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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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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their yolk sacs, the embryos were incubated for 1 h at 39 °C in 5 ml of a liquid culture medium consisting of bovine serum, chicken embryo extract ultrafiltrate and a phosphate-Ringer buffer in a ratio of 3:1:1 and containing ^{14}C-valine at a concentration of 0.02 μCi/ml. (For a more complete description of the incubation medium and the embryo-in-yolk-sac preparation see NETZLOFF et al.[9] and KERNIS and JOHNSON[1].) After incubation, the yolk sacs were separated from the embryos and each was rinsed and individually dissolved in 0.25 ml HNO_3. The resulting solutions were placed on tared, stainless steel planchets that were air-dried overnight and then at 120 °C for 24 h. The planchets were weighed and counted for radioactivity with a thin-window gas-flow β-detector. Results were expressed as cpm per mg dry weight of tissue and as cpm per embryo per mg dry weight of yolk sac. The student's t-test was used for statistical analysis and a P value of less than 0.05 was considered significant.

Results. The dry weights of yolk sacs and embryos are shown in the Table and in Figure 1. Chlorambucil-treated yolk sacs at days 13 through 14 and embryos at days 12.5 through 14 weighed significantly less than the corresponding untreated and sesame-oil-treated controls. Though dry weights were decreased, the relative growth rates as indicated by the slopes were similar beginning on day 13. While teratogen-treated embryos weighed less than controls at day 12.5, the weights of the yolk sacs from these animals were not significantly different.

Specific activities expressed in terms of cpm per mg dry weight of tissue are shown in Figure 2. No specific pattern of isotope uptake by yolk sacs from each of the three groups could be established. On day 13 chlorambucil-treated yolk sacs absorbed significantly more amino acid than did the control, but on day 13.5 the yolk sacs exposed to sesame oil contained greater amounts of valine than either of the other groups. Embryonic concentrations of valine, however, were more revealing. All groups showed decreasing uptakes as a function of gestational age. Abnormally developing embryos absorbed significantly greater amounts of isotope than untreated controls on days 12.5 through 13.5 and sesame-oil-treated animals on day 13.5 only.

When the results were calculated in terms of the amount of label present in each embryo as a function of the dry weight of its yolk sac, an indication of the transfer capacity of the membrane was achieved. Figure 3 demonstrates that the yolk sacs from chlorambucil-treated animals permitted a significantly increased amount of label to penetrate into the embryo than did those from either set of controls. By day 14 an apparent recovery had occurred. The differences between chlorambucil and sesame oil were significant on days 12.5, 13.5 and 14, whereas no differences between the latter and the control group were apparent on any day of gestation.

Discussion. Though the functions of the yolk sac have not been clearly elucidated as yet, it appears that the organ is important not only as the first site of blood and germ cell development, but also for protection of the embryo[10–13] and transfer of nutritional elements[1, 7, 14–18].

That the transfer function could be affected by a teratogen was proposed by BECK, LLOYD and GRIFFITHS[2]. These investigators suggested that trypan blue by virtue of its being stored in the vitelline epithelium interferes with yolk sac transport thereby exposing the embryo to a nutri-

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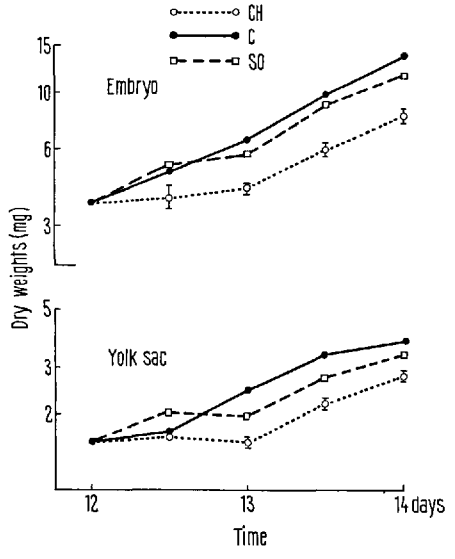

Fig. 1. Semilogarithmic graph of dry weights of yolk sacs and embryos as a function of gestational age. Vertical bars represent standard errors and are present when a significant difference ($P < 0.05$) may be demonstrated. Numbers of samples are indicated in the Table. C, control; CH, chlorambucil; SO, sesame oil.

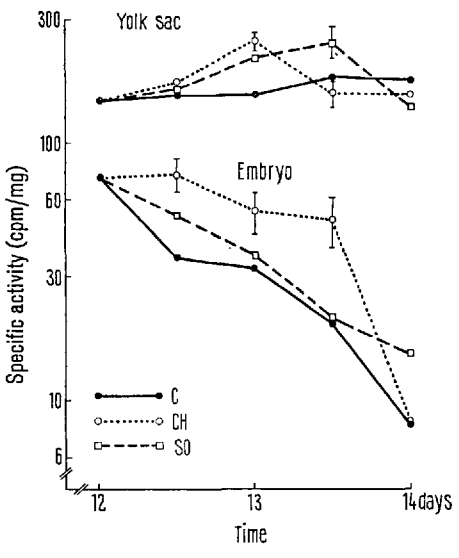

Fig. 2. Semilogarithmic plot of cpm/mg dry weight of valine after incubation for 1 h. Vertical bars represent standard errors and are present when a significant difference ($P < 0.05$) may be demonstrated. Numbers of samples are indicated in the Table. C, control; CH, chlorambucil; SO, sesame oil.

