TRANSLOCATIONS, THE PREDOMINANT CAUSE OF TOTAL STERILITY IN SONS OF MICE TREATED WITH MUTAGENS[1]

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ABSTRACT

Histological and cytological analyses of the testes were carried out in 42 sterile sons of males treated in the spermatozoal or spermatid stage with 250 mg/kg ethyl methanesulfonate (EMS) alone or after prefeeding with butylated hydroxytoluene (BHT); or treated with 200 R X-rays. Of the 42 sterile males, 17 had some mature spermatids, nine were blocked at diakinesis, 15 were blocked in pachytene, and one lacked spermatogenic cells altogether, having Sertoli cells only. Mitotic (spermatogonial) metaphases could therefore be analyzed in 41 of the males and meiotic configurations in 26.——(1) None of the males showed abnormalities in chromosome number, such as monosomy, trisomy, or mosaicism for either of these conditions. Certain classes of chromosome abnormalities that have been found associated with male sterility in other investigations, namely trisomies, XXY's, and X-autosome translocations, are not expected from treatment of 19A + Y cells when F_1 males are studied. (2) A very high percentage of the sterile males carried translocations. Direct meiotic evidence for this was found in 22 of the animals. In addition, 11 of the 16 that were blocked (or virtually blocked) in pachytene, and thus could be analyzed in mitosis only, consistently showed one abnormally short chromosome (or, one short plus one long), which presumably had resulted from unequal exchange (or sizable deficiency). Of the meiotically detected translocation males, 1 carried a T(A;Y), 17 had single autosomal translocations, and 4 had multiple autosomal rearrangements involving three, four, four, and six breaks, respectively. In addition, three males showed failure of X-Y pairing. (3) Translocations that cause sterility, rather than partial sterility, in males appear to be those in which at least one of the breaks occurs close to one end of a chromosome. The mitotic and meiotic evidences for this were found to be correlated. (4) It is proposed that many cases of induced F_1 male sterility may be the result of position effects produced when paracentromeric regions are translocated to euchromatic regions of certain other chromosomes. Since many translocations that produce partial sterility in the female cause complete sterility in the male, the male must be assumed to be more susceptible to disturbances of fertility by the postulated mechanism. (5) There is evidence that EMS, especially in the lower dose range, more often breaks chromosomes near one of their ends than does X-irradiation.

WHEN male mice are treated with X-rays or mutagenic chemicals, their F_1 offspring show various degrees of fertility. Most are fully fertile, but a cer-

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tain portion, depending upon the dose and treated stage in spermatogenesis, are either "partially sterile" or completely sterile. W. L. RUSSELL's review (1954) indicated a frequency of about 10% F_1 sterile male offspring from presterile-period matings of males irradiated with 700 R, or slightly less than one-half the rate of induction of partially steriles. Relatively high frequencies of sterility have also been found in the sons of males treated in post-spermatogonial stages with mutagenic chemicals, such as triethylenemelamine (TEM) (CATTANACH 1959) and ethyl methanesulfonate (EMS) (CATTANACH, POLLARD and ISAACSON 1968; GENEROSO *et al.*, in preparation). In the various experiments reported by those investigators, the relative frequency of F_1 steriles ranged from about one-half that of partially steriles to approximate equality with the partially sterile group.

"Partial sterility" has long been known to be due to the presence of translocations (reviews by W. L. RUSSELL 1954; L. B. RUSSELL 1962) and results from the death of segregants derived from unbalanced gametes. On the other hand, sterile F_1 males have not been as well studied, although there are some indications that induced complete sterility of F_1 males, like partial sterility, may be due primarily to the presence of translocations (LYON and MEREDITH 1966; CATTANACH *et al.* 1968).

Certain classes of translocations in mice have been known for some time to be associated with male sterility. Thus, all of the eight known reciprocal X-autosome translocations were shown by RUSSELL and BANGHAM (1961), RUSSELL and MONTGOMERY (1970), L. B. RUSSELL (1972), and LYON *et al.* (1964) to be male sterile and female partially sterile in the heterogous state. Subsequently it was shown by LYON and MEREDITH (1966) that a similar sex difference can also exist for the effects of some autosomal translocations. A few translocations involving the Y chromosome have been described and found to cause male sterility (GRIFFEN and BUNKER 1967; LÉONARD and DEKNUDT 1969).

Certain types of chromosome imbalance, where additional sex chromosomes (RUSSELL and CHU 1961; CATTANACH 1961a; CATTANACH and POLLARD 1969) or autosomal portions (CATTANACH 1964; GRIFFEN and BUNKER 1964; GRIFFEN 1967) are present, have also been related to male sterility. In addition to numerical abnormalities or structural rearrangements of chromosmes, male sterility in mice can also result from gene mutations (GREEN 1966).

The present study involves populations of completely sterile F_1 males resulting from X-ray and EMS treatments of male mice. Testes were examined histologically to determine the nature of the sterility and analyzed cytologically for abnormalities in chromosome number, morphology, and behavior. The results provide additional information on the causes of male sterility in mice.

MATERIALS AND METHODS

The males used in this study were F_1 sons from three experimental series. One was an X-ray series (by L. B. RUSSELL), in which the sterile sons came from matings in the second week, corresponding to early spermatozoa and late spermatids (OAKBERG and DIMINNO 1960), after 200 R given to 3-month-old (101 × C3H)F_1 males. Two series (by R. B. CUMMING) involved EMS treatments of 250 mg/kg given by intraperitoneal injection to 3-month-old (C3H × 101)F_1 males. In one of these series (to be referred to as EMS), this was the only treatment; in the

other (to be referred to as BHT + EMS), the animals were pre-fed with 0.75% butylated hydroxytoluene (BHT), which was incorporated in the pellets of Purina Lab Chow, 1 month prior to EMS treatment. In the two EMS series, as in the X-ray series, the sterile sons came from treated spermatozoa and spermatids. Altogether, 42 sterile sons were studied—13, 17, and 12 from the X-ray, EMS, and BHT + EMS series, respectively.

