilien, 1979), are just two examples. Data collected over the past ten years, using a broad spectrum of techniques, show that glycosyltransferases also exist on the cell surface (Shur & Roth, 1975; Pierce, Turley & Roth, 1980; Shur, 1977*b*). In this location, these enzymes could participate in cellular interactions by binding their specific carbohydrate substrates on adjacent cell surfaces or in the extracellular matrix. In fact, one previous study (Shur, 1977*a*, *b*) showed that cell-surface glycosyltransferases do indeed exist on chick embryo cell surfaces. More interestingly, their activities correlated with a variety of well-defined morphogenetic phenomena.

To further probe the role, if any, of cell surface glycosyltransferases in cellular interactions, these enzymes were analysed on mouse cells bearing mutant haplotypes of the *T*/*t*-complex (Shur & Bennett, 1979). Mutant *T*/*t*-haplotypes interfere with normal embryonic development and fertilization as evidenced by the lethality of some *t*/*t* embryos and the increased transmission frequency of many *t*-bearing sperm (Bennett, 1975; Gluecksohn-Waelsch & Erickson, 1970). There are data (Artzt & Bennett, 1977; Yanagisawa *et al.* 1974) which support the hypothesis that these genes specify cell-surface antigens which are crucial for normal cell behaviour. Other investigators have shown (Ginsberg & Hillman, 1975) that some of these haplotypes affect intermediary metabolism which could account for some of the characteristic phenotypic abnormalities. In either case, it is clear that these genes interfere with the same processes in which the involvement of cell-surface glycosyltransferases has been hypothesized.

Since it had been shown previously (Durr, Shur & Roth, 1977) that normal mouse sperm were able to glycosylate egg carbohydrate acceptors, *t*-bearing sperm were assayed for specific enzymic defects relative to wild-type controls. When nine different enzymes were assayed, only one activity proved significantly different (Shur & Bennett, 1979). *N*-acetylglucosamine:galactosyltransferase was approximately twice as active in heterozygote (+/*t*) preparations relative to normal (+/+). Little galactosyltransferase activity towards acceptors endogenous to the sperm was detectable, but zona pellucida digests served as a good acceptor source. Furthermore, when equal aliquots of +/+ and +/*t* sperm were mixed and then assayed, galactosyltransferase activity was inhibited by 80% down from the theoretical intermediate level. These results suggested (Shur & Bennett, 1979) that +/*t* sperm had a specific increase in galactosyltransferase activity due to a deficiency of some inhibitory component normally made by +/+ sperm. This, in turn, could result in higher levels of *N*-acetylglucosamine binding either *in vitro* (transferase assays) or *in vivo* (segregation-distortion during fertilization).

The present work addresses three additional points. First, past studies (Shur & Bennett, 1979) do not allow an accurate calculation of *t*-sperm enzyme levels relative to wild-type, since the +-sperm component of the +/*t* heterozygote mixture must be inhibiting *t*-sperm enzyme as it does when +/+ and +/*t* sperm are mixed together. Since most segregation-distorting *t*-haplotypes produce

lethality (t^1) when homozygous, sperm from t^1/t^1 males is unavailable. In this study, sperm homozygous for a semilethal haplotype (t^{w2}/t^{w2}) are assayed relative to heterozygous littermates ($+/t^{w2}$), which in subsequent litters are assayed relative to $T/+$ sperm. In this way, the actual level of t-sperm galactosyltransferase activity is shown to be 3·5–4·3 times wild-type.

Second, sperm heterozygous for two partially complementing lethal t-haplotypes (t^{lx}/t^{ly}) are assayed relative to single heterozygous and wild-type controls. Data show that these different t-haplotypes affect sperm transferase activity due to a common biochemical lesion. Additionally, both of these assays (t^{w2}/t^{w2}, t^{lx}/t^{ly}) entail measuring t-sperm activity in the absence of wild-type alleles. In so doing, the presence of a wild-type sperm galactosyltransferase inhibitor is clearly shown, since in all of these instances the observed t/t sperm activity is nearly twice the theoretical level expected of t/t sperm.

