STUDIES ON THE MECHANISM OF ABNORMAL TRANSMISSION RATIOS AT THE *T*-LOCUS IN THE HOUSE MOUSE

III. TEST FOR PHYSIOLOGICAL DIFFERENCES BETWEEN *t*¹- AND *T*-SPERM MANIFESTED DURING STORAGE PERIOD IN THE *EPIDIDYMIS* AND *VAS DEFERENS*

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The experimental results of the *in vitro* observations on sperm viability suggested that in moderately high *t*-ratio males, T/t^1 , the ratios has been altered, to a certain extent, before the sperm were ejaculated (Yanagisawa 1965). The next experiment were designed to investigate whether *t*-ratios are altered by ageing in the male genital organs,

Following meiosis, the spermatid undergoes a long period of spermiogenesis and then sperm pass into *epididymis* and from there the *vas deferens*. During this period they complete the process of maturation (Young 1929, 1931). Conditions within the *epididymis* are exceptionally favorable for survival of the sperm, but there is indication of some change in the sperm with age at the end of passage through the *epididymis* (Austin and Bishop 1957). When the sperm are not ejaculated, they gradually degenerate and are absorbed in the *cauda epididymis* and *vas deferens* (Simoene et al. 1931). It is a matter of practical experience that the first ejaculated semen of bulls after a period of rest contains a high proportion of non-motile sperm (De Groot 1961), and that some physiological characters of the sperm, such as the resistance to a cold shock, are lost after a long period of storage. Thus the quality of sperm in ejaculated semen seems to be directly related to mating frequency.

The present experiments were planned to test whether ageing affects *t*- and *T*-sperm differently during this long period of maturation and storage. The *epididymis* of males which had been tested the ratios was ligated, and the effect of ligation of the *epididymis* on *t*-transmission ratios was tested. When the *epididymis* is ligated, the sperm degenerate after a certain period of time. Preliminary experiments were performed to test the duration of maintenance of sperm fertility under such conditions.

MATERIALS AND METHODS

Sixteen fertile males in our laboratory stock were operated on to disconnect the

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caput- from the *cauda-epididymis*. Disconnection was done completely by ligating twice and cutting between the ligatures without any significant interference with the blood supply. The males operated on usually healed within a day. Each of these males was then put in a pen of 4 or 5 females at different intervals of 5, 7, 10 and 12 days after the operation, and the fertility was tested. Four males were used for tests at each interval.

Table 1. Fertility of the males with a disconnection between the *caput-* and the *cauda-epididymis*

1) 5 days after the operation				
Male	I	II	III	IV
Number of offspring	0	9	0	8
2) 7 days after the operation				
Male	X	VI	VII	VIII
Number of offspring	6	0	8	6
3) 10 days after the operation				
Male	IX	X	XI	XII
Number of offspring	0	0	6	0
4) 12 days after the operation				
Male	XIII	XIV	XV	XVI
Number of offspring	0	0	0	0

The results of these tests are given in Table 1. Five days after the operation, 2 out of 4 males tested produced offspring; 7 days after the operation, 3 out of 4 males gave offspring; after 10 days, 1 out of 4 males still gave offspring; however, after 12 days interval, none of 4 males tested fertile. These results lead to the conclusion that sperm in mice can maintain their fertility at least for 7 to 10 days in the *upper epididymis* and the *vas deferens*.

If the fertility of *t-* and *T*-sperm are lost at different rates in the *epididymis*, the *t*-ratio of the male heterozygotes should be altered in mating occurring 7 or 10 days after the operation. This was tested in the following experiment.

Fifteen fertile tailless male heterozygotes, T/t^1 or T/t^{12} which gave moderately high *t*-ratios and about 100 normal tailed females were used. The transmission ratios of the *t*-allele in each of these heterozygous males was tested under normal mating conditions, then the males were operated to disconnect between the *cauda* and the *caput-epididymides*. Seven days after the operation, each male was introduced into a pen of 6 females. Since the estrous cycle of a female is about 4 to 6 days, the male introduced will mate probably in that day. Thirteen out of 15 males tested gave offspring.

