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PRODUCTION OF HERITABLE PARTIAL STERILITY IN THE MOUSE BY METHYL METHANESULPHONATE

BY

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Partial sterility, in the sense of reduced reproductive capacity, has been demonstrated in the F_1 progeny of male mice mated in the second week after a single dose of methyl methanesulphonate (50 mg/kg, intraperitoneally) with females of proved fertility. Sterility, both partial and complete, was encountered in the F_2 generation obtained by mating F_1 males and females with fertile partners. These results show that the compound, in a substerilizing dose, is capable of producing transmissible genetic damage. It is suggested that the procedure used is a practicable method of testing drugs for possible genetic effects.

Certain simple alkane sulphonic esters produce characteristic episodes of sterility in male rats and mice (Jackson, Fox & Craig, 1961). For example, a single administration of methyl methanesulphonate ($CH_3.SO_2.OCH_3$) to male mice will interfere partially or completely—according to the dosage—with their capacity to fertilize females during the first and second week after treatment, after which normal fertility returns. Mating is unaffected by these compounds; the results observed are related exclusively to effects on certain stages of spermatogenesis, that is spermatid development and on morphologically mature sperm in the epididymis. The precise nature of their action—presumably an alkylation of cellular material —is undecided. The possibility of application of compounds of this type to the control of fertility in man raises the problem of toxicity. The more conventional aspects of the toxicology of methyl methanesulphonate have been studied in connection with its possible use in the treatment of malignant disease (Duvall, 1962). The fact that this compound is known to be mutagenic for *Drosophila* (Fahmy & Fahmy, 1957, 1961), barley (Minocha & Arnason, 1962) and certain bacteria (Strauss, 1961), and the possibility that its effects upon fertility in laboratory rodents involves mutation in the germ cells, has prompted this present study. An attempt has been made to assess this possibility by an examination of the reproductive capacity of offspring from male mice given a substerilizing dose of this compound.

METHODS

Randomly mated albino mice (Alderley Park Strain I) from the specific-pathogen free colony at I.C.I. Ltd., Pharmaceuticals Division, were used throughout the present experiments. Sixty males of proved fertility were each given a single intraperitoneal injection of methyl

methanesulphonate (50 mg in 1 ml. of arachis oil per kg body weight) and a similar number of control male mice of similar age and weight were given arachis oil (1 ml/kg).

Six days after treatment, each male was paired with a fertile female; successful matings were identified by vaginal plug formation and the females were allowed to bear their litters. Approximately one-half of the males given methyl methanesulphonate fathered litters of reduced size and the F_1 males from these litters were kept for testing. An equivalent number of F_1 males were selected from the control series, firstly from small litters, then at random from other F_1 litters. All F_1 females were discarded. Control data were further supplemented by results obtained with untreated mice from the breeding colony. The F_1 males of both experimental and control groups were tested as follows:

Fertility tests

(*i*) *By litter size.* The fertility of each F_1 male was initially determined by mating with a fertile female. Those fathering litters below average size (less than nine, see Table 3) were remated with a minimum of three females which were allowed to bear their litters, to decide whether the males were consistently subfertile.

(*ii*) *By autopsy of pregnant females.* All males suspected of partial sterility on the basis of the litter-size tests were subsequently paired with two fertile females which were killed on the 13th day of pregnancy. Their uteri were examined for living and dead embryos and the numbers of corpora lutea were also recorded. Diagnosis of partial sterility was based on the criteria of Carter, Lyon & Phillips (1955).

Histology

After tests by mating and autopsy, suspected males were killed by cervical dislocation. The testes were removed, dissected free from fat, fixed in formol-saline followed by Helly's fluid, embedded in paraffin wax, sectioned at 7 μ and stained in periodic acid-Schiff followed by haematoxylin.

The F_2 generation

At least one litter fathered by every F_1 male suspected of partial sterility was kept and both male and female offspring were subjected to fertility tests. F_2 males were each provided with three fertile females and two of these were subjected to autopsy as previously described. F_2 females were allowed to have three successive litters by a normal male.

RESULTS

Control series

From the control series four mice fathered litters of nine or less (Table 1). Of the males from these litters, one died during a first fertility test. Twelve of the remainder fathered litters of normal size. Further matings of the other two males (16/2 and 4/1) gave results suggesting that their reduced fertility was not of genetical origin (Table 2,A).

