STUDIES OF EFFECTS OF *t*-ALLELES IN THE HOUSE MOUSE ON SPERMATOZOA

I. MALE STERILITY EFFECTS

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Summary. Males bearing certain combinations of alleles at locus *T* (linkage group IX in the house mouse) have been tested and shown to be mating-sterile. The numbers, motility and location of spermatozoa of such males have been examined in the reproductive tracts of females inseminated by them. Similar observations on the spermatozoa of fertile males bearing other combinations of *t*-alleles served as controls. The observations indicate that this genotypic sterility is related not to numbers of spermatozoa entering the female tract but to lowered motility which results in their failure to remain near the site of fertilization.

INTRODUCTION

The extensive series of alleles at one complex locus (*T*) in the mouse has provided interesting material for studying the genetic control of early developmental processes (Bennett, 1964), the behaviour of such alleles in natural populations (Dunn, 1964), recombination (Lyon & Meredith, 1964) and other properties. A remarkable feature of many of these alleles has received less attention. This is the effect expressed in the male only upon fertility and upon the proportion of spermatozoa bearing a given allele which effect fertilization (transmission ratio).

We propose to devote several papers to a study of these latter properties.

Male mice with certain combinations of *t*-alleles have been shown to be sterile or quasi-sterile whereas females of the same genotypes are fertile (Dunn, 1950; Braden & Gluecksohn-Waelsch, 1958). Various explanations of the failure of all or most of the spermatozoa of these genotypes to effect fertilization have been suggested, but as yet no satisfactory theory has been established. We have, therefore, tested the fertility of many additional males bearing different combinations of *t*-alleles. After classifying the different genotypes according to their relative fertility, a sample of those that were 'mating-sterile' was selected and in-vivo examinations made of their spermatozoa in the reproductive tracts of females. The results suggest that some allele combinations

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resulting in sterility affect functions expressing themselves in sperm-motility and ability of spermatozoa to reach or remain at the site of fertilization.

In this paper we describe methods of testing mating-fertility of males, list the *t*-genotypes associated with complete mating-sterility and those giving quasi-sterility, and report in-vivo observations on spermatozoa of sterile genotypes compared with those of fertile males. In a second paper we shall describe the effects of combinations of alleles leading to quasi-sterility upon relative fertility, in which certain alleles show characteristic differences and can thus be distinguished by this property. A third paper will deal with the question whether these alleles interact in compound males t^n/t^x with respect to a second property in which *t*-alleles differ, namely, the ratio in which such alleles are transmitted through the spermatozoa.

MATERIALS AND METHODS

The fertility of male mice was tested by mating with females of proven fertility. A mating of 1 month's duration constituted a 'mating unit (m.u.)'. Usually a male was kept with five fertile females for 2 months and thus accumulated ten mating units which is a standard minimum test. A male is judged to be 'mating-sterile' when no offspring have resulted from ten mating units.

If the male is not entirely sterile each pregnant female is removed as detected and replaced by another fertile female. The young born in isolation are classified and credited to the male under test whose fertility can then be described as number of young sired/mating unit (n.y./m.u.). Control observations on the stock (Brachy = $T/+$) from which the test females were usually drawn indicated that 'normal fertility' was about four offspring/mating unit (actually 3.8, with range for sixteen different males 2.4 to 4.7). We have referred to males having less than one offspring/mating unit, that is less than one quarter of 'normal fertility,' as 'quasi-sterile' (Dunn, 1950). Further discussion of quasi-sterility will be given in the second paper of this series. As Gluecksohn-Waelsch, Segal & Fitch (1950) have shown, mating sterility or quasi-sterility thus measured is confirmed by examination of cleaved eggs showing that it is not due to death of zygotes before birth. There are some minor exceptions which do not alter the usefulness of the mating test for the purposes of this paper.

A large series of recessive *t*-alleles is maintained in balanced lines $T/tf/t^n+$, *tf* being a marker near to *T*. The genotype T/t^n is tailless; since *T* is lethal in homozygous condition, when t^n/t^n is also lethal only tailless offspring are born. Six different *t*-lethals are maintained as true breeding balanced lethal lines. Crosses between lines containing different *t*-lethals produce, in addition to tailless offspring T/t , some which have normal tails. These prove to be compound for the different *t*-lethals of the parental lines, e.g. $T/t^0 \times T/t^9$ yields normal-tailed offspring t^0/t^9 . Females of such compound genotypes are fertile; but males are completely sterile. (One exceptional fertile male will be discussed in a following paper.)