Sterility of these sons of treated males had been established as follows. For the X-ray experiment, each F_1 male was mated to one female of the FR or RAPA stocks.* If no pregnancy occurred after four weeks, each male was mated to three new females of the same stock for three months, for confirmation of sterility. In the EMS and BHT + EMS experiments, each F_1 male was caged with a T-stock* female. The females were checked for signs of pregnancy for four weeks. If none were observed, the T-stock female was replaced by two $(C3H_f \times C57BL10)F_1$ females, which remained with the F_1 male for eight weeks. None of the 42 sterile males showed any abnormalities in size or external phenotype.

The testes of the sterile males were removed when the mice were 6 months of age in the X-ray experiment and 18 months in the EMS and BHT + EMS experiments. One testis from each male was fixed in Bouin's, sectioned at 8 μm, and stained with hematoxylin and eosin. These sections were used to evaluate the stages of spermatogenesis present and to determine where the blockage occurred. Squash preparations were made from the other testis as follows.

The testis was placed in a petri dish with isotonic NaCl solution (0.85%), the tunica was removed, and the tubules were spread out under a dissecting microscope, taking care not to break them. The tubules were then placed in hypotonic sodium citrate solution (0.7%) for 7 minutes, and subsequently fixed in a mixture of 3 parts methanol and 1 part glacial acetic acid for at least 30 minutes. Strands of fixed tubules were placed on a slide with one or two drops of 50% acetic acid and were cut into small pieces with the aid of dissecting needles. With this treatment, the cells ooze out and the tubules become transparent. A silicon-coated coverslip was placed on top of the material and squashed with thumb pressure or with a Franco Arbor Press. The degree of chromosome dispersion was checked under a phase-contrast microscope. The slide was quickly immersed in liquid nitrogen; and, once frozen, the cover slip was removed with a scalpel. The material which remained on the slide was then placed in fixative (3 parts methanol : 1 part glacial acetic acid) for 15 to 30 minutes. After this second fixation, the slides were air-dried, stained with aceto orcein for 15 minutes, washed through 95% alcohol (three times) and absolute alcohol (two times), and finally mounted in "Euparal", using a new cover slip.

The squashes were used for mitotic and meiotic analyses, using spermatogonial metaphases for the former and spermatocytes in diakinesis for the latter. Karyotype analysis of spermatogonial metaphases can reveal numerical abnormalities and certain kinds of structural rearrangements. Owing to the acrocentric nature of mouse chromosomes, the only interchanges that can be detected at mitosis with the stains used here are those that result in the formation of metacentrics or submetacentrics, or of an unmatched long or an unmatched short (in addition to the Y) chromosome, or both. Meiotic analysis, which can reveal interchanges through abnormal chromosome associations, could be carried out only in testes in which no block in spermatogenesis occurred prior to diakinesis.

To determine whether chromosomal differences could be detected between sterile and partially sterile males, some partially sterile males from the same experiments were also analyzed cytologically.

RESULTS

(1) Mitotic studies: Results of the analysis of 1128 spermatogonial metaphases are shown in Tables 1 and 2. It is clear from these results that no case of sterility among the 42 F_1 males studied could be attributed to any anomaly in chromosome number. The great majority of cells scored for each F_1 male have the diploid num-

* FR, RAPA, and T are non-inbred stocks carrying various combinations of recessive markers that were required for other purposes of these experiments.

TABLE 1

Chromosome counts and observations in spermatogonial metaphases of sterile sons of X-irradiated males

F_1♂ no.	No. of cells with chromosome number					Observations in most cells			
	< 39	39	40	41	> 41*	1 short chromos.	1 short + 1 long chromos.	1 long chromos.	No abn. detectable
141	1	0	32	0	2	+	..	..	..
142	0	1	24	0	2	..	..	..	+
143	3	1	29	0	1	..	..	..	+
155	2	3	26	3	3	..	+†	..	..
156	2	1	26	1	3	..	..	..	+
157	0	0	23	0	2	..	..	..	+
185	1	0	23	0	1	..	..	..	+
268	2	1	29	0	2	..	..	..	+
269	1	2	27	0	1	..	..	..	+
270	0	1	28	0	1	..	..	+	..
271	2	2	27	1	2	..	..	..	+
314	2	2	25	2	6	..	..	..	+
315	1	1	19	0	4	..	..	+	..

* Mostly tetraploid cells.
† The unmatched short chromosomes is very short.

ber of 40 chromosomes. The few cells with aberrant counts are presumably artefacts of the technique used.

Morphological anomalies observed in spermatogonial metaphases consisted of the presence of one abnormally long and/or one abnormally short chromosome, presumed to result from translocations. Both of these elements were consistently present in every cell of each animal carrying them. Examples of mitotic chromosomal abnormalities are shown in Figure 1.

Of the 13 F_1 males from the X-ray series (Table 1), only four showed obvious chromosome anomalies in spermatogonial metaphases. Among the remaining nine, four were revealed to be translocation heterozygotes by meiotic studies (see below).

Of 28 males in the EMS and BHT + EMS series (excluding ♂ 519, which had Sertoli cells only), 23 showed obvious chromosome abnormalities in spermatogonial metaphases (Table 2). Three of the remaining five were proved to be translocation carriers on the basis of meiotic studies (see below).

Results of the analysis of spermatogonial metaphases of partially sterile F_1 males from the two EMS series were compared with results from complete steriles in the same series (Table 3). Among 28 partially sterile males, only four had visibly abnormal mitotic chromosomes, while among 28 sterile males, 23 had obviously abnormal karyotypes.