Third, our past results (Shur & Bennett, 1979) have not rigorously analysed which portions of the T/t-complex were responsible for the elevated transferase activity on t-sperm. By appropriate recombinations, the T/t-complex can be subdivided into a proximal segment allelic to T and responsible for taillessness, and a distal segment responsible for embryonic lethality and segregation-distortion (Bennett, 1975; Bennett, Dunn & Artzt, 1976; Lyon & Mason, 1977). Therefore, by assaying sperm bearing various recombinant chromosomes, the alleles governing t-sperm galactosyltransferase stimulation are determined. Only sperm bearing the lethal/segregation-distorting portions of the T/t-complex show elevated enzyme activity, i.e. recombinant, viable t-haplotypes show no effect on sperm galactosyltransferases. These results further support our working hypothesis for sperm surface galactosyltransferase involvement in fertilization.

2. MATERIALS AND METHODS

(i) *Mouse colony and genetic crosses*

Animals on the BTBRTF/Nev background carrying specific T/t-complex haplotypes were bred in closed colonies. The lethal haplotypes t^{12} and t^{w5} were maintained in balanced lethal crosses ($T/t^1 \times T/t^1$) where T/t^1 (no-tail) is the only viable genotype (T/T and t^1/t^1 are embryonic lethals). t^{12}/t^{w5} males were generated from $T/t^{12} \times T/t^{w5}$ crosses, since t^{12} and t^{w5} belong to two different lethality complementation groups. $T/t^{w2} \times T/t^{w2}$ crosses generated normal-tailed animals (t^{w2}/t^{w2}), in addition to T/t^{w2}, since t^{w2} is an example of a haplotype producing semilethality when homozygous (51% viable) (Bennett, 1975).

In order to re-examine the enzyme activity on $+/t^1$ preparations relative to control litter-mates ($T/+$), $T/t^1 \times +/+$ crosses were made. Only segregation-distorting, normal-tailed ($+/t^1$) and non-segregation-distorting, short-tailed ($T/+$) progeny were produced. All $+/+$ animals were derived from crosses between inbred BTBRTF/Nev mice.

The t^{w111} and t^{w82} haplotypes are examples of viable, recombinant t-haplotypes

(Bennett, 1975; Silver, White & Artzt, 1980). Ttf/t^{w82} tf (no-tailed, tufted) × $++/t^{w111}$ tf (normal-tailed, non-tufted) crosses generated four phenotypes representing non-t-bearing animals (T $tf/++$), single t-heterozygotes (T tf/t^{w111} tf, $++/t^{w82}tf$) and double t-heterozygotes ($t^{w82}tf/t^{w111}tf$). Sperm from these males were assayed for galactosyltransferase activity as below.

(ii) *Sperm preparation*

Sperm from 10-week-old males was prepared as described (Shur & Bennett, 1979). Briefly, the cauda epididymis was removed and minced, and viable sperm were collected, washed twice by centrifugation and assayed for galactosyltransferase activity. All manipulations were conducted in the standard incubation buffer (NaCl, 7·5 g/l; KCl, 0·4 g/l; Hepes buffer (Sigma Chemical Co.), 4·76 g/l; pH 7·2). The final suspension was composed of essentially pure cauda epididymal sperm with less than 5 % contamination (erythrocytes). Previous results (Shur & Bennett, 1979) showed that this small blood population contains undetectable levels of galactosyltransferase activity.

t^{w2}/t^{w2} males were generally aspermic (Bennett, 1975; Dooher & Bennett, 1974). Of the 28 t^{w2}/t^{w2} males examined, two were good sperm producers, which when prepared as described above, resulted in sperm suspensions indistinguishable from normal (Plate 1). The galactosyltransferase data presented here were collected from the two litters which generated these two males.

(iii) *Galactosyltransferase assay*

Sperm galactosyltransferase activity towards N-acetylglucosamine was measured as previously described (Shur & Bennett, 1979). Briefly, 50 μl assays contained 210 μM uridine diphosphate (UDP)-^{3}H-galactose (197 mCi/mmole; New England Nuclear), 30 mM-N-acetylglucosamine (Sigma Chemical Co.), 10 mM-$MnCl_2$, and approximately $0{\cdot}5 \times 10^6$ sperm. After the desired incubation time at 37 °C, the reaction was terminated with Na EDTA and subjected to high-voltage borate electrophoresis to separate the product, N-acetyl-lactosamine, from unused UDP-galactose and any UDP-galactose degradation products. The reaction components were shown in previous results (Shur & Bennett, 1979) to be optimal for all parameters.