Dry weights[a]

	Day of gestation 12	12.5	13	13.5	14
Number females					
Control[b]	5	4	8	4	4
Chlorambucil[c]		4	3	3	3
Sesame oil		3	3	3	3
Number samples					
Control	26	17	40	15	18
Chlorambucil		21	15	17	17
Sesame oil		15	12	17	12
Yolk sac weight					
Control	1.51 ± 0.08	1.65 ± 0.11	2.43 ± 0.11[d]	3.34 ± 0.17[d]	3.76 ± 0.18[d]
Chlorambucil		1.64 ± 0.07	1.53 ± 0.07	2.16 ± 0.11	2.77 ± 0.13
Sesame oil		2.00 ± 0.14	1.94 ± 0.12[e]	2.78 ± 0.12[e]	3.32 ± 0.17[e]
Embryo weight					
Control	3.64 ± 0.19	4.82 ± 0.35[d]	6.54 ± 0.41[d]	9.52 ± 0.42[d]	13.31 ± 0.54[d]
Chlorambucil		3.78 ± 0.33	4.14 ± 0.20	5.85 ± 0.38	7.60 ± 0.36
Sesame oil		5.05 ± 0.25[e]	5.61 ± 0.14[e]	8.66 ± 0.33[e]	11.50 ± 0.33[e]

[a] Dry weight in mg, mean ± standard error. [b] Control animals left untreated. [c] A single, intraperitoneal injection on day 12 of gestation. [d] Significant difference ($P < 0.05$) between control and chlorambucil. [e] Significant difference ($P < 0.05$) between sesame oil and chlorambucil.

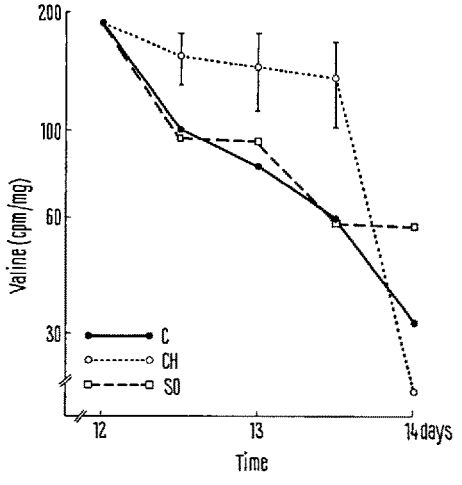

Fig. 3. The amounts of valine transferred to the embryo by each unit weight of yolk sac. Vertical bars represent standard errors and are present when a significant difference ($P < 0.05$) may be demonstrated. Numbers of samples are indicated in the Table. C, control; CH, chlorambucil; SO, sesame oil.

tional deficiency. Though their theory has been questioned with respect to trypan blue[19], the results of the present study suggest that at least one component of the mechanism of chlorambucil-induced teratogenicity may be interference with normal yolk sac function.

Two aspects to yolk sac function were indicated by the data: absorption of material and its transfer to the embryo. Analysis of Figure 1 will show that the weights of the embryos treated with chlorambucil were significantly decreased within 12 h after treatment. However yolk sac weight during this time period was not affected. It is significant that during this initial 12 h period the absorption of valine by the treated yolk sac was not significantly different from the controls, while the amounts of label actually penetrating the embryo proper (Figure 2) and being transferred by each unit weight of yolk sac (Figure 3) were significantly increased. As gestation continued the weights of the chlorambucil-treated tissues remained significantly lower than controls, while the transfer to the embryo for the next 24 h remained high. Actual absorption by the yolk sac was more variable perhaps indicating a drug-induced separation of the two functions of absorption and transfer.

The precise site of drug action or mechanism by which chlorambucil caused teratogenesis was not determined. Chlorambucil has a short serum half-life and reacts very rapidly with nucleophilic groups[20]. In addition, its presence has been associated with the induction of cytotoxicity[19], altered protein synthesis[21], and inhibited enzyme function[22]. Any or all of these changes could account for the apparent separation of the yolk sac's absorbing and transferring properties that in turn may have resulted in an altered transfer of material to the embryo. The embryo, therefore, may have been exposed to an abnormal microenvironment to which it responded by developing abnormally[23].

Zusammenfassung. Es wird gezeigt, dass Chlorambucil eine teratogene Wirkung, die durch Störungen der Nahrungsübertragung des Dotterbläschens verursacht wird, besitzt, was die Annahme nahe legt, Missbildungen seien durch ein abnormales «Mikromilieu» bedingt.

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