(2) Histological findings: Results of the histological analysis of the testes are summarized in Table 4. In only 26 of the 42 sterile males did spermatogenesis proceed to diakinesis or farther, and 17 of these also had at least some mature spermatids. However, these spermatids, where observed, were present in low

TABLE 2

Chromosome counts and observations in spermatogonial metaphases of sterile sons of EMS- and BHT + EMS-treated males

Father's treatment	F_1♂ no.	No. of cells with chromosome number					Observations in most cells			
		< 39	39	40	41	> 41*	1 short chromos.	1 short + 1 long chromos.	1 long chromos.	No abn. detectable
	333	0	1	18	0	1	..	+	..	..
	334	0	4	35	1	2	+	..	..	..
	335	1	2	18	0	2	..	..	+	..
	336	1	2	21	0	5	+†,‡	..	..	..
	499	4	2	21	0	6	..	..	..	+
	500	1	1	20	0	3	..	+	..	..
	501	2	2	25	0	9	..	..	..	+
EMS	502	5	5	18	0	4	+†	..	..	..
	503	2	2	19	0	4	+†	..	..	..
	504	3	3	18	1	2	..	+	..	..
	505	1	1	15	1	2	..	+	..	..
	506	3	3	22	0	3	..	+	..	..
	507	2	2	16	1	4	..	+	..	..
	508	2	3	22	0	5	+†	..	..	..
	509	1	2	15	0	1	..	..	..	+
	510	1	0	25	0	6	+†	..	..	..
	523	1	2	19	1	4	..	+	..	..
	511	1	0	12	0	2	+†	..	..	..
	512	3	2	10	1	2	+†	..	..	..
	513	5	1	28	0	4	+	..	..	..
	514	2	1	6	0	2	..	..	+	..
BHT	515	2	0	6	0	0	..	..	..	+
+	516	2	1	8	0	0	..	+	..	..
EMS	517	1	0	17	0	6	+	..	..	..
	518	1	1	15	0	3	..	..	+	..
	519	0	0	0	0	0	..	..	..	..
	520	3	1	28	0	6	..	..	+	..
	521	2	3	27	0	4	..	..	..	+
	522	0	1	17	0	2	+†	..	..	..

* Mostly tetraploid cells.
† The unmatched short chromosome is very short.
‡ In addition to the very short chromosome, this male also had a submetacentric chromosome.

numbers and occasionally were abnormal. [Note: In agreement with conventional usage, all stages of spermiogenesis are referred to as spermatid stages as long as the cells are in the seminiferous tubules (CLERMONT 1968). Since the present analysis was confined to the testis, no information is available on whether spermatozoa were present in epididymis or vas deferens]. In 15 of the males, pachytene was the latest stage present, and one male had Sertoli cells only.

Figure 2, which shows examples of arrest at different stages of spermatogenesis, illustrates clearly that the sterile males were not a homogenous group. The latest

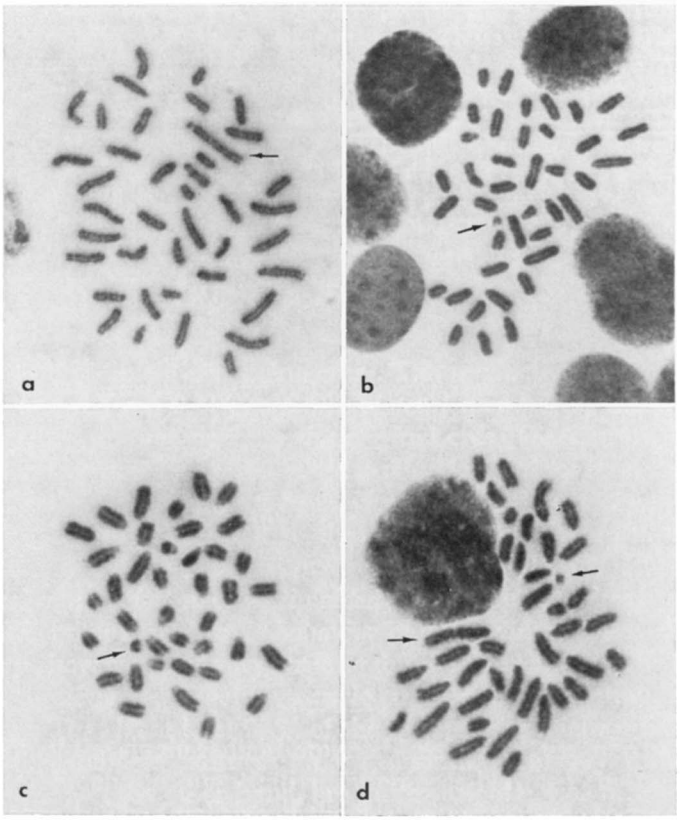

FIGURE 1.—Spermatogonial metaphases showing abnormal chromosomes (marked by arrows). (a) One chromosome excessively long. X-ray series, ♂ 270. (b) One chromosome very short. EMS series, ♂ 334. (c) One chromosome short. BHT + EMS series, ♂ 517. (d) One chromosome excessively long, one chromosome extremely short. X-ray series, ♂ 155.

stages present in any given male generally contained a considerable number of abnormal cells.

(3) Meiotic studies: Meiotic studies could be carried out only in those 26 males in which spermatogenesis proceeded at least as far as diakinesis. Of the 26, eight came from the X-ray series, eight from the EMS, and ten from the BHT + EMS series (Table 4). In general, the number of cells in diakinesis or meiotic meta-

TABLE 3

Relation of sterility or partial sterility to mitotic abnormalities in sons of EMS-treated males

	No. of males with mitotic complement	
	Visibly abnormal	Not visibly abnormal
Sterile males	23	5
Partially sterile males	4	24

TABLE 4

Results of histological analysis: latest spermatogenic stage present in the testis of 42 F_1 sterile males

Latest spermatogenic stage present	No. of sterile sons of males treated with			Total
	X-rays	EMS	BHT + EMS	
Late spermatids*	6	6	5	17
Diakinesis	2	2	5	9
Pachytene	5	9	1	15
None (Sertoli cells only)	—	—	1	1
Totals	13	17	12	42

* Since epididymis and vas deferens were not examined, it is not known whether spermatozoa were present.

phase was very low in these males, even when—as had to be done in some cases—the whole testis was used in making cytological preparations.

