STUDIES ON THE MECHANISM OF ABNORMAL TRANSMISSION
RATIOS AT THE *T*-LOCUS IN THE HOUSE MOUSE
IV. SOME MORPHOLOGICAL STUDIES ON THE MATURE SPERM
IN MALES HETEROZYGOUS FOR *t*-ALLELES

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Received November 30, 1964

The previous studies on the mechanism of abnormal transmission ratios of *t*-allele suggested that in certain heterozygous males *t*- and *T*-sperm would differ in their physiology *in vitro*, but that they would not differ in it in the male genital organs (Braden 1958, Yanagisawa et al. 1961, Yanagisawa 1965).

The evidence presented seems to give a clue to explore further some possible mechanisms for the effect of *t*-alleles on the male transmission ratios. We may presume as one of the possibilities that a physiological difference between the two kinds of sperm would relate to difference of their motility, and that this is the reason why the two kinds of sperm show their physiological difference only *in vitro*. If this is so, difference of motility between these two kinds of sperm might depend on some thing like different amounts of their mitochondria, difference of their cellular metabolic activities or difference of their tails functions.

Depending on this presumption, first of all, gross morphology and fine structure of the mature sperm from the heterozygotes, especially their mitochondrial and tail's parts were examined. No significant morphological difference at the gross level could be found in the mature sperm. However, studies on the fine structure of the mature sperm revealed a significant evidence on the tail structure.

MATERIALS AND METHODS

Small pieces of the *vasa deferentia* (2 mm) were taken from heterozygous mice of genotypes *T/t*¹, *T/+* and from normal mice *+/+* as control. Two animals were examined in each genotype group. The tissues were fixed in 1% osmium tetroxide buffered to pH 7.4 with vernal-acetate buffer. After 2.5 hours fixation the tissues were dehydrated in increasing concentrations of ethyl alcohol (70, 90, 95 and 100%) and embedded in Epon. Polymerization was carried out at a temperature of 60°C for 4-5 days. Sections 25 mμ in thickness were cut on a microtome equipped with a glass knife; they

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were stained with lead acetate. Electron micrographs were taken with an RCA electron microscope at original magnification 10,000 to 40,000 and were enlarged photographically to the desired size.

OBSERVATIONS

1. *Fine structure of the mature sperm from normal mice (+/+)*

The head of the mouse sperm consists of the nucleus, the acrosome, the acrosomal cap (or head cap) and the postnuclear cap, like the sperm head of most other mammals. All of these parts are enclosed in a continuous cell membrane. The front of the head is covered by an acrosomal cap which stretches from the tip down to a transverse plane (Fig. 1 A) about 2/3 of way down the head. This cap consists of two membranes, an inner membrane and an outer one. These two membranes are continuous with one another at their posterior margin. The size of the acrosome is relatively small. A peculiar crevasse-like structure is usually seen in the acrosomal region.

The middle-piece is a cylindrical structure and consists of the axial filaments covered by a mitochondrial sheath (Fig. 2). The mitochondrial sheath is covered by a thin outer membrane. The internal structure of the mitochondrial helix differs from that of mitochondria in other tissues.

The tail fibrils are arranged in a well known characteristic pattern, 9+2 (Fig. 3A), that is, 9 double peripheral fibrils and 2 central ones. Outside of the row of 9 double fibrils there is a second row of 9 coarse fibers. Three out of the 9 coarse fibers are larger than the others. These coarse fibers seem to arise in the neck region and continue back ward through the middle piece and diminish gradually in size and then disappear at the posterior part of the tail (Fig. 1 B). The bundle of fibrils is surrounded by a dense fibrous sheath (tail helix). The tail helix disappears at the tip part of the tail where only the 11 fibrils and membrane remain (Fig. 1 B). The fine structure of the mature sperm from *T/+* males showed no difference from that of the sperm from normal males (+/+).

2. *Fine structure of mature sperm from T/t^1 mice*

In the observation of the sperm from 2 males heterozygous for *t*-alleles (*T/t¹*), however, striking evidence of abnormality was found in the cross sections of the tail at different levels. The sperm from these heterozygotes showed quite frequent abnormalities in the fibril configurations in their tail (Figs. 4 A and 4 D). Some cross sections through the middle piece of the sperm indicated the presence of 2 extra fibrils besides the normal 9+2 fibril pattern (Fig. 4 A). Some others demonstrated completely disorganized fibril pattern (Figs. 4 B and 4 C). Some sperm showed only 6 fibrils in cross sections through the tail (Fig. 4 D). It is very unlikely that these abnormalities are artefacts.