In other balanced tailless lines both tailless and normal-tailed young are born. The latter prove to be homozygous, t^n/t^n . Where such a 'viable *t*-allele' was derived from a wild population, the normal-tailed females are fertile but

all such males are completely sterile. Large numbers of normal-tailed males from each of three 'wild viable' lines (t^{w2} , t^{w8} , t^{w36}) have been tested without finding an exception. Normal-tailed males carrying any two of these alleles are also sterile.

Males which carry a 'wild viable' allele and a lethal allele are normal-tailed and sterile (genotypes tested t^0/t^{w2} , t^1/t^{w2} , t^1/t^{w8} , t^{12}/t^{w8} , t^0/t^{w36}).

Thus there are four sources of genotypically sterile males: compounds of two lethals, homozygotes of 'wild viable' alleles, compounds of different 'wild viable' alleles, compounds of a wild viable and a lethal *t*-allele.

Males homozygous for other viable *t*-alleles derived as exceptions from balanced lethal lines (cf. Dunn, Bennett & Beasley, 1962) may have normal or reduced sterility while males compound for such alleles and certain lethal alleles may be sterile, quasi-sterile or fertile depending on the alleles combined. These will be discussed in a second paper.

Examination of spermatozoa in vitro

The physical basis for genotypic sterility in males was investigated by studying the number and motility of spermatozoa introduced into the female genital tract at mating. These studies were initiated using the following procedures: single males of two proven fertile genotypes (T/t^{w35} and t^v/t^v , where t^v indicates a viable *t*-allele producing fertile homozygous males) and males of the proven mating-sterile genotypes t^0/t^{w36} and t^9/t^{12} were caged continuously with five normal females. The females were examined for vaginal plugs as evidence of mating each morning. Females with plugs were killed and dissected between 10.00 hours and 13.00 hours of the same day. At this time, the entire uterus and both oviducts were removed intact to a dry Petri dish where the oviducts were cut free with scissors from the uterus just above the uterotubal junction. The compression of cutting had the effect of sealing off the cut end of the oviduct, so that apparently no fluid escaped. The uterine contents were then expressed into a small dish, and an equal volume of tissue culture medium 199 added. Samples were examined immediately under the phase contrast microscope and scored for quantity of spermatozoa present and motility. The oviducts were then removed to a dry slide, and the thin-walled expanded ampullae where the eggs are collected at this stage were slit open and the contents allowed to flow out on to the slide where the drop so formed was immediately covered with a vaseline-ringed cover slip. The oviducts were then removed to another slide where they were flushed out with a small volume of normal saline solution introduced with a micropipette first through the cut end and then through the fimbrial end. The washings were cover-slipped as above and both preparations were examined under phase contrast and actual counts of the spermatozoa observed were made. These observations could be expected to provide information on sperm number and motility in these various portions of the female genital tract at an average of about 12 hr after insemination, since mating in the laboratory mouse is thought to occur usually between about 22.00 hours and 01.00 hours (Snell, 1956). However, since plugs were checked only once a day, and considerable individual variation is known to exist amongst females, the time interval could have been as long

as 24 hr. Because of the rather long and possibly very variable period of time interval in the initial observations, a second series of experiments was done using females induced to ovulate at a known time by hormonal treatment according to the method of Fowler & Edwards (1957). In this series, females which were in oestrus, but not yet ovulated, were presented to the same panel of males at 10.00 hours, examined for plugs 3 to 5 hr later and dissected immediately. In this case the time interval between mating and sperm examination was shortened to a maximum of 6 hr, and potential variability was decreased.

RESULTS

The indices of the quantity of uterine spermatozoa are shown in Table 1. There is clearly no significant difference in quantity of uterine spermatozoa between fertile and sterile males. It is, however, obvious that the numbers found in the uterus after 'morning plugs' are highly variable, while they are rather uniformly high within 6 hr after mating. The low numbers of spermatozoa

TABLE 1
DISTRIBUTION OF SPERM NUMBERS IN UTERI OF FEMALES EXAMINED: (1) WITHIN 5 HOURS OF FINDING MORNING PLUG, AND (2) WITHIN 6 HOURS OF MATING