(iv) *Data analysis*

In previous studies (Shur & Bennett, 1979), almost all sperm galactosyltransferase activities were compared between age-matched males with the appropriate genetic constitution, rather than between litter-mates segregating for the t-haplotype of interest. As a result, these assays showed considerable variation within a given t-complementation group (i.e. large standard deviations) and between different t-complementation groups. Consequently, we could not determine whether different lethal t-haplotypes showed characteristically different levels of enzyme activity or whether strain and colony variation produced non-T/t-complex-dependent effects. In the present study, to alleviate these ambiguities and to ensure that only T/t-complex-dependent effects on enzyme activity were being assayed, all com-

Haemocytometer fields of cauda epididymal sperm prepared as described in Materials and Methods. Sperm characteristically lie in many focal planes, making photography difficult. (A) $+/+$, (B) t^{12}/t^{w5}, (C) t^{w2}/t^{w2} 300×.

BARRY D. SHUR

(*Facing p.* 228)

https://doi.org/10.1017/S0016672300020577 Published online by Cambridge University Press

parisons of enzyme activity were routinely made between litter-mates resulting from the crosses described above. The sperm galactosyltransferase activity for each male was assayed as a function of time, as in Fig. 1. Within any one litter, the enzyme rates were averaged for males of the same genotype, and the averaged rates characteristic for each of the segregating genotypes compared to one another as ratios. These ratios were pooled together from all similar litters from which the average ratio ± standard deviation was calculated. The numbers of individual males used are shown in the appropriate figures adjacent to each genotype.

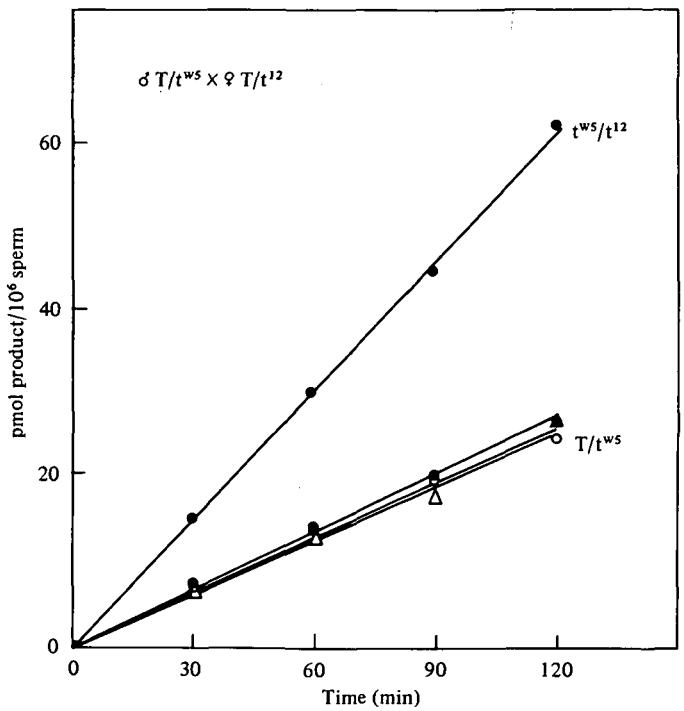

Fig. 1. Sperm *N*-acetylglucosamine: galactosyltransferase activities of male litter-mates resulting from a $\delta T/t^{w5} \times \varphi T/t^{12}$ cross.

3. RESULTS

(i) *Sperm homozygous for a semilethal haplotype* (t^{w2}/t^{w2})

As previously discussed (see 'Materials and Methods'), the t^{w2}-haplotype produces semilethality when homozygous and also shows strong segregation-distortion (0·95) when heterozygous ($+/t^{w2}$) (Bennett, 1975). Therefore, t^{w2}/t^{w2} sperm give us the opportunity to calculate the true galactosyltransferase level of segregation-distorting *t*-sperm without interference from +-sperm inhibition which occurs in $+/t$ assays.

