DISTURBANCE OF MEIOSIS IN STERILE MALE LATI:CFLP MICE

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Investigation of genetic variation in laboratory animals has been the source of new data on spontaneous mutation in mammals and of new mutants, which can be used to study genetic determination of particular traits. Sterility in male mice is a pathological state under active investigation at the present time and which, in most cases, is genetic in nature [6, 8, 11]. This trait, together with "semisterility," is a diagnostic sign in the search for carriers of reciprocal translocations (RT) induced in parental sex cells. The frequency of spontaneous RT is low and, as a rule, it is indeterminable in control groups of mutation experiments. Colonies of laboratory mice in specialized institutes and nurseries producing large numbers of animals under conditions of strictly controlled reproduction, could be another source of information. Previously the writers studied chromosomal mutations in inbred mice from the "Stolbovaya" nursery, by analysis of meiosis in sterile males [3].

This paper gives the results of a similar investigation conducted at the Hungarian Institute of Laboratory Animals (Lati). When planning the investigation, the aim was to determine the frequency of male sterility in a colony of random-bred CFLP mice and, by studying meiosis, to determine whether sterility is connected with chromosomal mutuations.

In the process of breeding CFLP mice, which was undertaken with strict observance of a rotating system of mating and recording of all manipulations with the mice and their reproduction, sterile males were discovered. Sterility was confirmed by absence of reproduction after a further exchange of females. The standardized nature of the conditions under which the animals were kept reduced the possible role of nongenetic factors in the genesis of male sterility. Animals in Lati were free from pathogenic microorganisms (the SPF state), they were kept under constant conditions of temperature and light, and they received a granulated balanced diet produced actually in Lati.

Sterile males were killed by cervical dislocation, the testes were removed and weighed on torsion scales, and air-dried preparations of meiotic cells were obtained by the method in [6]. The preparations were stained with aceto-orcein. Spermatocytes were analyzed in the same way as during investigation of RT carriers detected in experiments with mutagens [2]. Fifty cells in the metaphase I stage (MI) of meiosis were examined for each male, but in a high proportion of males this number of cells could not be found. On examination of the preparation the general state of spermatogenesis was estimated (the presence of particular cell stages, and of modified mitotic chromosomes in spermatogonial metaphases).

EXPERIMENTAL RESULTS

Random-bred CFLP mice, imported into Hungary in 1974 from England, are large and highly fertile animals, each female of which produces about 10 young mice per month. Against the background of such high fertility, the detection of sterile individuals is not difficult. Among 3330 young males tested for ability to reproduce, 13 sterile individuals were discovered. The frequency of appearance of sterile males was only 0.33 ± 0.11% and, consequently, male sterility cannot have a significant effect on reproduction of the animals of this colony. These mice were random-bred and, consequently, heterozygous. The frequency of sterile males in inbred line C57BL/6 Jy is 3 times higher (0.98 ± 0.21%) [3].

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TABLE 1. Disturbances of Meiosis in Sterile Male Mice .

Mouse No.	Weight of two testes, mg	Number of MI of meiosis examined	Chromosomal aberrations in MI	Spermatogenesis
1	63	0	O	F
2	106	36	O	D
3	106	50	O	D
4	123	15	O	D
5	126	4	G	D
6	131	50	O	D
7	137	50	O	C, D
8	150	26	O	D
9	159	50	O	C, E
10	165	50	O	No spermatozoa, few spermatocytes, block in late pachytene of I MI
11	166	18	O	B, D
12	169	26	G	B, D
13	181	50	O	B, D
14	187	32	O	B, D
15	201	50	O	A
16	208	50	O	A
17	221	50	O	A
18	239	50	O	A
19	2444	50	O	A
20	280	50	O	A
21	298	50	O	A
22	329	50	O	A

Legend. A) Normal picture of spermatogenesis, B) no spermatozoa, C) few spermatozoa, D) few dividing spermatocytes, block in late pachytene of MI, E) normal meiosis, F) no dividing spermatocytes, block to meiosis in early prophase, G) fragments in two MI.

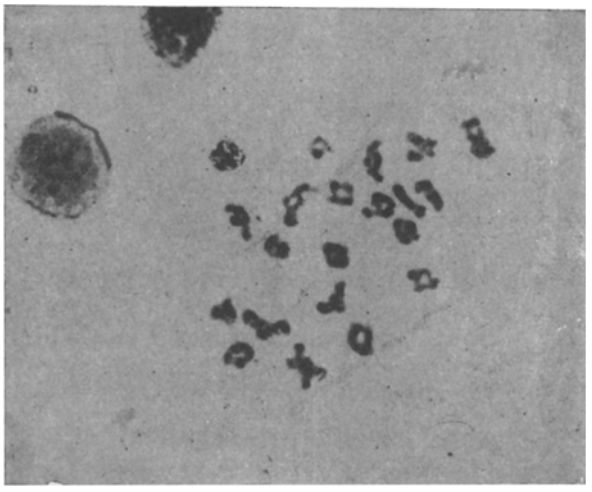

Fig. 1. Metaphase I of meiosis of a sterile male; normal formation of bivalents.

The mean weight of two testes in normal CFLP males is 261.4 ± 8.7 mg. The weight of these testes in the majority of sterile males was much less. Examination of preparations of meiosis of 13 sterile males revealed a disturbance only of spermatogenesis, expressed as the formation of spermatozoa with an abnormal shape of head, in five males which had normal testes.

