recovered in the lavage fluid. These could easily have been randomly excluded from the relatively small sample of cells which was examined.

The results suggest a marginally higher labelling index in conventional mice than in germ-free mice. This trend remained after examining a further sample of 3 conventional animals from the same colony (unpublished results), and could reflect an influence of the microbial flora on the mitotic activity of lymphocytes and mononuclear phagocytes functioning within the specific immune system.

The degree of 'physiological' stimulation experienced by peritoneal macrophages may be greater in conventional mice than in the germ-free animals[3,4]. By currently accepted criteria[11], however, the peritoneum of conventionally-reared mice is not inflamed relative to that of germ-free mice. In the first place, leukocyte numbers and differential counts are independent of microbial status in healthy mice (table). Moreover, inflammatory exudates initiate S phase in a large proportion of mature macrophages[12] whereas no clear-cut difference exists between the ^{3}H-thymidine labelling indices of peritoneal macrophages in germ-free and conventional mice.

The present study reveals an overall similarity, at the cytological level, between the free-floating peritoneal cell populations of germ-free and conventionally-reared mice. From this finding and earlier evidence[3,4] the germ-free mouse seems highly suitable for studying peritoneal inflammations in vivo.

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Spermatocyte selection during meiosis following mitomycin C treatment in mice

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Summary. In mice treated with mitomycin C, elimination of spermatocytes is observed during meiotic division, whereby an increase in number of the eliminated cells is closely related to an increase in the frequency of spermatocytes with chromosome aberrations at M-I.

In the study of genetic defects resulting from various chromosome aberrations, it is of interst to evaluate the cytogenetic effects of chemical mutagens on mammalian germ cells, in particular to examine whether cells with chromosome aberrations are able to complete spermatogenesis. Recently, Adler[1] has demonstrated the presence of chromosome aberrations at the stage of diakinesis-metaphase I (M-I) in early primary spermatocytes from mice treated with mitomycin C (MC). Kratochivl[2] has shown that a reduction of sperm number is found in mice treated with MC as compared to untreated animals. However, it is uncertain whether this reduction is related to the presence of chromosome aberrations in spermatocytes at M-I. In this study, the number of ^{3}H-labelled or unlabelled spermatocytes and the number of spermatocytes with chromosome aberrations were examined at M-I and N-II (secondary spermatocytes at metaphase II), as a function of MC treatment. This paper reports that, in mice treated with MC, elimination of spermatocytes occurs during meiotic division (between M-I and M-II), whereby the rate of elimination is closely related to an increase in the frequency of spermatocytes with chromosome aberrations at M-I.

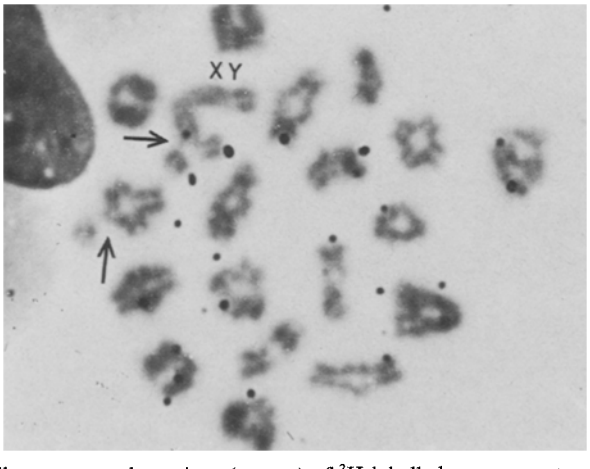
Material and method. Male mice of ddK strain, 8 weeks old, were injected i.p. with MC at doses of 3.75 mg/kg or 5.0 mg/kg, followed by i.p. injection of ^{3}H-thymidine with sp. act. 45 Ci/mM (The Radiochemical Center, Amersham) at dose of 10 μCi. The nontreated controls were also given ^{3}H-thymidine in saline. 2 groups of mice were killed by cervical dislocation at various intervals according to the Oakberg time-table[3], modified slightly. The primary and secondary spermatocytes were collected at 12-h intervals between 11 and 13 days after the treatment of MC. 6 mice in each group were used for each interval. Slides of spermatocytes were prepared according to the method of Meredith[4] with several modifications. Before staining, the slides were dipped in Sakura emulsion NR-M2 (Konishiroku Photo Ind. Co. Ltd, Japan). 2 weeks after dipping, the film was developed and stained with Giemsa solution. 50 primary spermatocytes at M-I were scored for each animal. Simultaneously with the M-I cells scoring, the number of all available secondary spermatocytes at M-II were scored. In addition, the proportion of labelled cells at M-I and M-II was noted.

Results. The table shows the number of labelled spermatocytes and the frequency of chromosome aberrations at M-I (figure) and M-II in mice treated with or without MC. In control mice, the number of labelled spermatocytes at M-II was higher by a factor of 1.2 than the number of labelled cells at M-I, but no significant difference was found in the proportion of labelled cells between M-I and M-II spermatocytes. This result implies that the meiotic process from M-I to M-II proceeds rapidly (within 12 h) in untreated animals. The number of labelled spermatocytes at M-I and M-II from MC-treated mice differed markedly from the number of labelled cells for untreated animals. In MC-treated mice, the number of labelled cells at M-II was about 0.5 in relation to the number of labelled cells at M-I. This decrease in the number of labelled spermatocytes at M-II from mice treated with MC might reflect either the elimination of a fraction of spermatocytes, or the prolonga-