Fig. 2(*e*, *f*) presents the ratio of sperm galactosyltransferase activities of litter-mates segregating from a $T/t^{w2} \times T/t^{w2}$ cross as well as the ratio of ($+/t^{w2}$)

https://doi.org/10.1017/S0016672300020577 Published online by Cambridge University Press

activity to $(T/+)$ litter-mates resulting from a $\sigma T/t^{w2} \times \varphi + /+$ cross. These values can be used to calculate the theoretical level of t^{w2}/t^{w2} activity and compare it to that observed. Substituting the values of 1·57 for $+/t^{w2}$, and 1·0 for $T/+$, into the formula $2\,(+/t)-T/+$, yields a theoretical t^{w2}/t^{w2} value of 2·14 relative to $T/+$. This value, however, is only 62 % of that observed for t^{w2}/t^{w2} sperm, which are actually 3·45 times as active as $(T/+)$ controls. Since, in the absence of wild-type alleles, t^{w2}/t^{w2} enzyme activities are nearly twice the theoretical levels, these results clearly show the presence of a normal sperm galactosyltransferase inhibitor, deficient on t^{w2}-sperm.

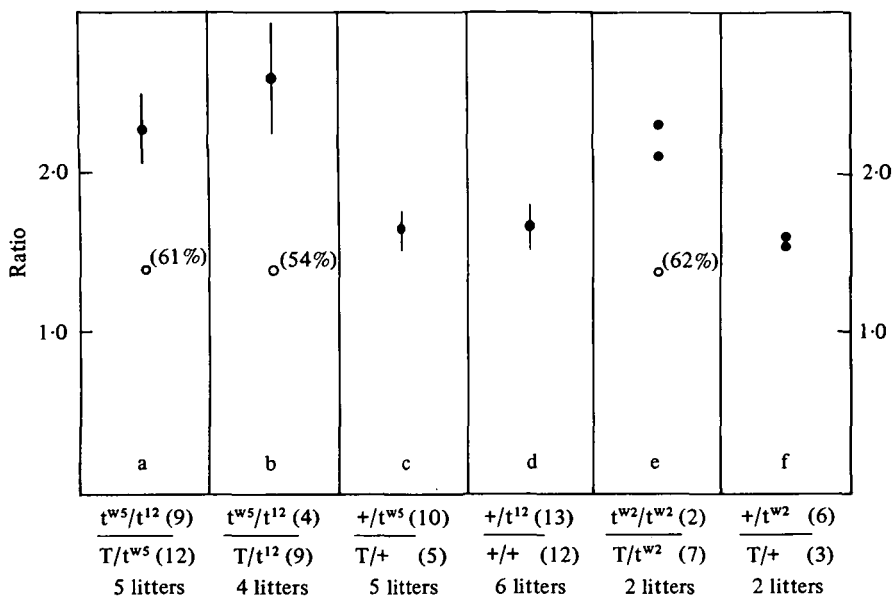

Fig. 2. Comparisons of sperm *N*-acetylglucosamine:galactosyltransferase activities between segregating genotypes within appropriate litters. The number of litters in each category are shown, as well as the number of males of each genotype. Bars represent ± standard deviations. Open circles give the theoretical value for $(t/t)/(T/t)$.

(ii) *Sperm heterozygous for two complementing, lethal* t-*haplotypes* (t^{1x}/t^{1y})

As mentioned above, t^{12} and t^{w5} belong to two partially overlapping *t*-complementation groups, since 74 % of the t^{12}/t^{w5} animals survive from a $T/t^{w5} \times T/t^{12}$ cross (Bennett, 1975). We assayed sperm galactosyltransferase activities from males resulting from such a cross and compared the $(t^{12}/t^{w5})/(T/+)$ ratio to that calculated above using t^{w2}/t^{w2} sperm. This analysis enables us to determine whether different segregation-distorting *t*-haplotypes (i.e. t^{12}, t^{w5}) behave as if they are homozygous for sperm galactosyltransferase stimulation.

The sperm galactosyltransferase activities from male litter-mates resulting from a $\sigma T/t^{w5} \times \varphi T/t^{12}$ cross are shown in Figs. 1 and 2*a*. As can be seen, the activity of the double heterozygote (t^{12}/t^{w5}) is 2·3 times that of the single *t*-heterozygote (T/t^{w5}) littermate.

https://doi.org/10.1017/S0016672300020577 Published online by Cambridge University Press

In $T/t^{w5} \times T/t^{12}$ crosses, the actual genotype of the no-tailed (T/t) progeny is unknown, since T/t^{12} and T/t^{w5} animals are indistinguishable. However, due to the high segregation-distortion of the t^{w5} haplotype originating from the male in this cross, the T/t progeny are assumed to be of the paternal, rather than maternal (t^{12}) type. Fig. 2*b* shows the cumulative data from the reciprocal cross, $\delta T/t^{12} \times \varphi T/t^{w5}$, which is similar to that when the sexes are reversed.