Cross sections through the sperm tail at different levels were chosen at random in the specimens and classified as to whether they were normal or abnormal in the fibril

Fig. 1 A. A longitudinal section of the anterior part of head of the normal mature sperm (+/+). Acrosome (Ac), Acrosomal cap (ACap), inner layer of the cap (IL), outer layer of the cap (OL), nuclear membrane (NuM), plasma membrane of the sperm (Pm) and sperm nucleus (SpN). $\times 140,000$.

B. Cross sections through the tail at different levels. $\times 85,000$.

Fig. 2. A logitudinal section and a cross section through the middle-piece in the normal mature sperm (+/+). Mitochondria (Mit), fibrils (Fib), coarse fibers (CFib) and superficial membrane (Mem). $\times 60,000$.

Figs. 3 A. and B. Longitudinal and cross sections through the principal piece of the normal mature sperm (+/+). Coarse fibers (CFib), fibrils (Fib), superficial membrane (Mem) and tail helix (THel). $\times 70,000$.

Figs. 4 A. - D. Cross sections through the middle-piece and the tails, at different levels, of the abnormal mature sperm from T/t^1 heterozygous males. The fibril patterns are abnormal. A. $\times 95,000$. B - D. $\times 70,000$.

pattern. In 5 specimens chosen at random from 3 heterozygotes T/t^1 , 35 out of 280 sperm tails examined were abnormal in the fibril pattern. On the other hand, one abnormal sperm was found out of 103 sperm tails observed in 4 specimens from 2 $T/+$ males and no abnormal sperm were found among 109 sperm examined in 3 specimens from 2 $+/+$ genotype males.

DISCUSSION

Studies on fine structure of the mature sperm in heterozygous males T/t^1 revealed an interesting fact that tail fibril pattern in some of the sperm was completely upset. These abnormalities of the tail structure seem to relate to males genotype. However, it is possible that the abnormality may be related to other conditions such as the health of the males. Further studies of electron microscopy on the mature sperm and the process of spermatogenesis in male heterozygous for various t -alleles would be required. If the relation between the frequency of the abnormal sperm formation and abnormal t -transmission ratios is established by further observations we will have another clue, besides the physiological hypothesis, bearing on the problem of the mechanism of abnormal transmission ratios of t -alleles.

There are very few descriptions of abnormal fibril patterns of motile flagella and cilia. It is not clear whether the flagella or cilia with an abnormal fibril patterns can be motile or not, but there is good evidence that all motile flagella and cilia of animals and protozoa have the characteristic 9+2 fibril pattern. This pattern seems to be the essential one for contraction of the flagella and the cilia. According to Satir (1961), non-motile cilia found in the ears of some insects have no central fibrils.

The evidence obtained would lead to make a hypothesis on the mechanism of the abnormal transmission ratios of t -alleles, if formation of the abnormal tail in the sperm is really related to the males genotypes, that none or less motile sperm with abnormal tail would be produced as effects of t -alleles, and that these sperm would lose a chance to fertilize eggs. Since t -bearing sperm are functionally in the majority, the abnormal sperm would be T -bearing.

The results of the present experiments, including the data of both the late mating and the *in vitro* tests of the sperm viability in the previous papers, seem to suggest a possibility that the behavior of the sperm can be affected, at least in some cases, by their genotype. There would be two possible ways for genotype to be expressed in the mature sperm. One would be direct expression of haploid gene activity in the mature sperm. However, this is very unlikely, because in the mature sperm most of the RNAs and the proteins are eliminated and the DNA is metabolically inactivated. There are a few reports which suggest the possibility of phenotypic expression of haploid gene activity in animal gametes, but this idea is not generally held. The other would be the effect of a gene activity expressed originally at some time during the early stage of

spermiogenesis and manifested it later in the mature sperm. During spermiogenesis young sperm cells are quite active in their metabolism and they undergo great changes in their morphology, accompanied by chemical and physiological alterations.

According to Fawcett (1959), a group of spermatids that came from one spermatocyte connects each other with big intercellular bridges. It is quite possible that *t*-alleles in the spermatids affect each other formation of their tail, and that *t*-bearing sperm form normal tails, but structure between these sperm would be manifested later as difference of their motility, and the difference of their motility would alter normal *t*-ratios to various abnormal ratios.

ACKNOWLEDGMENT

I would like to express my sincere gratitude to Professor L. C. Dunn and Professor D. Bennett for their guidance, encouragement and criticism through the whole course of this investigation. I would also like to express my appreciation to Professor L. D. Peachey, Dr. R. Goodman, Dr. Okano and Mr. D. Jasper for their great help in electron microscopy works.

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