the cusp is variable: they may run parallel or orthogonal to it. Their small size and limited number probably explain why they have not previously been reported. However, they are consistently present in all the specimens we have examined (5 cusps from 5 pigs), and they cannot be considered an occasional occurrence.

The presence of smooth muscle cells in the valvular cusps correlates with the embryonic origin of the cusps from the wall of the bulbus arteriosus, in the same way as the presence of striated muscle fibres[5] in the cusps of the atrioventricular valves correlates with the latters' origin and development. Further investigation is in progress to define the spatial arrangement of these contractile elements and their bearing on the mechanical properties of the valvular cusps.

1 Supported by grant CT76 011599.04 CNR, Roma.
2 G. Chiarugi and L. Bucciante, Istituzioni di anatomia dell'uomo. Ed. F. Vallardi, Milano 1975.
3 R.E. Clarck and E.H. Finke, J. thorac. cardiovasc. Surg. *67*, 792 (1974).
4 Y.F. Missirlis and C.D. Armeniades, Acta anat. *98*, 199 (1977).
5 A.L. Bassett, J.J. Fenoglio, A.L. Wit, R.J. Meyerburg and H. Gelband, Am. J. Physiol. *250*, 1366 (1976).

The peritoneal leukocytes of the germ-free mouse[1]

B. Woodward[2]

Department of Human Biology and Anatomy, University of Sheffield (England), 11 May 1978

Summary. The cytology of the lavage-recoverable peritoneal cell population of germ-free mice is similar to that of conventional controls. The microbial status has no effect on the total counts, differential counts or ^{3}H-thymidine labelling index of peritoneal leukocytes.

The germ-free mouse may be particularly useful for studying peritoneal inflammations in vivo[3,4], but only limited cytological information is available which characterizes the lavage-recoverable peritoneal cell population in germ-free animals[5,6]. The present study was therefore conducted as groundwork for an investigation of peritoneal macrophage stimulation in germ-free mice.

Materials and methods. Male and female NMRI strain mice were used at 8 weeks of age. Both the germ-free and the conventional animals were maintained in plastic isolators, and the germ-free mice were free from horizontally transmitted microbial associates including viruses[4].

Total leukocyte counts were obtained with an improved Neubauer chamber. Cells were recovered in Hanks' balanced salt solution and were immediately diluted in 2% (v/v) acetic acid containing a trace of toluidine blue and 15% (v/v) glycerol[7]. Differential counts were made from 0.5-µm sections of cell pellets which were prepared for transmission electron microscopy as described elsewhere[4]. Sections were stained in hot alkaline 1% (w/v) toluidine blue and 1000 randomly-chosen nucleated cell sections from each mouse were examined at a magnification of ×2500.

The ^{3}H-thymidine labelling index was estimated in cells recovered by lavage 30 min following a single i.p. injection of 6-^{3}H-thymidine (sp.act. 20.7 mCi/mg; The Radiochemical Centre, Amersham, England). Each mouse received 20 µCi of ^{3}H activity in 0.2 ml of Hanks' balanced salt solution. The cells were processed as for the differential counts, and 0.5-µm sections were hand-dipped into Ilford K-2 emulsion which was then exposed for 4 weeks at 4 °C. Developed autoradiographs were stained with alkaline 0.5% (w/v) toluidine blue and were scanned at ×2500 magnification. A nucleus was recorded as labelled if at least 5 developed silver grains were visible above it. 1000 nucleated cell profiles were examined from each mouse.

Results and discussion. Microbial status does not affect the total number, differential counts or ^{3}H-thymidine labelling index of lavage-recoverable peritoneal leukocytes in healthy mice (table). Macrophages, lymphocytes and mast cells predominate in the peritoneal fluid whereas neutrophils comprised less than 0.1% of the recovered cell population in the present investigation and eosinophils were also rarely seen. Similarly, in earlier studies of germ-free mice, neither total counts[5] nor (probably) differential counts[6] were affected by microbial status. However, a smaller proportion of peritoneal leukocytes was reported as macrophages even during an experimental inflammation in the previous work[6] than is indicated in the table. Differential counts vary considerably among strains and age-groups of conventional mice and such variation evidently also exists in germ-free mice.

The small ^{3}H-thymidine labelling indices shown in the table are consistent with earlier findings on conventional mice[8–10]. Radioactively-labelled nuclei were seen only within macrophages and lymphocytes but if a comparably small proportion of mast cells, neutrophils and eosinophils are in S phase, very few carrying ^{3}H-thymidine would have been

Numbers and ^{3}H-thymidine labelling index of leukocytes recoverable from the peritoneum of germ-free and conventional mice

	Cell numbers (× 10^6)		Labelling index (%)	
	Germ-free mice	Conventional mice	Germ-free mice	Conventional mice
Macrophages	3.5 ± 1.0 (10)*	3.3 ± 1.6 (10)	0.9 ± 0.3 (8)	1.4 ± 0.4 (8)
Lymphocytes	2.0 ± 0.4 (10)	1.6 ± 0.3 (10)	0.6 ± 0.3 (8)	1.2 ± 0.4 (8)
Mast cells	0.2 ± 0.07 (10)	0.1 ± 0.03 (10)	-	-
Total recoverable leukocytes	5.7 ± 1.5 (12)	5.0 ± 1.0 (22)	0.8 ± 0.3 (8)	1.4 ± 0.4 (8)

*Numbers in parentheses refer to the number of mice included in the determination. Means ± SD.

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.

1 This work was supported by the Association of Commonwealth Universities. I am also grateful to Professor R. Barer for his criticisms of this manuscript and to Mr O. Illman of the University Animal House for his support.

2 Present address: Room 12A LCDC, Health and Welfare Canada, Tunney's Pasture, Ottawa K1A OL2, Ontario, Canada.

3 B. Woodward, J. Physiol. *259*, 3P (1976).
4 B. Woodward, Cell Tiss. Res., in press.
5 G.D. Abrams and J.E. Bishop, Archs Path. *79*, 213 (1965).
6 E.H. Perkins, T. Nettesheim, T. Morita and H.E. Walburg, Jr, in: The Reticuloendothelial System and Atherosclerosis, p. 175. Ed. N.R. Diluzio and R. Paoletti. Plenum Press, New York 1967.
7 Ch.G. de Boroviczeny (ed.), in: Standardization in Haematology III, p.2. S. Karger, Basel and New York 1966.
8 I.J. Forbes, J. Immunol. *96*, 734 (1966).
9 R.J. North, J. exp. Med. *130*, 299 (1969).
10 D.G. More, J.M. Penrose, R. Kearney and D.S. Nelson, Int. Archs Allergy appl. Immunol. *44*, 611 (1973).
11 G.B. Ryan and G. Majno, Am. J. Path. *86*, 185 (1977).
12 M. Ando, A.M. Dannenberg and K. Shima, J. Immunol. *109*, 8 (1972).

Spermatocyte selection during meiosis following mitomycin C treatment in mice

S. Okoyama

Department of Anatomy, School of Medicine, Kanazawa University, Kanazawa 920 (Japan), 20 February 1978

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-