In cytological analysis of diakinesis and metaphase-I cells (Tables 5 and 6),

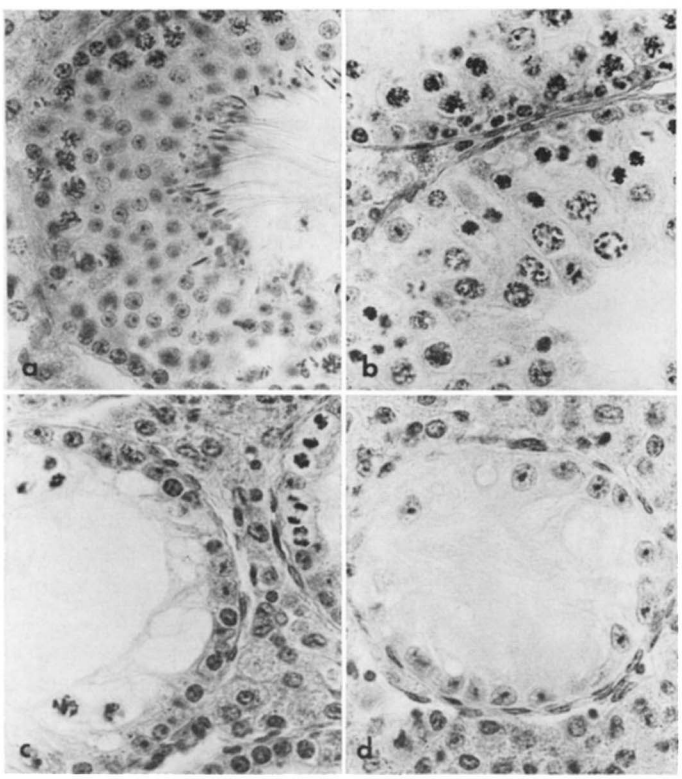

FIGURE 2.—Testis sections from sterile males, showing blockage of spermatogenesis in various stages. The latest spermatogenic stages present in typical cases are as follows: (a) some late spermatids (♂510); (b) diakinesis (♂518); and (c) pachytene (♂503). (d) ♂519 has Sertoli cells only.

TABLE 5

Analysis of spermatocytes of 26 F_1 sterile males in which meiotic analysis was possible

Treatment to father	F_1 ♂ no.	No. of cells analyzed	Percent cells with the following chromosome associations				
			20 II	18 II + Ch IV	18 II + Ch III + I	18 II + Ring IV	Others*
	143	41	64	36	—	—	—
	155	14	8	64	28	—	—
	185	5	20	80	—	—	—
X-rays	268	20	—	80	—	20	—
	269	24	12	33	21	5	29
	270	8	—	50	12	12	26
	271	50	2	—	4	—	94 (19 II + X + Y)
	315	24	8	75	—	17	—
	333	10	20	40	20	20	—
	335	41	—	90	3	7	—
	336	34	3	—	97†	—	—
EMS	499	71	5	—	—	—	95 (19 II + X + Y)
	501	49	—	—	—	—	100 (see Table 6)
	505	10	40	—	—	—	60 (see Table 6)
	506	1	—	(100)	—	—	—
	510	40	—	3	94	—	3
	511	3	—	33	—	—	67
	512	5	—	—	20	—	80 (19 II + X + Y)
	513	35	—	—	86	—	14
BHT	514	5	—	100	—	—	—
+	515	28	25	50	—	3	22
EMS	516	1	—	—	—	—	(10 II + 20 I)
	517	19	5	48	21	5	21
	518	9	—	—	—	—	100 (see Table 6)
	520	22	—	13	9	—	78 (see Table 6)
	521	47	85	15	—	—	—

* Where not otherwise indicated, most of the miscellaneous cells contain many chromosomes as univalents.

† T(Y;A), see text.

multivalent configurations were detected in 22 of the 26 analyzable males, indicating that these 22 were heterozygous for translocations. Only four males could not definitively be classified as carriers of translocations. In one of these (♂516), only one cell could be analyzed, and this had 10 bivalents and 20 univalents. The other three (♂♂271, 499, 512) had very high incidences of non-association between the X and Y chromosomes (Table 5). Two of these males also exhibited Chain III + I configurations and could therefore have carried translocations. However, the number of such cells was small (two in ♂271, one in ♂512); and in all three cells, the presumed X (which, in the present experiment, is derived from the non-treated parent) was distally apposed to an autosomal bivalent without obvious chiasma formation. These males will therefore be conservatively included in the nontranslocation classification.

TABLE 6

Analysis of meiotic cells from four sterile males with more than two chromosome breaks

F_1 ♂ no.	No. cells analyzed	Percent cells with the following chromosome associations												Percent configurations that are	
		20 II	18 II + Ch IV	18 II + R IV	17 II + Ch VI	16 II + 2 Ch IV	16 II + 2 R IV	16 II + Ch IV + R IV	16 II + Ch IV + Ch III + I	16 II + R IV + Ch III + I	15 II + R IV + Fig. VI†	15 II + Ch IV + Fig. VI†	Others*	Chains	Rings
501	49	—	—	—	—	8	8	25	6	2	20	31	—	60	40
505	10	40	—	—	—	—	—	40	—	—	—	—	20	50	50
518	9	—	—	—	78	—	—	—	—	—	—	—	22	100	0
520	22	—	13	9	—	68	5	5	—	—	—	—	—	87	13

* Where not otherwise indicated most of the miscellaneous cells contain many chromosomes as univalents.
† "Figure of VI" includes Ch VI as well as "ring-plus-chain" of six configurations. The latter constituted 64% of all Fig VI's of ♂ 501.