Disturbances in the earlier stages of spermatogenesis and a decrease in the number of cells at the MI stage were found in the remaining eight males. It was often impossible to

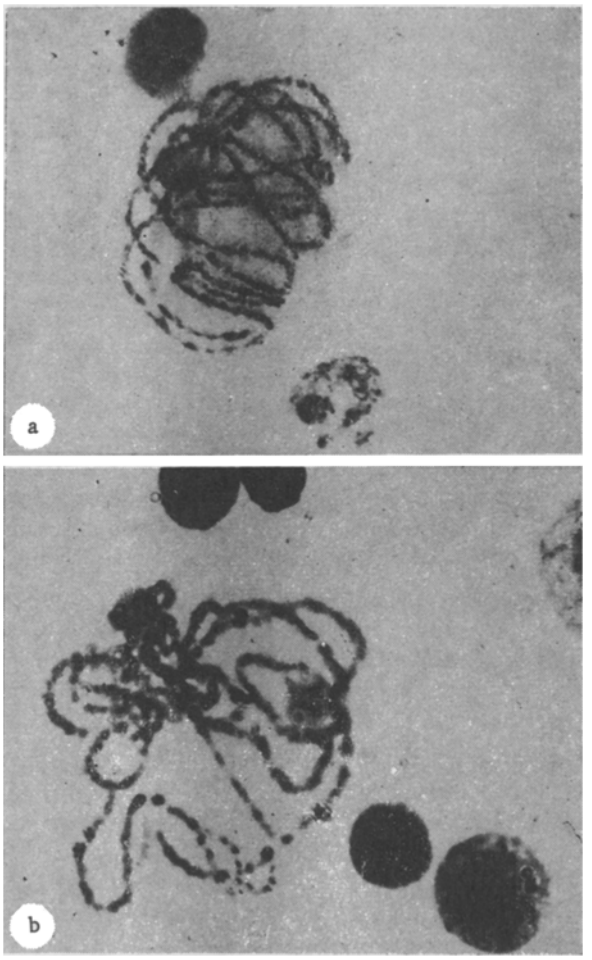

Fig. 2. Pachytene chromosomes in spermatocytes of sterile male mice (1000 ×).

find 50 cells in preparations from these males, although normally several hundred MI can be found always in preparations obtained by this method.

A further nine sterile males were discovered in addition, but they were found among an unknown number of parents, and for that reason they are not included in the calculation of the frequency of male sterility in the colony.

The minimal weight of the testes (63 mg) was found in male No. 1, in whose testes spermatocytes were totally absent, probably because of death in the early prophase or spermatogonial stage (Table 1). The decrease in weight of the testes in sterile males heterozygous for RT on account of death of spermatocytes is well known [10]. This unique male possibly had a chromosomal aberration.

The remaining males were divided into two groups: 13 males (Nos. 2-14) with disturbances of meiosis and eight (Nos. 15-22) with no visible disturbances (Table 1). The mean weight of the testes in the animals of group 1 (146.6 ± 7.5 mg) was significantly ($P < 0.001$) lower than that of the males of group 2 (252.5 ± 16.3 mg). Since the males of group 2 had no visible disturbances of meiosis, the possible cause of their sterility could be the effect of recessive t-alleles [4], which is a usual feature of populations of laboratory and wild mice, or developmental defects.

Animals of one group, which had similar cytological disturbances, probably due to one cause, are very interesting. Examination of cells found in the MI stage revealed no associations of chromosomes specific for heterozygotes for RT (Fig. 1). No marker chromosomes (clearly altered in size, or metacentric) could be found in the spermatogonial mitotic metaphases. The unusual morphology of the pachytene bivalents is noteworthy: the have an extremely heterogeneous structure, with regions differing sharply in the degree of condensation of their chomatin. The bivalents were "connected" by their ends into groups (in most cases into three groups), into which no sex vesicle is incorporated (Fig. 2), probably by nucleolar materi-

al. These males had very few cells in the MI stage, and degenrating, palely stained metaphases with bivalents still connected with bands of some kind of material, were present.

Pachytene chromosomes with similar morphology were found previously in a sterile male with chemically induced RT [1]. The males studied probably also had chromosomes with a very minor aberration, not interfering with the formation of bivalents. A second possible cause of sterility could be a gene mutuation affecting meiosis. In any case, some mice in the population are carriers of a genetic factor with an unusal effect. The pattern of disturbance of meiosis suggests a disturbance of the time sequence of activity or disintegration of the nucleolus and coiling of the chromosomes in sterile males. A disturbance of metabolism in meiotic cells of sterile males heterozygous for RT and, in particular, of DNA metabolism, has been reported [8, 9].

The presence of bands forming connections between bivalents, consisting most probably of nucleolar matieral, is possibly due to a disturbance of functioning of one region of the nucelolar organizer. Some chemically induced RT also perhaps caused damage to chromosomes in these regions. Chemical mutagens, especially if used in small doses, are known to injure the ends of chromosomes more frequently and to induce RT responsible for male sterility. Chemical mutagens may perhaps act primarily on the ends of chromosomes near the nuclear membrane, pass through the membrane into the cell, and relatively often (compared with radiation) damage regions of the nucleolar organizer, and these injuries lead to a block of meiosis.

The genetic load of the population in a colony of Lati:CFLP mice thus includes a factor (chromosomal abberation or gene mutuation) disturbing processes taking place in prophase of meiosis in spermatocytes, causing their death and total sterility of males. These animals may be used as a model for cytogenetic research into meiosis in mammals.

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