RESULTS AND DISCUSSION

The transmission ratios of *t*-alleles in the heterozygous males before and after the

Table 2. Male transmission ratios of alleles *t* and *T* before and after ligation of the *epididymis*

Male	before the ligation		after the ligation	
	<i>t</i> /+ : <i>T</i> /+	% of <i>t</i> /+	<i>t</i> /+ : <i>T</i> /+	% of <i>t</i> /+
<i>T/t</i> ¹				
I	23 : 6	79.3	1 : 6	14.3
II	26 : 6	78.1	4 : 1	80.0
III	29 : 4	87.9	7 : 1	87.5
Total	78 : 16	83.0	12 : 8	60.0
<i>T/t</i> ¹²				
I	39 : 0	100.0		
II	38 : 0	100.0	6 : 0	100.0
III	40 : 4	90.9	2 : 1	66.7
IV	37 : 4	86.0	6 : 0	100.0
V	33 : 3	91.7	9 : 2	81.8
VI	34 : 5	87.2	4 : 1	80.0
VII	36 : 2	94.8	5 : 0	100.0
VIII	38 : 5	88.4	6 : 1	85.7
IX	39 : 5	88.6	4 : 1	80.0
X	29 : 6	82.9	5 : 1	83.3
XI	36 : 5	87.8		
XII	35 : 5	87.5	7 : 1	87.5
Total	434 : 44	90.8	54 : 8	87.1

operation are given in Table 2. The average *t*-ratios before the operation in the heterozygotes, *T/t*¹ and *T/t*¹² were 83.0 and 90.8 percent, respectively. After the operation, the mean *t*-ratios in these males were reduced to 60.0 and 87.1 percent, respectively. The differences between *t*-ratios in these males and after the operation were not statistically significant.

The above results show that ageing does not affect *t*- and *T*-sperm differently during a period of storage in the *epididymis* and the *vas deferens*.

In order to reexamine this point, an effect of male mating frequency on the *t*-transmission ratios was also tested. Because, male mating frequency was assumed to relate to ageing of the sperm. If *t*- and *T*-sperm did not really differ their physiology during the storage period in the *epididymis*, the male mating frequency would not affect the *t*-transmission ratios.

The result of this test, however, showed that the *t*-alleles had a tendency to be transmitted lower than expected into the offspring that were born after frequent mating. This result should be examined again. This experiment has a weak point, because, in this experiment, a continuous supply fresh sperm from the *testis* to the *epididymis* would make the experimental result indistinct. We might expect, therefore, to obtain more clear cut results from the test by the ligation of the *epididymis*. At present, we

may say there would be no physiological difference between *t*- and *T*-sperm manifested during the storage period in the male genital organs.

In the previous papers (Yanagisawa, Dunn and Bennett 1961, Yanagisawa 1965), the experimental results indicated that *t*-sperm would have higher viability than *T*-sperm would *in vitro*, and that the difference in their viability after the ejaculation would alter the *t*-ratios. The results suggested also that *t*-ratios had been altered before the sperm were ejaculated. Experiments presented in this paper were carried out to investigate whether there was any physiological difference between *t*- and *T*-sperm manifested during storage period in the male genital organs, and the results obtained showed that there would be no physiological difference between these sperm in the male genital organs.

Questions are still left for further study. If there is no physiological difference between these sperm in the male genital organs, what causes would alter the ratios before the sperm are ejaculated? Why does *t*-sperm have higher viability than *T*-sperm have *in vitro*? Why does the viability of *t*-sperm differ from that of *T*-sperm *in vitro* but not differ from it in the male genital organs? In order to answer these questions and to solve the problem of abnormal transmission ratios of *t*-alleles, some other possible mechanisms should be explored for the effect of *t*-alleles on the male transmission ratios.

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