

Table 2. Proteolytic activity of *L. casei* recovered after exposure of X-ray irradiation (mg of tyrosine/g of curd)

Irradiation trial	<i>L. casei</i> *	
	Parent strain	Mutants
1	0.320	0.655
2	0.375	0.610
3	0.300	0.625
4	0.380	0.670
5	0.350	0.600
Mean	0.345	0.632

*No. of colonies of parent strain or mutants tested in each trial = 50.

activity of the colonies was established (table 1a). Out of 50 colonies of the parent culture, 45 with 0–4 mm clearance zones showed poor proteolytic activity. A similar trend was observed in case of 5–7 mm. However, clearance zones of 8–10 mm exhibited higher proteolytic activity. The analysis of variance data (table 1b) in regard to the proteolytic activity of 3 types of colonies selected on the basis of diameter of clearance zones showed the significance of F-ratio between the types. The relationship of proteolytic activity with the zonal diameter was tested in a 3×3 contingency table, and it was observed that the proteolytic activity is highly associated with the diameter of the zone in mm

$$(\chi^2 = 39.49; p < 0.05).$$

A linear regression of tyrosine liberated with clearance zone was fitted as

$$(Y = 0.1322 + 0.0192 X) (r^2 = 75.87\%),$$

where Y = mg of tyrosine liberated/g,
X = clearance zone (mm).

The above equation is presented in figure 1. The correlation between tyrosine liberated and clearance zone was 0.871 ($p < 0.01$).

The X-ray irradiation survival curve of *L. casei* (RTS) is illustrated in figure 2. An X-ray dose of 18,000 R gave less than 1.0% survival and mutants (only those with more than 10 mm clearance zone) were isolated from such petri dishes. 100 isolates from each of the 5 trials exhibited an appreciable increase in proteolytic activity in milk (table 2) as compared to the unirradiated parent culture. These mutants retained their altered characteristics even after several subcultures. These results on enhanced proteolysis are comparable to earlier observations of Dilanyan et al.^{3–5}, who demonstrated increased proteolytic activity of X-ray mutants of lactic acid bacteria.

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The peritoneal fluid cytology of adult female dogs

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Summary. The percent distribution of peritoneal fluid cellular content of adult female dogs was recorded and found to be dissimilar to all other species examined, especially in regard to mesothelial cell, polymorphonuclear leukocyte and lymphocyte proportions.

Peritoneal fluid cytologic specimens obtained from different mammalian species provide basic data to study the cellular response of serous abdominal fluid in health and disease. In normal women, pelvic peritoneal fluid may be considered a function of cell renewal and degeneration whose balance changes in pathological conditions so that cul-de-sac cytologic aspirations may be used to detect early ovarian cancer^{1,2}. Peritoneal fluid also provides a useful tool for studying inflammation^{3,4}, endocrine factors^{5,6}, estrous and menstrual cycles⁷ and pregnancy^{8,9}. The effect of human pregnancy on peritoneal fluid cytology was found to be quite similar to that observed in mice. In the present study, we analyzed the percent distribution of peritoneal fluid in adult female dogs to extend our species knowledge of peritoneal fluid as well as to determine how the cellular count compared with women and other species so that we might consider this animal as a future model for experiments involving peritoneal fluid cytology.

23 adult female mongrel dogs (3–4 years, 9.9 ± 0.2 kg) in good health and nutrition with normal heart rates, blood pressure and electrocardiogram readings were used.

The animals were tranquilized with 0.5 ml acepromazine maleate administered i.m. A cytologic specimen was then aspirated through the ventral side of the manually restrained, prone animal using a tuberculin (1.0 ml) syringe with a 23 gauge needle. The aspirated specimen was placed on an albumin coated slide and stained by

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Papanicolaou's procedure¹⁰. 200 consecutive cells were randomly counted and grouped as mesothelial cells, polymorphonuclear (PMN) leukocytes, lymphocytes, histiocytes, monocytes, mast cells, bare nuclei (light to dark staining nuclei without cytoplasm) and daisy cells. Randomly counting a fixed number of cells rather than counting the number of cells in an absolute volume eliminates the extreme variation resulting from counting

The normal peritoneal fluid cellular content of adult female dogs as compared to women