Since eighty-two F_1 males were tested in the experimental series and only fifteen in the initial control series (Table 1), a further sixty-seven F_1 males were selected at random from the normal-sized F_1 control litters. These males were each paired with one female. Fifty-two produced normal-size litters and fourteen produced six or less offspring, or litters which were eaten. Each of these fourteen was subsequently mated to two or more fertile females (Table 2,B). Two (13/2 and 20/2) died during the test and, of the remainder, all but three (1/1, 20/1 and 44/2) produced one or more normal-size litters. Autopsy tests on females mated with

TABLE 1

REDUCTION IN LITTER SIZE CAUSED BY METHYL METHANESULPHONATE

Matings were 6 to 12 days after treatment with methyl methanesulphonate, which was given intraperitoneally in arachis oil, in a dose of 50 μg/kg. The results are contrasted with those for control animals given vehicle alone (1 ml./kg). Litters of normal size had more than nine offspring, those of reduced size had nine or less. Forty-eight males produced litters in each of the control and treated groups

	Treated	Control
A. Litters of normal size		
Number of litters	16	44
Total offspring	184	500
Average litter size	11·5	11·3
B. Litters of reduced size		
Number of litters	32	4
Total offspring	184	28
Average litter size	5·7	7·0
Number of F_1 males from these litters tested	82	15

these three males showed that the frequency of dead implantations did not exceed the number expected by chance.

In a supplementary control series, 23 out of a total of 128 litters from untreated stock mice contained nine offspring or less (Table 3,*A*). The males fathering these small litters were remated with fertile females (two females per male) which were autopsied on the 13th day of gestation. Forty-one pregnancies and two pseudopregnancies were recorded, one male mated once and one other failed to mate. Approximately 5% of the embryos were found to have died soon after implantation and less than 0.5% during later stages of gestation. The average number of implantations per mating was 11.5 and the fertility of individual males showed

TABLE 2

BREEDING PERFORMANCE OF F_1 MALE OFFSPRING OF CONTROL MICE ASSESSED BY LITTER SIZE AND AUTOPSY TESTS

* One male lost; E, litter eaten. † Male died after the first mating

A. Males from small (control) litters				B. Males from normal (control) litters			
F_1 male no.	Litter size in 2 or 3 additional matings	Implants at autopsy, two additional matings		F_1 male no.*	Litter size in 2 to 3 additional matings	Implants at autopsy, two additional matings	
		Live	Dead			Live	Dead
6/2	13, 7			1/1	6, 8, 6	18	1
4/3	13, 8			50/2	6, 12		
16/3	13, 9			1/2	6, 13		
16/6	13, 10			13/2†	6		
6/1	12, 11			44/2	5, 8	21	3
19/2	12, 13			41/1	5, 15		
16/2	11, 2, 7	23	2	20/1	4, 5, 10	19	2
16/1	11, 10			33/2	3, 14		
16/4	11, 14			20/2†	E		
4/2	10, 13			43/2	E, 0, 14		
4/3	10, 12			30/1	E, 9, 14		
16/5	9, 12			52/2	E, 12, 8		
19/1	7, 12			41/2	E, 13, 15		
4/1	4, 14, 5	16	2	49/2	E, 14, 9		

TABLE 3

SUMMARY OF BREEDING PERFORMANCE OF UNTREATED MALES FROM NORMAL BREEDING STOCK

A.	*Initial performance*	
	No. of matings studied	128
	Average litter size	9·7
	No. of males fathering litters of nine offspring or less	23
B.	*Autopsy tests on low litter males*	
	No. of fertile matings at autopsy tests of these 23 males	41
	No. of pseudopregnancies	2
	Total live implants	449
	No. of dead implants: Early	23
	Late	2
	Average no. of implants per mating (live and dead)	11·5

little deviation from this figure (Table 3,*B*). Two males in this group showed reduced fertility but autopsy tests (results not given) showed that this could not be attributed to hereditary partial sterility.

Experimental series

Of the sixty males paired during the 2nd week after treatment with methyl methanesulphonate, thirty-two fathered litters of nine or less, providing a total of eighty-two F_1 males for fertility tests (Table 1). Small litters (six or less) resulted from pairing thirteen of these F_1 males, and further matings of the same animals indicated that two were fertile and five partially sterile, whilst results from the remaining six were inconclusive (Table 4).