In order to calculate the actual increase in enzyme activity for t^{lx}/t^{ly} relative to non-segregation-distorting control ($T/+$, $+/+$) populations, we need to know the relative increases in activity for $+/t$ versus $+/+(T/+)$. By the appropriate matings, such litter-mates were obtained and their relative sperm enzyme activities are given in Figs. 2*c* and 2*d*.

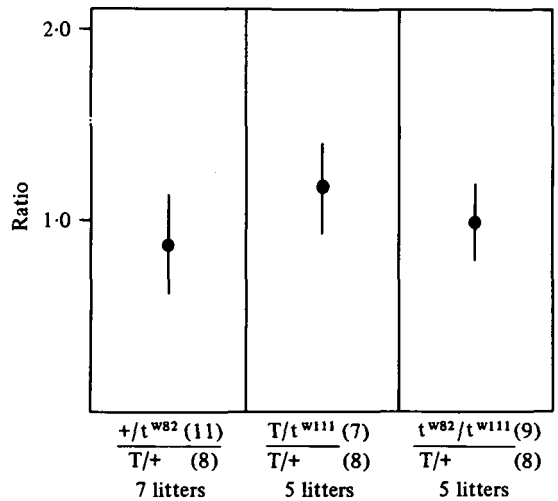

Fig. 3. Comparison of sperm *N*-acetylglucosamine:galactosyltransferase activities between segregating genotypes within appropriate litters. The number of litters in each category are shown, as well as the number of males of each genotype.

As before, the values presented in Fig. 2 can be used to calculate both the theoretical and observed levels of t^{12}/t^{w5} enzyme activity relative to wild-type. Results show that t^{12}/t^{w5} sperm are 3·67–4·30 times as active as normal sperm, yet theoretically should be only 2·26–2·30 times as active. As is the case with t^{w2}-assays, t^{12}/t^{w5} sperm actually show nearly twice the expected, theoretical enzyme level. Despite the fact that t^{12} and t^{w5} belong to two different 'lethal' complementation groups (Bennett, 1975), these haplotypes fail to correct for each other's sperm galactosyltransferase inhibitor deficiency. These data are basically indistinguishable from those using t^{w2}/t^{w2} sperm, suggesting that t^{12} and t^{w5} behave as if they are homozygous for this particular biochemical lesion.

(iii) *Sperm heterozygous for one or two viable* t-*haplotypes* ($+/t^{v}$, t^{vx}/t^{vy})

To further clarify the relationship between sperm galactosyltransferase activity and *t*-sperm segregation-distortion, galactosyltransferase activity was assayed on *t*-sperm bearing recombinant, viable, *non*-segregation-distorting *t*-haplotypes. For this analysis, t^{w82} and t^{w111} were chosen as examples of such recombinant viable