Number of ^{3}H-thymidine labeled cells and frequency of chromosome aberrations in spermatocytes after treatment of male mice with mitomycin C (MC)[a]

Intervals (days)	No. of M-I cells scored		No. and (%) of labeled cells at M-I		No. of M-II[b] cells scored		No. and (%) of labeled cells at M-II		SDR[c]		Difference in SDR values	Frequency (%) of M-I cells with aberration	
	Control	MC	Control	MC	Control	MC	Control	MC	Control (A)	MC (B)	(A–B)	Control	MC
11	300	300	192 (64)[d]	210 (70)	390	247	226 (58)	126 (51)	1.18	0.60	0.58	0.3	21.0
11.5	300	300	225 (85)	264 (88)	388	231	330 (85)	155 (67)	1.29	0.59	0.70	0.0	31.5
12	300	300	261 (87)	282 (94)	398	193	334 (84)	135 (70)	1.28	0.48	0.80	0.0	37.0
12.5	300	300	198 (66)	-[e]	402	-	261 (65)	-	1.32	-	-	0.0	-
13	300	300	168 (56)	-	375	-	206 (55)	-	1.09	-	-	0.2	-

[a]6 male mice in each group were used for each interval. Male mice were injected i.p. with MC at doses of 3.75 mg/kg or 5.0 mg/kg, followed by i.p. injection of ^{3}H-thymidine at dose of 10 µCi. The proportions of labeled spermatocytes at M-I and M-II, and the relation between the values of SDR and the frequency of chromosome aberrations from mice treated with 3.75 mg/kg, were quite similar to those observed in animals treated with 5.0 mg/kg of MC, with the difference of the absence of dividing meiotic cells and the frequency of chromosome aberrations. Dividing spermatocytes were absent at day 12.5–13 after the treatment, in mice treated with a high dose of MC, and at day 13 in animals treated with a low dose of MC. The frequency of chromosome aberrations in the spermatocytes at M-I from mice treated with MC was found to depend on the dose of MC; [b]The number of all available secondary spermatocytes at M-II were scored simultaneously with the M-I cells scoring; [c]SDR: spermatocyte dividing ratio (number of labeled spermatocytes at M-II/number of labeled spermatocytes at M-I); [d]Each value is given as labeling proportion of examined cells; [e]No analyzable meiotic dividing cells were found in the slides of examined animals.



Chromosome aberrations (arrows) of ^{3}H-labelled spermatocytes at M-I from mice treated with mitomycin C (5 mg/kg).

tion of the cell cycle between M-I and M-II. The spermatocyte dividing ratio (SDR = number of ^{3}H-labelled spermatocytes at M-II/number of labelled spermatocytes at M-I) was then calculated from the number of spermatocytes at M-I and M-II in both groups of mice. The value of SDR for MC-treated and control mice was 0.48–0.60 and 1.09–1.32, respectively. Differences in the SDR values between MC-treated and control mice are also presented in the table. From these values it is estimated that, in control mice, 55–65% of the labelled spermatocytes at M-I divide further to become labelled cells at M-II, whereas 24–30% of labelled spermatocytes at M-I divide into the labelled cells at M-II in MC-treated animals. On the other hand, the incidence of chromosome aberrations in spermatocytes at M-I from mice treated with a high dose of MC (5.0 mg/kg) reached approximately 37% of examined cells and the labelling proportion of the cells with chromosome aberrations was 100% at 12 days after the treatment, whereas chromosome aberrations could not be found in the spermatocytes at M-II. Comparison between the difference in SDR values and the frequency of chromosome aberrations at M-I indicates that the difference in SDR value was closely related to the frequency of spermatocytes with chromosome aberrations. This result may also provide some suggestion about the cause of decrease in number of labelled spermatocytes at M-II.

Discussion. The present result indicates that, in MC-treated mice, the number of labelled spermatocytes is increased at M-I and decreased at M-II as opposed to untreated animals. These 2 phenomena result in the decrease in value of SDR from MC-treated mice. The increase in the number of labelled spermatocytes at M-I from mice treated with MC indicates the absence of spermatocyte elimination during the prophase of meiosis. It also indicates that delayed spermatocytes at M-I were present as a result of the prolongation of cell cycle from M-I to M-II. On the other hand, a decrease in the number of labelled cells at M-II could result either from the prolongation of cell cycle or from spermatocyte elimination in meiotic division between M-I and M-II.

As described above, the values of SDR for MC-treated animals were considerably lower than those for untreated animals. The value of SDR = 1.22 signifies that 60% of labelled spermatocytes at M-I were capable of proceeding from M-I to M-II, whereas a value of SDR = 0.45 implies further division of 25% among the labelled spermatocytes. Therefore, the difference in SDR values between the 2 groups of mice strongly indicates that 35% of labelled spermatocytes is eliminated, or else that development from M-I to M-II spermatocytes is considerably delayed by the treatment. However, as the present observations revealed the complete absence of meiotically dividing cells at day 12.5–13 after the treatment, it is evident that many delayed spermatocytes at M-I were eliminated before entering further meiotic division. As noted earlier, the decrease of SDR values was accompanied by an increase in the frequency of chromosome aberrations at M-I, and chromosome aberrations could be found in the spermatocytes at M-II in the MC-treated animals. Therefore, the decrease of SDR value in the mice treated with MC indicates spermatocyte elimination concomitant with chromosome aberration. Hence, it is suggested that the reduction in sperm number in MC-treated mice is closely related to the occurrence of chromosome aberrations at M-I.

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