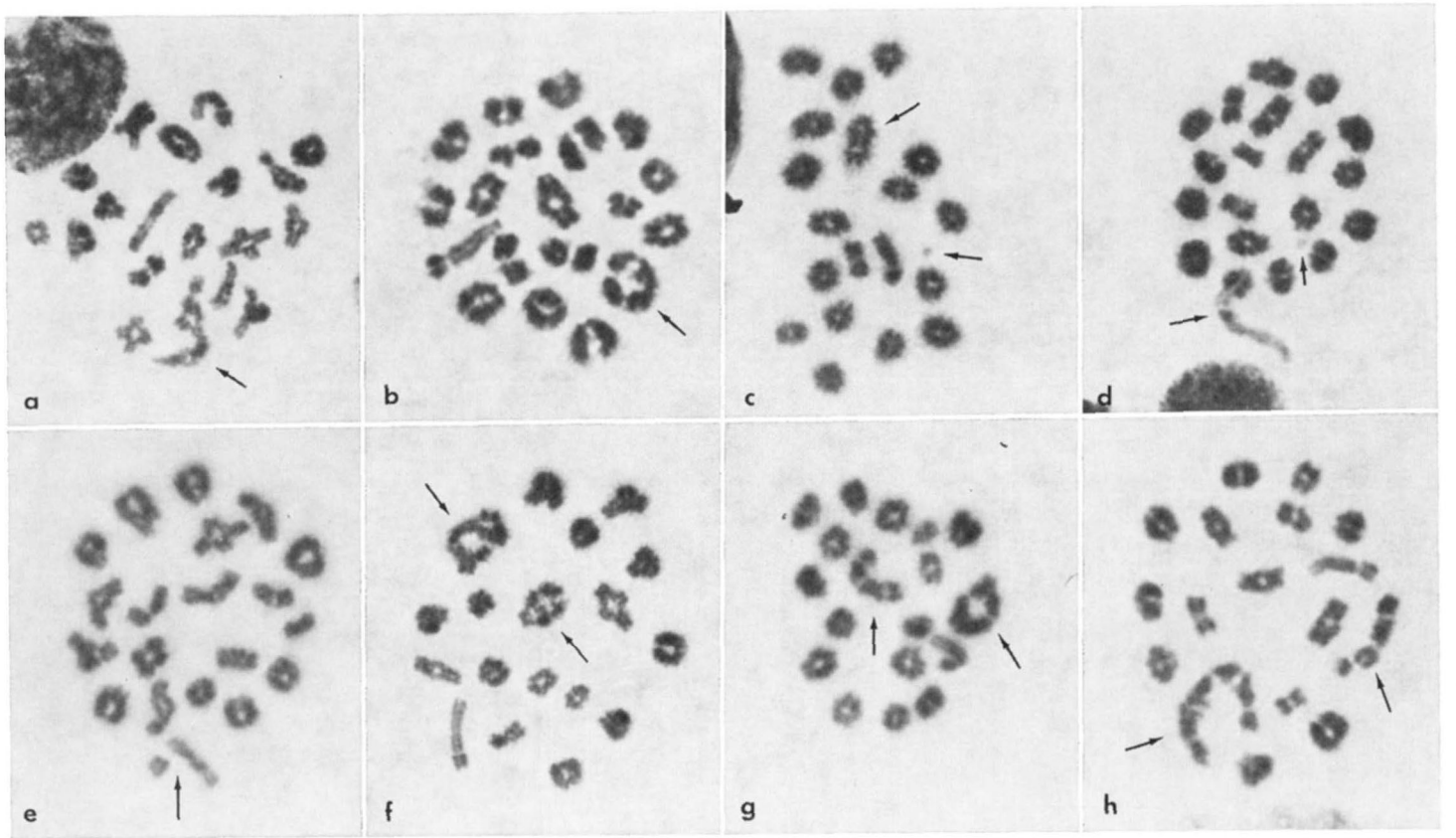

FIGURE 3.—Abnormal meiotic configurations in primary spermatocytes of sterile males. (a-d) Single reciprocal translocations; (e) failure of pairing; (f-h) multiple rearrangements. (a) 18 bivalents and a chain of 4 chromosomes (X-ray series, ♂270); (b) 18 bivalents and a ring of 4 chromosomes (X-ray series, ♂268); (c) 18 bivalents, a chain of 3, and a univalent (EMS-series, ♂510); (d) presumed Y-autosome translocation, showing 18 bivalents, a chain of 3 (including the sex chromosomes), and a very small univalent (EMS series, ♂336). (e) Failure of X-Y pairing, showing 19 II + X + Y (X-ray series, ♂271); (f) 16 II + Ring IV + Ring IV (EMS series, ♂501); (g) 16 II + Chain IV + Ring IV (EMS series, ♂501); (h) 15 II + Chain IV + Chain VI (EMS series, ♂501).

Further analysis of animals with multivalent configurations revealed that 18 of the known heterozygous translocation males carried a reciprocal exchange between two nonhomologous chromosomes (Table 5), and the remaining four carried translocations involving more than two chromosomes (Tables 5 and 6). Examples of various abnormal chromosome associations are shown in Figure 3.

Of the 18 males that showed meiotic evidence of translocation involving two chromosomes, most exhibited more than one of the three observed types of multivalent configuration (18 II + Chain IV, 18 II + Chain III + I, 18 II + Ring IV). In 15 of the 18 males, the prevalent or sole type was 18 II + Chain IV (and six of these also had Chain III + I configurations); in three males, 18 II + Chain III + I was the most frequent type. The Ring IV configuration occurred in only eight animals of the former group; and in all of these, cells with this configuration were in the minority. It is of interest that some cells with 20 bivalents were found in 11 of the 22 presumed translocation males, and in two of these (♂143, ♂521) such cells were in the majority.

The results of cytological analysis of the four males in which exchanges involving more than two chromosomes had occurred are shown in Table 6. Two of the males exhibited several different types of multivalent associations; only a single type was observed in each of the other two. A figure-of-six found in 78% of the cells of ♂518 indicates a serial translocation involving breaks in three chromosomes; and the presence of two quadrivalents in each of ♂♂505 and 520 indicates breaks in four chromosomes (two reciprocal translocations). The fourth animal, ♂501, appeared to have a multiple translocation involving four breaks in three chromosomes (as indicated by occasional "ring + chain" hexavalents), as well as a separate reciprocal translocation (i.e., six breaks in all), with the former giving rise to less-than-hexavalent associations in about half the cells. A breakdown according to type of multivalents in these various cases of multiple translocations (Table 6) again shows rings to be in the minority.