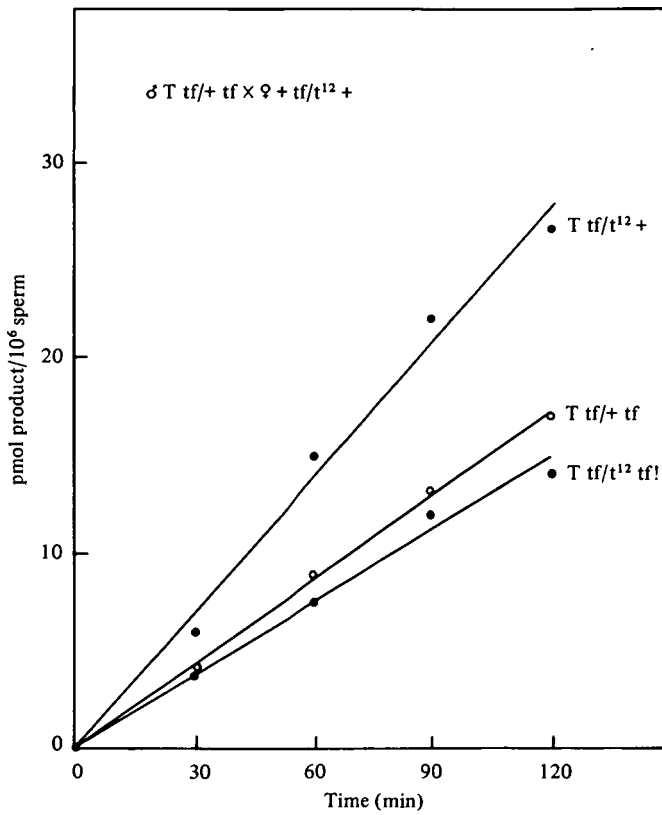

Fig. 4. Sperm *N*-acetylglucosamine : galactosyltransferase activities of male litter-mates resulting from a $\delta Ttf/+tf \times ♀ + tf/t^{12}+$ cross. The recombinant $Ttf/t^{12}tf$ is signified by the ! notation.

haplotypes (Bennett, 1975; Silver, White & Artzt, 1980). The relative galactosyltransferase activities of litter-mate sperm resulting from $♂T/t^{w82} \times ♀ + /t^{w111}$ crosses are shown in Fig. 3. In neither a single dose ($+/t^{w82}$, T/t^{w111}) nor a double dose (t^{w82}/t^{w111}), do viable, recombinant *t*-haplotypes affect sperm galactosyltransferase activity. These results are in marked contrast to the effects of segregation-distorting *t*-haplotypes on sperm enzyme activity. t^{12}, t^{w5} and t^{w2} all elevate sperm transferase activity in heterozygous form, and the degree of enzyme stimulation is approximately fourfold over wild-type in compound heterozygous (t^{12}/t^{w5}) or homozygous (t^{w2}/t^{w2}) form.

https://doi.org/10.1017/S0016672300020577 Published online by Cambridge University Press

During the course of these studies, a rare recombinational event occurred in our animal colony which resulted in a litter containing both parental type (segregation-distorting) and recombinant chromosomes (non-segregation-distorting), in addition to controls ($T/+$). Fig. 4 presents the sperm galactosyltransferase activities from these littermates, which resulted from a $\delta Ttf/+tf \times \varphi +tf/t^{12}+$ mating. The $Ttf/t^{12}tf$ male is the result of a recombination event similar to that described above, which produces non-segregation-distorting, viable *t*-haplotypes. Sperm from this male has enzyme activity characteristic of wild-type levels, rather than of the parental type(t^{12}).

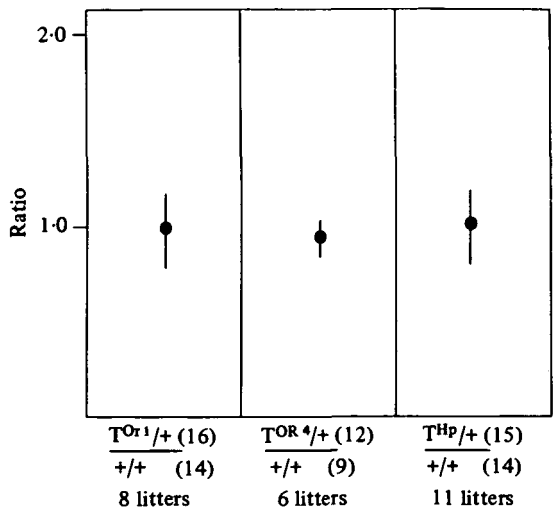

Fig. 5. Comparisons of sperm *N*-acetylglucosamine:galactosyltransferase activities between segregating genotypes within appropriate litters. The number of litters in each category are shown, as well as the number of males of each genotype.

(iv) *Sperm heterozygous for three dominant* T/t-*complex mutations*

Past (Shur & Bennett, 1979) and present results show that the *T* mutation does not affect sperm galactosyltransferase activity. Fig. 5 shows the sperm enzyme activities associated with two other mutants of the T/t-complex, T^{OR4} and T^{Or1}, relative to wild-type litter-mates. Neither mutation affects sperm transferase activity and both lie outside the lethal/segregation-distorting elements of the *t*-haplotype (Alton *et al.* 1980).

In addition to these three *T*-mutations, the T^{hp}-deletion also lies within the proximal portion of the T/t-complex and has no effect on gamete transmission frequency. *T*-, T^{OR4}- and T^{Or1}-sperm all show galactosyltransferase levels indistinguishable from wild-type when assayed exclusively by litter-mate comparisons. Since our previous analysis (Shur & Bennett, 1979) of T^{hp}-sperm activities was based almost entirely on age-matched *non*-litter-mate assays, we therefore decided to re-examine T^{hp}-sperm activities solely against wild-type litter-mates. The cumulative results from eleven litters segregating for T^{hp} are shown in Fig. 5.

