Effects of Mycoplasma Contamination on the Radiosensitivity of Rat Glial Cells

Reports have already been made of the widespread occurrence of mycoplasma (pleuropneumonia-like organisms or PPLO) as a contaminant of cell cultures[1,2]. These organisms have been shown to affect cell growth[3]. In addition, the metabolism of arginine[3-5] as well as that of nucleic acids[3,6,7] can be altered in infected cells.

In view of the widespread occurrence of PPLO in tissue culture and its somewhat unpredictable effects on cell metabolism, doubts have been raised concerning the interpretation of the data obtained while ignoring the presence of any cell contamination[8]. This is particularly true in the case of radiation studies since research in this field has frequently been performed in vitro. Results concerning cell radiosensitivity and ability to repair radiation damage could very well be affected by metabolic disturbances induced by PPLO.

The data reported in this paper concern the effects of PPLO contamination of the radiosensitivity of rat glial cells growing in monolayer cultures. The capacity to recover from sublethal damage was also considered.

A cell line (C-6 strain) established from a brain tumour induced in the Wistar rat by N-Nitrosomethylurea was used[9]. Stock cultures were grown in Blake bottles containing 20 ml of Ham's F-10 medium supplemented with 15% foetal calf serum. Growth was the same in contaminated as well as in normal cultures with a doubling time of approximately 30 h. However, in the presence of PPLO, cells contained granules which appeared to be indicative of PPLO infection.

Cultures became accidentally infected with a strain of PPLO which was identified as *Mycoplasma laidlawii*. Since it is a bovine strain, it was probably present originally in the foetal calf serum used to supplement the culture medium. Mycoplasma detection was done by using the stained agar technique as described by DIENES[10]. Uncontaminated cultures were checked for PPLO before and after the experiments and, at both times, results proved to be negative.

No antibiotics were added to stock cultures in order to rule out the possibility that mycoplasma contamination might be due to the induction of L-form bacteria by the continuous culturing of cells in the presence of antibiotics[2]. However, medium containing 100 units of Penicillin and 100 µg of Streptomycin per ml was used in experiments.

Experiments were carried cut by culturing cells in 15 × 60 mm plastic petri dishes. Approximately 20,000 cells were plated as a feeder layer and irradiated 2 days later with 4,000 rads of ^{60}Co γ-radiation in order to inactivate them. Immediately after this irradiation cells from stock culture were plated in appropriate dilutions and the dishes were kept at 37 °C for a period of time long enough for the cells to become attached (approximately 3 h). Afterwards, irradiations were performed at room temperature. In order to study recovery from sublethal damage a first dose of 500 rads was given followed by a second and final one, 6 h later.

Following irradiation, surviving cells were allowed to proliferate for a period of 14 days at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air. Afterwards cultures were fixed and stained. Clones of more than 50 cells were counted. The ratio of the number of clones in treated cultures to that in unirradiated ones was considered to represent the surviving fraction. For each point, an average value was obtained from 4 dishes.

X-irradiations were carried out with a Picker X-ray machine operated at 280 kVp and 18 mA. The half value layer of the radiation was 1.40 mm Cu. The beam was filtered with 1.0 cm Al and 0.5 cm Cu. Dose measurements were made with a Victoreen ionization chamber and the dose rate, measured at 76 cm from the target, was 44.6 rads/min.

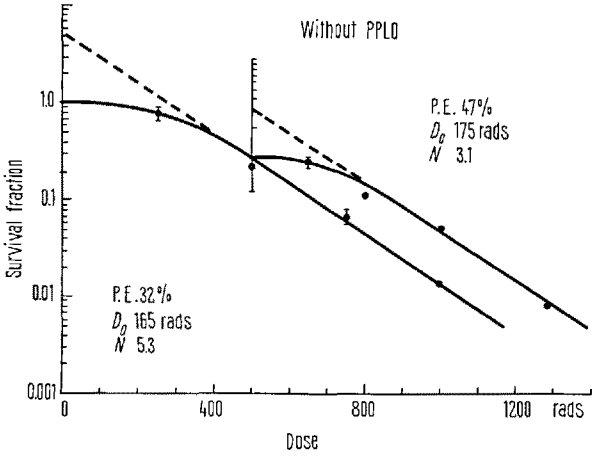

Fig. 1. Survival curves of rat glial cells irradiated with single doses of X-rays (left curve) and with fractionated doses (right curve). For the latter, an initial dose of 500 rads was given which was followed by a second one, 6 h later. Each point represents an average value obtained from 4 dishes. When larger than the points, standard deviations are indicated.

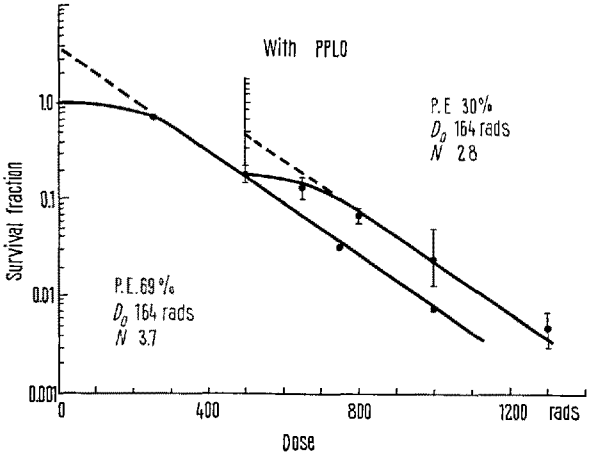

Fig. 2. Survival curves of rat glial cells contaminated with M. *laidlawii*. Further explanation in Figure 1.