Males	No. of samples	Estimate of sperm quantity				
		0	+	++	+++	++++
(1) Control T/t	49	8	15	4	14	8
Fertile t/t	11	8	2	—	1	4
Sterile t^x/t^n	32	5	7	4	6	10
(2) Control T/t	12	—	1	1	1	9
Fertile t/t	1	—	—	—	—	1
Sterile t^x/t^n	7	—	—	1	3	3

found in some samples of the first series are therefore probably largely due to a long time interval between mating and examination, which, as mentioned above, could be as much as 24 hr. For this reason, the motility estimates in Table 2 are presented only for samples in which uterine sperm counts were scored as 3+ or 4+, the argument being that this would reduce heterogeneity both with respect to time and to other possibly male-dependent factors. Table 2 shows unequivocally that, in spite of their normal numbers, spermatozoa from sterile males are only rarely found to be normally and fully motile in the uterus. For example, whereas thirteen samples in a total of twenty-three fertile males observed after an average time of 12 hr showed maximum motility, only one sample in seventeen from sterile males scored in this class. Conversely only five of the twenty-three samples from fertile males were completely non-motile at this time, while twelve of the seventeen specimens from sterile males were non-motile. The difference in motility was already apparent when the specimens were examined within 6 hr after insemination. As seen in Table 2 part (2),

although no spermatozoa from any group were recorded as being completely non-motile, ten of eleven fertile males produced spermatozoa of maximum

TABLE 2

DISTRIBUTION OF SPERM MOTILITY SCORES IN MALES SCORING 3+ OR 4+ ON QUANTITY OF UTERINE SPERM: (1) WITHIN 5 HOURS OF FINDING MORNING PLUG, AND (2) WITHIN 6 HOURS OF MATING

	<i>No. of samples</i>	<i>Non-motile</i>	<i>Moving slowly and/or abnormally</i>	<i>Motile</i>
(1) Control <i>T/t</i>	18	3	5	10
Fertile <i>t/t</i>	5	2	—	3
Sterile <i>t^x/tⁿ</i>	17	12	4	1
(2) Control <i>T/t</i>	10	—	1	9
Fertile <i>t/t</i>	1	—	—	1
Sterile <i>t^x/tⁿ</i>	6	—	5	1

TABLE 3

DISTRIBUTION OF SPERM NUMBERS COUNTED IN AMPULLAE AND IN OVIDUCTS FOR MATINGS DETECTED BY 'MORNING PLUGS' AND SCORED AS 3+ OR 4+ FOR QUANTITY OF UTERINE SPERM

<i>Male</i>	<i>No. of sperm. in ampullae</i>	<i>No. of sperm. in oviduct</i>	<i>No. of sperm. in ampullae</i>	<i>No. of sperm. in oviduct</i>
Control <i>T/t</i>	9 (1) 0 (2) 42 (2) 4 (1) 285 (2) 0 (1) 3 (1) 4 (1) 7 (2) 50 (2) 19 (2)	354 (1)	12 (2) 30 (2) 47 (2) 208 (1) 47 (1) 53 (2) 6 (1) 24 (2) 20 (1) 24 (1) 32 (2) 10 (1)	620 (1) 286 (1) 25 (1) 83 (1)
Fertile <i>t/t</i>	16 (1) 153 (1) 60 (2)	2500 (1)	0 (1) 12 (1) 14 (1) — (1) 0 (1) 1 (1) 0 (1) 8 (1) 0 (1)	19 (1) 37 (1) 38 (1) 14 (1) 0 (2) 12 (1) 0 (1) 5 (1) 2 (1) 1 (2) 8 (2)
Sterile <i>t^x/tⁿ</i>	0 (1) 8 (1) 0 (1) 1 (1) 1 (1) 21 (1) 2 (1) 1 (1) 0 (1) 21 (1) 16 (1)	12 (1) 24 (1) 46 (1) 4 (1) 1 (1) 5 (1) 9 (1) 7 (1) 14 (1) 1 (1)	0 (1) 12 (1) 14 (1) — (1) 0 (1) 1 (1) 0 (1) 8 (1) 0 (1) 0 (2) 0 (2) 0 (2)	19 (1) 37 (1) 38 (1) 14 (1) 0 (2) 12 (1) 0 (1) 5 (1) 2 (1) 1 (2) 8 (2)

(1) signifies one ampulla or oviduct, (2) signifies pooled contents of both ampullae or oviducts.

motility, while five of the six samples from sterile males were recorded as moving slowly or abnormally with a peculiar twitching motion which did not serve to propel them forward. These data thus tend to indicate that sterility

in males of the type examined here is related not to numbers of spermatozoa introduced at insemination, but to their reduced motility in the female genital tract.