Clearly, the T^{hp}-deletion fails to influence sperm galactosyltransferase activity within the limits of our resolution. In addition, surface galactosyltransferase activity was compared on spermatogenic cells isolated from $T^{hp}/+$ and wild-type testes. No T^{hp}-dependent differences were detectable (data not shown). These results clearly show the need to compare sperm enzyme activities exclusively between litter-mates segregating for the allele of interest. Age-matched, non-litter-mate comparisons fail to remove strain and colony influences on sperm galactosyltransferase assays.

(v) *Discussion*

Three main observations result from this study. First, in the absence of any wild-type (+) sperm, t^1/t^1 sperm galactosyltransferase activity is higher than that expected from the formula $2\ (+/t^1) - +/+$. These, and other (Shur & Bennett, 1979) results, clearly show the presence of a galactosyltransferase inhibitor on normal sperm which is deficient on t^1-sperm. We can now explain the 'trans' sperm inhibition seen in $+/t$ assays, since the inhibitor is released from +-sperm during a 37 °C incubation (unpublished results). In the incubation medium, this inhibitor is free to interact with +-, as well as t^1-sperm galactosyltransferases.

Secondly, t^{12}/t^{w5} sperm suspensions behave identically to t^{w2}/t^{w2} homozygote sperm. These three t-haplotypes all produce segregation-distortion and have similar galactosyltransferase elevations in heterozygous ($+/t$) form. Even though t^{12} and t^{w5} belong to different 'lethal' complementation groups, they fail to correct for each other's galactosyltransferase inhibitor deficiency. Previous results (Shur & Bennett, 1979) show that sperm bearing t-haplotypes from each of the six known complementation groups of lethal t-haplotypes all produce elevated galactosyltransferase activities. The results in this study demonstrate that at least two of these complementation groups (t^{12}, t^{w5}) show stimulated galactosyltransferase levels due to a similar biochemical lesion. It seems highly unlikely that the two complementation groups chosen, at random, for this analysis are the only two to affect galactosyltransferase regulation in the same way. Presumably, t-sperm from males belonging to each of the remaining four groups show elevated enzyme levels due to a similar defect. However, we have no data concerning this other than those presented here.

Third, the only parameter that correlates, without exception, with elevated galactosyltransferase activity on sperm is t-sperm segregation-distortion. Sperm bearing either one or two non-segregation-distorting viable t-haplotypes, or four different dominant mutants of the T/t-complex, all have enzyme levels equal to their wild-type litter-mates. Only when the distal portions of the mutant T/t-complex are present, which are responsible for t/t embryonic lethality and increased t-sperm transmission frequency, is sperm galactosyltransferase activity likewise elevated. These results eliminate, therefore, the possibility that this galactosyltransferase inhibitor is related to p63/6.9 – a protein identified by 2-D gel fluorography as a product of the proximal (i.e. viable) portion of the T/t-complex (Silver, Artzt & Bennett, 1979).

On the other hand, these results strengthen our hypothesis that sperm surface galactosyltransferases are involved in egg binding. However, if this is true, then it is difficult to reconcile the high levels of sperm enzyme activity present in sterile t^{lx}/t^{ly} and t^{w2}/t^{w2} sperm suspensions. It is clear, though, that these males are sterile for reasons other than their sperm's ability to bind eggs. Specifically, t^{w2}/t^{w2} males are usually aspermic, which accounts for their sterility. The fertilizing capabilities of the rare t^{w2}/t^{w2} sperm-producing male have not yet been analysed *in vitro*. t^{lx}/t^{ly} males are also sterile, despite the fact that they produce normal numbers of sperm. However, these sperm are non-motile, and only a few reach the site of fertilization (Tucker, 1980). It has been shown recently (McGrath & Hillman, 1980) that if given the opportunity *in vitro*, t^{lx}/t^{ly} sperm are fully capable of binding the zona pellucida similar to $+/t^{l}$ sperm. These observations are entirely consistent with our working model for gamete interactions.