In Table 7, a number of partially sterile F_1 males from the EMS experiments are compared with the complete steriles with respect to multiple and single translocations. There are no obvious differences.

In all but one of the males in which multivalents were present, a clear X-Y bivalent, or, alternatively, separate X and Y chromosomes, could also be observed. The exception was ♂336 (Table 5), presumed to be a Y-autosome translocation,

TABLE 7

Distribution of sterile and partially sterile sons of EMS-treated males with respect to number of translocations present

Class of F_1 males	Number with	
	Single reciprocal translocations	Multiple or compound translocations
Sterile	18	4
Partially sterile	25	3

TABLE 8

Summary of attributes of 42 sterile males

Latest spermatogenic stage present	Listing of sterile sons* of males treated with		
	X-rays	EMS	BHT + EMS
Late spermatids	143 T	† 335 T (R)	‡ 513 T (U)
	268 T (R)	‡ 336 T (Y;A) (U)	513 T (R)
	269 T (R,U)	‡ 506 T? (1 cell)	‡ 517 T (R,U)
	† 270 T (R)	‡ 510 T (U)	521 T
	† 315 T (R)	‡ 505 TM (R)	† 520 TM (R)
	271 X-Y uni	499 X-Y uni	
Diakinesis	‡ 155 T (U)	‡ 333 T (R,U)	‡ 511 T (?)
	185 T	501 TM (R,U)	† 514 T
			† 518 TM
			‡ 512 X-Y uni
			‡ 516 ? (1 cell)
Pachytene	‡ 141	‡ 334	‡ 522
	142	‡ 500	
	156	‡ 502	
	157	‡ 503	
	314	‡ 504	
		‡ 507	
		‡ 508	
		509	
		‡ 523	
None (Sertoli cells only)			519

* Symbols following male's number denote the following: T = translocation, single reciprocal; TM = multiple exchanges (3, 4, or 6 breaks); R = some ring quadrivalents observed; U = significant number of Chain III + I configurations; X-Y uni = failure of X-Y pairing; ? = not enough cells for decision.

†, ‡ Recognizably abnormal karyotype in spermatogonial metaphase; ‡ denotes those cases where (one of) the abnormal chromosome(s) is smaller or very small.

in which one translocation product is a metacentric chromosome. This case will be described in more detail in another publication.

The various attributes, meiotic and mitotic, of each of the 42 sterile males studied are summarized in Table 8.

DISCUSSION

Information on the cytology of sterile progeny of mice treated with mutagens has become available over the past 10 years (LYON and MEREDITH 1966; GRIFFEN 1963; GRIFFEN and BUNKER 1967; CATTANACH, POLLARD and ISAACSON 1968). The present investigation adds data on a large number of sterile males derived from different mutagenic treatments and subjected both to analysis of the stage of spermatogenic "block" and to detailed cytological study.

None of the 41 males in which cytological analysis was possible showed any evidence of abnormality in the number of chromosomal elements present. Similarly, LÉONARD and DEKNUDT (1968) found no monosomics or trisomics in 47

males that showed cytological or histological anomalies of the testis, and whose fathers had received 300 R X-rays to spermatocytes or spermatozoa. On the other hand, in a population of 303 sterile sons of males X-irradiated with 350 or 700 R in spermatogonial and later stages, GRIFFEN and BUNKER (1964, 1967) and GRIFFEN (1967) found six trisomics. CATTANACH (1964) reported a case of autosomal trisomy in a sterile son of a male treated with TEM during the spermatocyte stage. It has been suggested (LYON and MEREDITH 1966) that at least some of these cases represent, in fact, tertiary (rather than primary) trisomy, i.e., a condition that can result from abnormal segregation in animals or stem cells already heterozygous for a translocation. Tertiary trisomies, which have been reported by various authors (LYON and MEREDITH 1966; CATTANACH 1967; EICHER and GREEN 1972; WHITE *et al.* 1972; EICHER 1973), would not be expected to be induced in the present experiment, since haploid cells were treated and first-generation offspring studied. The induction of primary trisomy, similarly, would not be expected, unless one assumes that a delayed effect on a given chromosome leads it to nondisjoin at the first cleavage (with the monosomic blastomere subsequently dying). Tertiary trisomy in mice can greatly reduce viability (EICHER 1973; CATTANACH 1967), or lead to neonatal death (WHITE *et al.* 1972). The tertiary trisomy that produces duplications for small portions of chromosomes 14 and 15 may be male sterile (EICHER 1973). However, it seems clear that trisomics constitute only an insignificant fraction of sterile F_1 males, even where spermatogonia are irradiated, as in GRIFFEN's work. Reliable figures on the proportion of trisomics among partially sterile and fertile offspring of mutagenically treated animals are not available.

While autosomal trisomy may only rarely make mice sterile, certain sex-chromosome trisomies are apparently invariably associated with sterility of males. The XXY condition, first reported by RUSSELL and CHU (1961) in an aspermic male with small testes, has since then been repeatedly found to be sterile (L. B. RUSSELL, unpublished), regardless of whether it is detected in animals carrying X-linked markers or X-autosome translocations (see also CATTANACH 1961a; RUSSELL and MONTGOMERY 1970). An XYY male was reported by CATTANACH and POLLARD (1969) to be sterile and to possess only very few spermatids and spermatozoa. The XXY condition would not be expected to be induced in the present experiment, and XYY only under the special circumstances assumed above for primary trisomics (which conditions would lead to XYY///XO mosaics).