TABLE 4

BREEDING PERFORMANCE OF F_1 MALE OFFSPRING FROM MALE MICE TREATED WITH METHYL METHANESULPHONATE

Results relate to males from low litters, also giving small (F_2) litters at first mating. E, litter eaten; ‡, no matings recorded (females of proved fertility present for 6 to 8 weeks); 0, plug recorded but no litters; †, mating with only one female; *, offspring tested, see Table 5

F_1 male no.	First test Litter size	Subsequent tests: Litter size	Subsequent tests: Implants, two additional matings: Live	Subsequent tests: Implants, two additional matings: Dead	Diagnosis
4/3*	4	12, 7, 7	17	2	Fertile (see Table 5)
33/3*	5	12, 7, 7, 15	20	4	Fertile (see Table 5)
6/2	E	8, ‡	2	6†	Partially sterile
9/2*	E	7, 7, 5, 4	‡	‡	Partially sterile
24/1*	E	10, 7, 3, 5	7	14	Partially sterile
55/1*	5	5, 8, 1, 1	9	12	Partially sterile
55/2	3	10, 1, ♂ died	—	—	Partially sterile
4/1	E	13, 9, ‡	‡	‡	Results inconclusive
4/2	E (8)	12, 10, E (6)	‡	‡	Results inconclusive
4/6	E	‡	10	1†	Results inconclusive
32/1	E	0, 0, 13	‡	‡	Results inconclusive
1/1	4	♂ died	—	—	Results inconclusive
6/4	2	♂ died	—	—	Results inconclusive

The testes of ten of these thirteen F_1 males (all in Table 4 except for 1/1, 6/4 and 55/2) were examined histologically. Spermatogenesis was normal in seven of these and also in the five "low fertility" males of the control series previously mentioned (16/2, 4/1, 1/1, 44/2 and 20/1, Table 2). In three of the F_1 males from the treated series (4/1, 9/2 and 24/1, Table 4), two of which were diagnosed as partially sterile, the testes were somewhat reduced in size although spermatogenesis seemed normal in most tubules. There was some evidence of retarded spermatid development and degeneration of these cells in some testis tubules. In general, the histological picture provided little information regarding the functional state of the testes.

The F_2 generation

Twenty-six offspring of F_1 males 4/3, 33/3, 9/2, 24/1, and 55/1 (see Table 4) were kept for mating tests and the results are shown in Table 5. Progeny from 6/2 were not available for study. From the animals considered partially sterile (namely 9/2, 24/1 and 55/1 in Table 4), there was a sterile male among the progeny of 24/1, for no offspring were produced during 8 weeks with four fertile females (Table 5). From male 9/2, one F_2 male (No. 3) was partially sterile; from male 55/1, one male (No. 5) and one female (No. 8) were also consistently subfertile (Table 5).

Both the F_1 males considered to be fertile (4/3 and 33/3, Table 4) provided suspicious results in the F_2 generation. Thus male 4/3 produced some partially sterile offspring, whilst three of four males from 33/3 produced low litters, although

TABLE 5

BREEDING PERFORMANCE OF F_2 MALE AND FEMALE DESCENDANTS OF MALE MICE TREATED WITH METHYL METHANESULPHONATE

*, Only one of two females inseminated; †, F_2 male or female diagnosed as partially sterile

	F_2 males				F_2 females	
			Implants at autopsy, two additional matings			
F_1 male no.	Male no.	Litter size	Live	Dead	Female no.	Litter sizes
9/2	3	6	7	12†	1	11, 13
					2	11, 11
24/1	20	0	0	0	17	14, 10
					18	12, 11
					19	11, 12
55/1	5	6	12	9†	8	4, 2, 7†
	6	12	10	2*	9	12, 9, 13
	7	12	19	1		
4/3	13	6	13	8†	10	12, 10
	14	8	11	14†	11	9, 14
	15	♂ died			12	9, 12
33/3	25	0	15	3	21	9, 14
	26	3 (4)	19	1	22	6, 8, 16
	27	13	—	—	23	7, 9, 14
	28	8	14	2	24	6, 0, 0

the autopsy results from subsequent matings were not abnormal. The reproductive capacity of the F_2 females from these two animals appeared generally inferior, particularly in one animal (female No. 24, Table 5).