	Adult female dogs	Menstrual women
No. of subjects	23	34
Body weight (kg)	9.9 ± 0.2	—
Cell type	Percent distribution of cells ± SE	
Mesothelial cells	25.0 ± 1.6 (6.0)	51.8 ± 3.2
PMN leukocytes	51.4 ± 1.9 (3.0)	11.9 ± 2.2
Lymphocytes	12.9 ± 0.8 (6.0)	23.0 ± 1.9
Histiocytes	6.7 ± 1.1 (16.0)	8.8 ± 1.1
Monocytes	3.6 ± 0.5 (13.0)	—
Mast cells	—	—
Bare nuclei	—	—
Daisy cells	—	—

Percent coefficient of standard error variation in parenthesis.

cells per unit volume; often only a few drops were aspirated. By dividing the average cell count by 2, the percent distribution of each individual mean cell count was obtained. The SE for each mean cell count was calculated, and the percent coefficient of SE variation, a rough index of experimental error, was obtained by dividing the SE by the mean and multiplying by 100. The percent distribution of peritoneal fluid cellular content of adult female dogs is dissimilar to that of women or any other species studied. For example, mesothelial cell, PMN leukocyte and lymphocyte proportions were found to be 25.0%, 51.4% and 12.9% respectively, as compared with 55.4%, 16.7% and 7.3% in female rabbits and 51.8%, 11.9% and 23.0% in women. The dog PMN leukocyte distribution, in particular, was significantly greater than that recorded in other species (table). Peritoneal fluid specimens in the dog were cloudy and viscous, while specimens in most other species studied were found to be clear, colorless and of low viscosity. This data suggests that cellular exfoliation from the abdominal cavity in dogs is quite different from that found in other species. However, we do not feel this would prevent the dog from being used as an animal model in future studies examining the effects of inflammation, cancer, hormones, pregnancy and other factors of peritoneal fluid cytology. In any case, the present study does extend our knowledge of species differences and aids in our understanding the cellular response of abdominal serous fluid in health and disease.

Removal of 5-bromo-2-deoxyuridine incorporated in liver DNA of newborn and young adult rats¹

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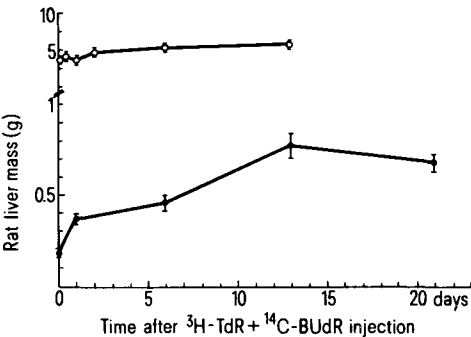
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Summary. 5-bromo-2-deoxyuridine (BUdR) removal from liver DNA in newborn and young adult rats has been demonstrated: it forms the basis of a reliable method to measure DNA repair in tissues provided with detectable DNA synthetic activity.

Recently, removal of 5-bromo-2-deoxyuridine (BUdR) incorporated in DNA of regenerating rat liver has been demonstrated and attributed to repair activity². Other evidence of excision repair of BUdR from DNA of mice embryo comes from Skalko's and Packard's work: BUdR,

but not 5-iodo-2-deoxyuridine (JUUR), is removed in a 2-day-period and this could explain the higher toxicity of equivalent dose of the latter halogenated compound³. Therefore we have searched for a similar BUdR removal in newborns and young adults.

Material and methods. 1-day-old Wistar rats were s.c. injected, in quadruplicate groups, with a mixture of ³H-thymidine (TdR) and ¹⁴C-BUdR (18.5 Ci/mmmole and 60 mCi/mmmole, respectively. The Radiochemical Centre, Amersham, England) in the ratio ³H/¹⁴C = 7 (7.0 μCi TdR plus 1.0 μCi BUdR/100 g b. wt). The same tracers were i.p. injected, in the same dose, in female rats (in groups of 5) weighing 100–120 g. In young adults, livers were removed at hours 1 and 8 and at days 1, 2, 6 and 13, and processed separately. In newborns, 14-liver pools in the case of 1 h and 1 d groups and 9-liver pools in the case of 6, 13 and 21 d groups were carried out. DNA was extracted using a phenol method⁴, as we previously reported², and



Time-course of liver mass (g) in 1-day-old (●—●) and young adult (100–120 g) (○—○) rats which were injected with a mixture of ³H-TdR and ¹⁴C-BUdR (7.0 μCi + 1.0 μCi/100 g b.wt). In young adults, data are the mean of 5 values ± SEM, while in newborns, the mean of 56 values for 1 h and 1 d groups, and of 36 values for 2, 6 and 21 d groups ± SEM is reported.

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