In addition to certain changes in sex-chromosome number, a specific type of sex-chromosome rearrangement, heterozygous X-autosome translocation, has been known for some time to cause male sterility in mice (RUSSELL and BANGHAM 1961; CATTANACH 1961b; RUSSELL and MONTGOMERY 1970; L. B. RUSSELL 1972; LYON *et al.* 1964). Spermatogenesis in males heterozygous for T(X;A)'s does not generally proceed past pachytene. Induced T(X;A)'s were not expected to be detected in the present experiment, since progeny of only Y-bearing sperm were observed. However, T(Y;A)'s could be produced and observed; and one case was, indeed, found among the 42 sterile males. Since its frequency relative to auto-

somal translocations was about as expected from a random distribution of breaks among all chromosomes, these data would not in themselves suggest that T(Y;A)'s are invariably or usually sterile. However, reports of sterile T(Y;A) males by other workers (GRIFFEN and BUNKER 1967; LÉONARD and DEKNUDT 1969) make it likely that this is the case. The T(Y;A) male observed in the present experiment produced some late spermatids, whereas, in the case described by LÉONARD and DEKNUDT, in which an autosomal translocation was also present, spermatogenesis stopped in diakinesis.

In the case of T(X;A)'s, males are sterile and females are partially sterile (due to embryonic death of unbalanced segregants). LYON and MEREDITH (1966) found a similar sex difference in 5 of 36 lines carrying purely autosomal translocations. The present results indicate that a very high percentage of the sterile sons of males treated in postspermatogonial stages with X-rays or EMS are carriers of autosomal translocations, and they implicate certain types of translocations.

In 22 of the 42 sterile males (Table 8) there was meiotic evidence of rearrangement, and in 21 of these (i.e., 50% of the total) the rearrangements were autosomal. An additional three that were meiotically analyzed showed failure of X-Y pairing, which could have resulted from a Y-chromosome terminal deletion or, possibly, a Y-autosome translocation: in one of these three, in fact, a very small chromosome (perhaps the deleted or translocated Y) was detectable in mitosis. Similar cases in mice and men have recently been discussed by BEECHEY (1973). Finally, in eleven males there was mitotic evidence for rearrangements or sizable deficiencies, but further meiotic analysis in these animals was precluded by blockage (or near blockage) in pachytene.

Only 6 of the 42 sterile males failed to yield cytological evidence for chromosome aberrations. All of these lacked meiotic cells. Male 519, which had Sertoli cells only, histologically resembled the "germinal cell aplasia" reported by KJESSLER (1966) in 7 of 137 infertile men studied. Six of those men did not exhibit any gross chromosome abnormalities in other tissues, and one was 47/XY, with the extra chromosome being a small metacentric. If ♂519 in the present experiment was, in fact, chromosomally normal, sterility might have been due to a dominant mutation. Male sterility associated with point mutations is known in a number of different mammals. The same possibility exists for the five males (142, 156, 157, 314, 509) that were blocked in pachytene and in which no positive proof of grossly abnormal chromosome morphology or behavior could be obtained. However, it should be remembered that chromosomal rearrangements can easily exist without being detectable in mitotic cells.

It is apparent that a very high percentage—if not all—of the sterile males in the present study had chromosome abnormalities, and that the majority of these consisted of exchanges, the remainder perhaps of deficiencies. CATTANACH, POLLARD and ISAACSON (1968) also detected chromosome aberrations in a high percentage (7 out of 10) of sterile sons of EMS-treated males.

There is obviously a basic difference between "partial sterility", which results from the death of offspring (see L. B. RUSSELL 1962 for review), and complete

sterility, where the effects are on the carrier himself. Yet, translocations are implicated in both phenomena. That the difference is not due to single *versus* multiple translocations is clear from our data, which show the frequency of the latter to be approximately the same in the "partially sterile" and sterile groups derived from the EMS experiment. LYON and MEREDITH (1966) observed only two lines with multiple translocations, and both of these were partially rather than completely sterile. Multiple translocations would, of course, in general yield a higher frequency of unbalanced segregants and thus a very small litter size, so that—unless fertility testing was thorough—such animals might occasionally be misclassified as sterile, even though their gamete production was normal.

The evidence seems to indicate that the difference between translocations that result in sterility and those that cause "partial sterility" may be correlated with the relative lengths of the chromosome segments that are exchanged, as revealed by cytological evidence. In very small segments, chiasmata will be rare, resulting in multivalent figures of the Chain IV type (one small translocated segment) or of the Chain III + I type (two small segments combined into one translocation product), or in a low percentage of multivalents (two small segments both distal or both proximal in the translocation products). LYON and MEREDITH (1966) found that in translocation lines where males were sterile virtually all the quadrivalents were chains rather than rings, and there was a relatively high frequency of Chain III + I configurations. By contrast, in "partially sterile" lines, almost two-thirds of the quadrivalents were rings, and there were very few Chain III + I figures. The results of the present investigation are in general agreement with these earlier findings: only a small percentage of the sterile males for which meiotic proof of translocation exists had Ring IV figures at all, and in none of the single translocations was the frequency greater than 20% (although the overall average frequency of rings is somewhat higher than that reported by LYON and MEREDITH).

Exchanges involving one or two small segments of chromosomes could, under certain circumstances, also be detectable in mitotic cells by the presence of an unmatched short and/or unmatched long chromosome. The probability of mitotic detection is greatest if both breaks are close to opposite ends of the two chromosomes involved; however, if one break is very close to one end, the second break can be somewhat farther from the opposite end of another chromosome and still produce a mitotically detectable aberration. In the present data, the recognition of an unmatched small element in mitosis is well correlated with the presence in meiosis of some Chain III + I configurations (Table 8). Thus, of the eight males that had a small or very small mitotic element and also had more than three meiotic cells analyzable, six had significant numbers of Chain III + I configurations; and of 15 meiotically analyzed males in which no small mitotic element was detected, only two had significant numbers of Chain III + I configurations. This would lend support to the assumption that a high proportion of the 13 males that showed an unmatched small element in mitosis, but in which spermatogenesis did not proceed far enough to allow adequate analysis of meiotic figures, were carriers of unequal translocations. This indirect evidence, added to the direct

evidence from meiotic analysis, leads to the conclusion that the great majority of the sterile males may have had at least one break close to the end of a chromosome.