DISCUSSION

Hereditary partial sterility was first described in male and female progeny of X-irradiated mice by Snell (1933, 1935). A number of partially sterile lines was established and bred for several generations. Hertwig (1938, 1939) confirmed Snell's findings and found that about half of the embryos in her semi-sterile lines usually died at or soon after implantation. If affected embryos came to term they were grossly abnormal, brain hernia being the most common lesion. Similar abnormalities were described in detail by Snell & Picken (1935). Later, Koller & Auerbach (1941) and Koller (1944) demonstrated cytologically that translocations were involved in some partially sterile lines and genetic evidence for this was obtained by Snell (1941, 1946) using genetic tags and laborious linkage tests. As the result of this work it is now accepted that translocation (interchange of segments between two chromosomes) is the cause of hereditary partial sterility.

The incidence of hereditary partial sterility in control populations was assessed concurrently in the majority of these studies. Snell (1935) found no clearcut cases of translocations in his controls after sampling 106 animals (male and female). The relatively high incidence reported by Hertwig (1938, 1940) was considered by Russell (1954) to be open to doubt as suspect animals were not tested for transmission of semisterility to their descendants. Charles (1950) found only two cases of semisterility in 2,755 control animals.

In the present control series, five of eighty-two F_1 males produced low litters (Table 2) but the autopsy technique showed that none could be classified as partially sterile. This was also true of the twenty-three untreated stock males selected for further test because they had fathered rather small litters.

Thus it appears that, in general, the incidence of hereditary partial sterility in breeding stocks is low.

Among seventy-one progeny of male mice given near-lethal doses of nitrogen mustard (HN_2), Falconer, Slizynski & Auerbach (1952) found two sterile males, two partially sterile animals and two suspected of partial sterility. Cytological study revealed associated translocations. This is a low incidence compared with a figure of 35% reported after X-irradiation (600 *r*) of the testes of mice (Russell, 1950). Tretamine seems to be the only other compound studied in this respect (Cattanach, 1957, 1959). Sterile and semisterile F_1 animals were found, the majority were examined cytologically, and the partial sterility was correlated with the presence of translocations. Of seventy-four animals tested, 10 to 13 days after treatment, twelve were sterile and ten partially sterile.

The present experiments do not justify a conclusion as to the precise frequency of occurrence of partially sterile animals in the F_1 progeny of mice treated with methyl methanesulphonate, since only eighty-two " low litter " offspring of a total of 151 F_1 males were tested. However, five cases of partial sterility (about 6%)

were confirmed (Table 4), a much lower incidence than after tretamine, although similar to that reported after nitrogen mustard (Falconer *et al.*, 1952).

In both the latter investigations, approximately equal numbers of sterile and partially sterile individuals were found and in this respect our results differ significantly, for no sterile animals were found in the F_1 generation after treatment with methyl methanesulphonate. This may be a question of dose, although twice the amount used would produce sterility in treated males for the next 2 weeks. No obvious toxic effects resulted from the dose used (rather less than the 0.5 LD50), and animals were subfertile only during the most sensitive period (5 to 11 days). In the nitrogen mustard experiments referred to above, the animals suffered severe toxic effects and thirteen of the eighteen treated males died or had to be killed during the first 9 days after treatment. On the other hand, the dose of tretamine used by Cattanach (1959) was well below the LD50, yet a considerable number of sterile animals was produced. It thus appears that tretamine is unique among mutagens so far tested, in producing high numbers of both sterile and partially sterile animals at low doses.

It was suggested by Cattanach (1959) that the mechanisms by which sterile and partially sterile animals are produced are similar. Thus, chromosome breakage and reunion, known to cause translocations and hence partial sterility, may also cause sterility when the result is deficiency of genes necessary for some stage of spermatogenesis. On this basis, however, it is difficult to explain the absence of sterile F_1 animals after methyl methanesulphonate.

It would appear that the study of partial sterility in the F_1 progeny of treated animals is a practicable method of testing drugs for possible genetic effects. The incidence of partial sterility in control stocks is so low that the recovery of even small numbers of partially sterile animals is highly significant.

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