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The sensitivity of uncontaminated rat glial cells to X-rays is shown in Figure 1. The survival curve of cells irradiated with single doses is characterized by a D_0 (the dose increment which reduces the surviving fraction to 0.37 in the exponential section of the curve) of 165 rads and an extrapolation number (N) of 5.3. As shown in the same figure, after fractionated doses the values are 175 rads and 3.1 respectively. These data show that recovery from sublethal damage after an initial dose of 500 rads appears to be complete by the time the second irradiation was performed. Repetition of experiments involving single-dose irradiations gave N values smaller than 5.3 thus showing that the difference between the 2 values of N given here is not significant.

The radiosensitivity of contamined cells is shown in Figure 2. The survival curve after single dose irradiations is characterized by a D_0 of 164 rads and an extrapolation number of 3.7. When cells are allowed to recover from sublethal damage, these parameters are found to be 164 rads and 2.8 respectively. The presence of PPLO does not affect the capacity of the cells to repair sublethal damage, at least when the ability to form clones is the end-point.

The data reported in this paper show that the presence of *M. laidlawii* in irradiated cultures of rat glial cells does not modify significantly the parameter D_0 when compared to normal cultures. This indicates that the intrinsic radiosensitivity is not altered due to the presence of PPLO. However, the values of N obtained after single irradiations of contaminated and normal cultures might be different. This would mean that the number of targets which must be inactivated by radiation in order to kill cells could be modified as a result of the presence of PPLO. Such a difference is not likely to be significant in view of the fact that repair of radiation damage appears to be as efficient in contaminated as well as in control cells. As a matter of fact, this difference is statistically not significant (p 0.55) with the data presently available.

The conclusion that *M. laidlawii* does not significantly affect cellular ability to survive after irradiation is somewhat surprising. In addition to metabolic disturbances which have been noticed for proteins[11] or nucleic acids[7] in contaminated cells, the formation of chromosomal aberrations has also been reported[8,12,13]. Since one of the effects of radiation is the production of chromosomal aberrations, one should then expect that the presence of PPLO would result in an increased number of chromosomal abnormalities and indirectly in a higher radiosensitivity. However, evidence of a synergistic effect of PPLO concerning cell mortality induced by radiation was not shown. It is true that contradictory effects of PPLO on mammalian cells have been reported with the result that a clear-cut conclusion cannot be reached concerning the mode of action of this contaminant. Among other things it has been shown that various strains of PPLO can result in different cellular effects[7]. It can be assumed that one strain of PPLO may probably affect differently various types of mammalian cells. It is of interest that *M. laidlawii* does not alter the response of C-6 cells to irradiation because this strain is more likely to contaminate mammalian cell cultures due to the presence of bovine serum in the medium. However, this finding cannot be extended to every mammalian cell line as long as there is still a possibility that the response to PPLO might be cell-line dependent[14].

Résumé. L'effet de la présence de *M. laidlawii* sur la sensibilité aux rayons-X des cellules provenant d'un glioblastome de rat a été étudié. Aucune différence significative n'a pu être mise en évidence entre la radiosensibilité des cellules infectées et celle des cellules saines. Ces résultats sont discutés en tenant compte des donnés bibliographiques.

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[14] This research was supported by a grant No. CA-07368 from the National Institutes of Health. The culturing and identification of *M. laidlawii* was made by Dr. L. DIENES.
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Abnormal Yolk Sac Function Induced by Chlorambucil

Altered yolk sac function in rodents often has been implicated as a possible mechanism through which various treatments may induce congenital abnormalities[1-3]. The present experiment was designed to determine if a positive correlation between teratogenesis and yolk sac function could be elucidated in the presence of the known teratogen, chlorambucil[4,5,6]. The amino acid valine, uniformly labeled with ^{14}carbon, was chosen as the indicator of yolk sac function since it has been demonstrated to be actively transported by the rabbit visceral yolk sac[7].

Material and methods. On day 12 of gestation (day 0 being the day on which sperm were found in the contents of a vaginal smear), Long-Evans black-hooded rats were given a single, i.p. injection of chlorambucil (donated by Burroughs Wellcome and Company) in sesame oil at a dosage of 6 mg/kg. This dose results in 90% of the surviving fetuses at day 20 having at least 1 structural abnormality[8]. Other pregnant animals either received sesame oil alone or were left untreated.

Gravid females from each of the 3 groups were killed by cervical dislocation on days 12 (control only), 12.5, 13, 13.5 and 14 of gestation. The embryos were dissected free, leaving the yolk sacs intact, and the umbilical vessels were ligated as they entered the chorioallantoic placenta. After the placentas were removed and while still surrounded by

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