The defective motility of uterine spermatozoa from sterile males appears, however, to be reflected in considerably reduced numbers of spermatozoa elsewhere in the female tract. Table 3 gives individual counts of spermatozoa at the site of potential fertilization in the ampulla, and in the entire oviduct after 'morning plugs' where uterine sperm quantity was again scored as 3+ or 4+; Table 4 summarizes these data in terms of ranges and averages. Although there is considerable variability there is no question but that spermatozoa from sterile males are not present in the tubes or ampullae in the numbers typical of control males. An average of only four spermatozoa/ampulla was obtained from sterile males, whereas the fertile controls averaged over thirty

TABLE 4
RANGES AND AVERAGE NUMBERS OF SPERMATOZOA IN AMPULLAE AND OVIDUCTS
FROM TABLE 3

Male	In ampulla			In oviduct		
	Range of sperm No.	Average	No. of samples	Range of sperm No.	Average	No. of samples
Control <i>T/t</i>	0 to 208	28	32	286 to 620	416	3
Fertile <i>t/t</i>	10 to 153	39	7	25 to 2500	869	3
Sterile <i>t^x/tⁿ</i>	0 to 21	4	26	0 to 46	11	24

TABLE 5
SPERM COUNTS IN OVIDUCTS OF FEMALES
STUDIED WITHIN 6 HOURS OF INSEMINATION

Male	No. of sperm./oviduct
Control <i>T/t</i>	227, 675
Fertile <i>t/t</i>	28, 42
Sterile <i>t^x/tⁿ</i>	0, 0, 1, 1, 4, 7, 12, 19, 23, 92, 119, 124, 325

Range for sterile males: 0 to 325.

Average for sterile males: 58.

at that site. The observations on sperm number in the whole oviduct were equally significant, where fertile males averaged over 500, with a maximum of 2500 spermatozoa recorded for one oviduct, but sterile males averaged only eleven/oviduct, with the high count for any one oviduct being only forty-six.

The numbers of spermatozoa present in the oviduct are to some extent a function of the time elapsed between insemination and examination. In Table 5 are data obtained within 6 hr of mating, which show, particularly for the sterile males, that sperm numbers in this region tend to be higher closer to the time of insemination. Although sperm counts from sterile males in the oviducts are far higher than in those examined at the later time they still do not approach the numbers found from fertile males in either series.

DISCUSSION

This type of genotypic sterility appears to be correlated not with reduced numbers of spermatozoa inseminated but with reduced motility, and low numbers at the site where fertilization should occur. The reason for the impaired motility is not known, but, whatever its cause, it seems reasonable to assume that the small numbers of spermatozoa found in the oviduct are in some way correlated with it. Considerable evidence, however, indicates that spermatozoa do not reach the site of fertilization by virtue of their own motility, but, whether alive or dead, are possibly carried there very rapidly by mechanical and/or fluid forces within the genital tract of the female (Howe & Black, 1963). We might suggest, therefore, that motility on the part of spermatozoa is necessary for them to remain in the oviduct or ampulla and to avoid being swept out at the fimbrial end. The fact that reducing the time interval between insemination by sterile males and examination results in finding both higher motility of uterine spermatozoa and also increased numbers in the oviduct would support this speculation.

The results recorded above are, in general, confirmatory of the similar studies on genotypically sterile or quasi-sterile males reported by Braden & Gluecksohn-Waelsch (1958). However, we have obtained clear-cut evidence for differences in sperm motility, whereas their findings were equivocal and their final conclusion was that the 'quality and duration of motility could not be shown to be significantly lower in infertile males'. Strangely enough, however, they found no spermatozoa at the site of fertilization in seven females 4 to 6 hr after mating with sterile males and essentially none in the oviducts in four females examined 4 to 6 hr after mating with quasi-sterile males, whereas for completely sterile males we found numbers in the ampulla ranging from none to twenty-one, at an average of 12 hr after insemination and much higher numbers (0 to 325) when entire oviducts were examined within 6 hr of mating. It is difficult to explain these differences in results, but even more difficult to explain why, since we did often find at least some spermatozoa at the site of fertilization, these males are completely sterile. The conclusion must be drawn that, even though some spermatozoa of genotypically sterile males are capable of reaching the site of fertilization and remaining there, and may even show motility, they are totally incapable of effecting fertilization.

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