(vi) *Acknowledgements*

The excellent technical assistance of Mary Gullak is deeply appreciated. I am indebted to Dr Dorothea Bennett for supplying the animals which generated my *T*/*t*-complex colony, and to Drs Sherwin Cooperstein and Robert Kosher for their suggestions regarding this manuscript. This work was supported by grants from the University of Connecticut Research Foundation, The Anna Fuller Fund, The American Cancer Society (CD-28) and by a Basil O'Connor Starter Research grant (5-253) from the March of Dimes Birth Defects Foundation.

REFERENCES

Alton, A. K., Silver, L. M., Artzt, K. & Bennett, D. (1980). Molecular analysis of the genetic relationship of *trans* interacting factors at the T/t complex. *Nature* **288**, 368–370.

Artzt, K. & Bennett, D. (1977). Serological analysis of sperm of antigenically cross-reacting *T*/*t*-haplotypes and their recombinants. *Immunogenetics* **5**, 97–107.

Bennett, D. (1975). The T-locus of the mouse. *Cell* **6**, 441–454.

Bennett, D., Dunn, L. C. & Artzt, K. (1976). Genetic change in mutations at the T/t-locus in the mouse. *Genetics* **83**, 361–372.

Dooher, G. B. & Bennett, D. (1974). Abnormal microtubular systems in mouse spermatids associated with a mutant gene at the T-locus. *Journal of Embryology and Experimental Morphology* **32**, 749–761.

Durr, R., Shur, B. & Roth, S. (1977). Sperm-associated sialyltransferase activity. *Nature* **265**, 547–548.

Frazier, W. A. & Glaser, L. (1979). Surface components and cell recognition. *Annual Review of Biochemistry* **48**, 491–523.

Ginsberg, L. & Hillman, N. (1975). ATP metabolism in t^{n}/t^{n} mouse embryos. *Journal of Embryology and Experimental Morphology* **33**, 715–723.

Gluecksohn-Waelsch, S. & Erickson, R. P. (1970). The T-locus of the mouse: implications for mechanisms of development. *Current Topics in Developmental Biology* **5**, 281–316.

Lyon, M. F. & Mason, I. (1977). Information on the nature of *t*-haplotypes from the interaction of mutant haplotypes in male fertility and segregation ratio. *Genetical Research* **29**, 255–266.

McGrath, J. & Hillman, N. (1980). Sterility in mutant (t^{Lx}/t^{Ly}) male mice. III. *In vitro* fertilization. *Journal of Embryology and Experimental Morphology* **59**, 46–58.

Pierce, M., Turley, E. A. & Roth, S. (1980). Cell surface glycosyltransferase activities. *International Review of Cytology* **65**, 1–47.

RUTZ, R. & LILIEN, J. (1979). Functional characterization of an adhesive component from the embryonic chick neural retina. *Journal of Cell Science* **36**, 323–342.

SHUR, B. D. (1977*a*). Cell-surface glycosyltransferases in gastrulating chick embryos. I. Temporally and spatially specific patterns of four endogenus glycosyltransferase activities. *Developmental Biology* **58**, 23–39.

SHUR, B. D. (1977*b*). Cell-surface glycosyltransferases in gastrulating chick embryos. II. Biochemical evidence for a surface localization of endogenous glycosyltransferase activities. *Developmental Biology* **58**, 40–55.

SHUR, B. D. & BENNETT, D. (1979). A specific defect in galactosyltransferase regulation on sperm bearing mutant alleles of the T/t-locus. *Developmental Biology* **71**, 243–259.

SHUR, B. D. & ROTH, S. (1975). Cell surface glycosyltransferases. *Biochimica et Biophysica Acta* **415**, 473–512.

SILVER, L. M., ARTZT, K. & BENNETT, D. (1979). A major testicular cell protein specified by a mouse T/t complex gene. *Cell* **17**, 275–284.

SILVER, L. M., WHITE, M. & ARTZT, K. (1980). Evidence for unequal crossing over within the mouse T/t complex. *Proceedings – National Academy of Sciences USA* **77**, 6077–6080.

TUCKER, M. J. (1980). Explanation of sterility in t^x/t^y male mice. *Nature* **288**, 367–368.

YANAGISAWA, K., BENNETT, D., BOYSE, E. A., DUNN, L. C. & DIMEO, A. (1974). Serological identification of sperm antigens specified by lethal *t*-alleles in the mouse. *Immunogenetics* **1**, 57–67.