It may be suggested that when the break that is close to the end of a chromosome is in or near the paracentromeric heterochromatin, and translocation occurs to a euchromatic region of another chromosome, position-effect inactivations are produced that could lead to sterility. Position effects as one possible cause of selection against male germ cells carrying translocation has been suggested by FORD *et al.* (1969). Since chromosome banding techniques were not used in the present material, it is not certain that the breaks we observed near chromosomal ends in the sterile males were in fact predominantly at the centromeric end. Experiments are now underway to check this. Conversely, it will be important to determine whether among *partially* sterile lines derived from mice treated with mutagens, there are some with breaks near the distal end—a position that should not lead to complete sterility on this hypothesis, unless telomeric translocations can lead to position effects. Since many translocations that produce partial sterility in the female cause complete sterility in the male, the male must be assumed to be more susceptible to disturbances of fertility through position effects or other possible mechanisms. In humans, too, translocation-associated subfertility affects the male only (CHANDLEY *et al.* 1972). If position effect is the explanation, it could, in some cases, exert its fertility-disturbing action within the affected cells themselves (germ cells); or it could alter a product vital to male fertility produced elsewhere in the body. The ultimately observed interruption in germ-cell formation can obviously occur at different stages in gametogenesis in different translocations.

It is of interest to examine whether the sterility of heterozygous X-autosome translocation males could also be explained in terms of position-effect inactivation. Since the X chromosome is differentially condensed in mouse spermatocytes (OHNO 1966), it could be postulated that translocated autosomal genes might be inactivated in these germ-cell stages. Soon after the discovery of the T(X;A) male sterility, RUSSELL (1962) suggested that this phenomenon might be the result of an upset in the timing of synapsis or subsequent events: since the X-Y bivalent is normally not in synchrony with autosomal bivalents, the translocated chromosome(s), by having affinity with both, could disrupt meiosis. Along similar lines, LIFSCHYTZ and LINDSLEY (1972) have suggested that infertility of T(X;A)'s may be the result of the X remaining active at a time in gametogenesis when inactivation normally occurs; and they have argued (on the basis of Drosophila findings) against the possibility that inactivation of autosomal genes causes T(X;A)-related disruption of gametogenesis. Any hypothesis must be reconciled with the finding that the insertional *fd* translocation in the mouse (autosomal segment inserted into X), while sterile in chromosomally balanced, "Type-I" males (carrying the correspondingly deleted autosome), has been found to be fertile in "Type-II" males in which the translocated autosomal portion acts as a duplication (CATTANACH 1961b). This clearly fits the autosomal inactivation hypothesis of male sterility. However, since chromosomal associations in meiosis

may be different in Type-I and Type-II males, it is also possible that conflicting control of the translocated chromosome may exist in the former but not the latter case.

Examination of the data reveals no very clear correlation between the spermatogenic stage at which the block occurs and some of the other findings. For example, in the group of nine males blocked in diakinesis and the group of seventeen with some mature spermatids (Table 8), there were two and two, respectively, with multiple translocations, five and thirteen with single translocations, and one and two with failure of X-Y pairing—obviously no significant differences in distribution. There is, however, some indication that males that exhibit a small or very small chromosome at mitosis are less likely to have spermatogenic stages beyond pachytene. In the group of 21 in which a small chromosome was noted in spermatogonia, there were 12 that had no diakinesis (or had fewer than two cells observed in diakinesis); among the remaining 20 mitotically analyzed males, only five were blocked in pachytene. There is also a slight indication that translocation carriers with ring quadrivalents among their multivalent figures may be more frequent among animals that have some mature spermatids than among those in which blockage occurs in diakinesis. Both of these findings suggest that classes of translocations with more nearly terminal breaks are more likely to produce blockage earlier in spermatogenesis.

If some or all the various spermatogenic blockages observed in the EMS and X-ray series are due to intracellular effects, the present results would indicate that a high percentage of spermatogonia in which similar changes were to be induced would not go on to produce functional sperm. Selection against rearrangements induced in spermatogonia has already been deduced on other grounds (SEARLE, EVANS and FORD 1965; FORD *et al.* 1969). By an extension of this reasoning, the types of sterility found in the present study should not be found in sterile F_1 of irradiated spermatogonia. FORD *et al.* (1969) studied four such animals and found three of them to be cytologically normal, while the fourth was probably an XO/XYY mosaic. On the other hand, GRIFFEN and BUNKER (1967) reported that about one-third of the sterile F_1 males derived from irradiated spermatogonia had "cytohistological anomalies" of various types; but it is not clear how many of these were translocation carriers.

Comparison between X-ray- and EMS-induced F_1 steriles shows that the percentage of cases that lack mitotic evidence for unequal translocation is higher in the X-ray-induced group (9 out of 13) than in the EMS-induced groups (5 out of 28, $P < 0.005$). Should the difference between the X-ray and EMS series be real, it would indicate that EMS has a higher probability of breaking chromosomes near one of their ends. If, in turn, exchanges from such break locations are, as the evidence seems to indicate, very likely to lead to male sterility, EMS should give a higher ratio of sterile to "partially sterile" F_1 males than does X-irradiation. Results of GENEROSO *et al.* (in preparation) indicate this to be indeed the case for lower EMS doses. Thus, 50 or 100 mg/kg EMS applied to spermatozoa or late spermatids actually produced slightly more sterile than partially sterile sons. On the other hand, higher doses of EMS (150 and 200 mg/kg)

gave a ratio more nearly resembling X-ray results, which typically show 2–3 times as many "partially steriles" as steriles (see W. L. RUSSELL 1954). Work by G. A. SEGA (personal communication) suggests that EMS-induced breakage is selective, but that saturation effects at higher doses (400 mg/kg in his experiment) may obscure this selective effect. Males in the present experiment were derived from fathers treated with 250 mg/kg EMS, i.e., from a dose level at which selectivity of breakage may be beginning to be obscured. It will be of interest to carry out cytological analyses of sterile males derived from lower EMS doses. The present X-ray results indicate that sterility can also result when the translocation products are not obviously unequal (though one or both of the breaks could still be very near the end), or possibly from causes other than chromosome rearrangement. Considerably more meaningful analysis will become possible with the use of the new chromosome banding techniques.

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