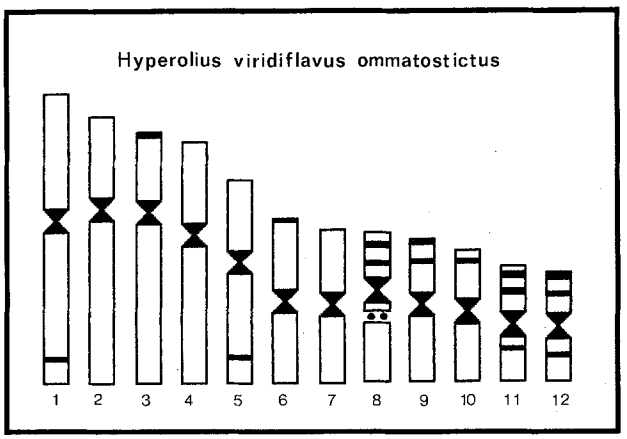

Figure 2. Diagrammatic representation of the maximum number of C-bands determined in the somatic metaphase chromosomes of male and female *Hyperolius viridiflavus ommatostictus*. Light areas: euchromatin; dark areas: constitutive heterochromatin; dark circles: nucleolus organizer regions.

Although none of the progeny from these breeding experiments could be raised and sexed we presume a male heterogametic sexual determination of the type XY based on the direction of sex change from female to male. This assumption is in accordance with successful breeding experiments with sex-reversed anurans using hormones[16]. In those experiments protandrous (male to female) sex reversal has been accomplished only in species showing a ZW system and protogynous (female to male) sex reversal in species with a XY system of determination.

Conflicting evidence comes from work by Richards on *Hyperolius viridiflavus viridiflavus*[17]. Breeding experiments showed a wide spectrum of sex ratios among different crosses. Richards interprets this high variance in sex ratio as perhaps indicating polyfactorial sex determination with environmental influence[17,18].

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2 Schiøtz, A., Vidensk. Meddr. dansk. Naturh. Foren. *134* (1974) 21.
3 Schiøtz, A., The Tree Frogs of Eastern Africa. Copenhagen: Steenstrupia 1975.
4 Drewes, R. C., Occ. Pap. Calif. Acad. Sci. *139* (1984) 1.
5 Duellman, W. E., and Trueb, L., Biology of Amphibians. McGraw-Hill Book Company, New York 1986.
6 Morescalchi, A., in: Cytotaxonomy and Vertebrate Evolution, p. 233. Eds A. B. Chiarelli and E. Capanna. Academic Press, London/New York 1973.
7 Morescalchi, A., Monitore Zool. Ital. (suppl.) *4* (1981) 41.
8 Blommers-Schlösser, R. M. A., Genetica *48* (1978) 23.
9 Bogart, J. P., and Tandy, M., Monitore Zool. Ital. (suppl.) *5* (1981) 55.
10 Schmid, M., Chromosoma *68* (1978) 361.
11 Schmid, M., Chromosoma *68* (1978) 131.
12 Sumner, A. T., Exp. Cell Res. *75* (1972) 304.
13 Schweizer, D., Chromosoma *58* (1976) 307.
14 Kubbies, M., and Friedl, R., Histochemistry *83* (1985) 133.
15 Grafe, T. U., and Linsenmair, K. E., Copeia (4) (1989) 1020.
16 Schmid, M., and Haaf, T., in: Evolutionary Mechanisms in Sex Determination, p. 37. Ed. S. S. Wachtel. CRC Press Inc., Boca Raton, Florida 1988.
17 Richards, C. M., (1990) unpublished manuscript.
18 Bull, J., Evolution of Sex Determining Mechanisms. Benjamin Cummings Publ. Company, Menlo Park, California 1983.

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Effect of low dose of 70 kVp X-rays on the intrauterine development of mice

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Summary. Pregnant Swiss albino mice were exposed to low doses of X-rays (~ 9 mGy) in the range used for diagnostic exposure, on day 3.5 of gestation (preimplantation period), day 6.5 (early organogenesis period) or day 11.5 (late organogenesis period). The fetuses were examined on the 18th day of gestation. Exposure at 3.5 days post coitus (d.p.c.) resulted in a significant increase in prenatal mortality, and an increased incidence of retarded fetuses was observed after exposure at 3.5 and 6.5 d.p.c. The major effect of exposure at 11.5 d.p.c. was a significant decrease in the fetal head size and brain weight.
Key words. Prenatal exposure; low dose X-rays; fetal anomalies.

Intrauterine development, particularly the period of organogenesis, is an especially radiosensitive phase in mammals. Even though the teratogenic effect of low doses of radiation has been demonstrated in children irradiated in utero by A-bomb radiation[1,2] and in laboratory animals[3–5], attempts to show a correlation between low doses in the range of diagnostic exposure and human fetal abnormalities have met with criticism[6]. Michel and Fritz-Niggli[7] showed that whole body exposure of pregnant mice to as little as 1 cGy of 140 kV X-rays and negative pions during organogenesis increased fetal abnormalities. However, data are lacking on the comparative response of the different stages of prenatal development to low doses at the levels that could result from

diagnostic radiation exposures. A study of this problem was undertaken in mice, selecting the early intrauterine developmental stages, and the results are presented here.

Materials and methods

Swiss albino mice were inbred under controlled temperature (22 °C ± 3 °C) and light (10:14 h light-dark) conditions and maintained on standard mouse feed and water ad libitum. Virgin females and males, 8–10 weeks of age, were mated overnight (3 ♀:1 ♂) and the day of observation of a vaginal plug was taken as day 0 of pregnancy. Three groups of pregnant females were exposed to a low dose of X-rays (Generay X-ray machine, Craniatame, Italy, 70 kV, 60 mAs, with inherent filtration of 1 mm Al without additional filter, SSD 91 cm) at a dose rate of approximately 15 mGy/s under ketamine (50 mg/kg b.wt) anesthesia on day 3.5 of gestation (preimplantation period), day 6.5 (early organogenesis period) or day 11.5 (late organogenesis period), and 3 groups were sham-irradiated to serve as controls. The dose per X-ray for whole body exposure was ~9 mGy, as calculated by the method of Edmonds[8]. The fetuses were dissected out on the 18th day of gestation and were examined for fetal death, sex ratio and morphological anomalies. The data were statistically analyzed by the 'Z' test[9].

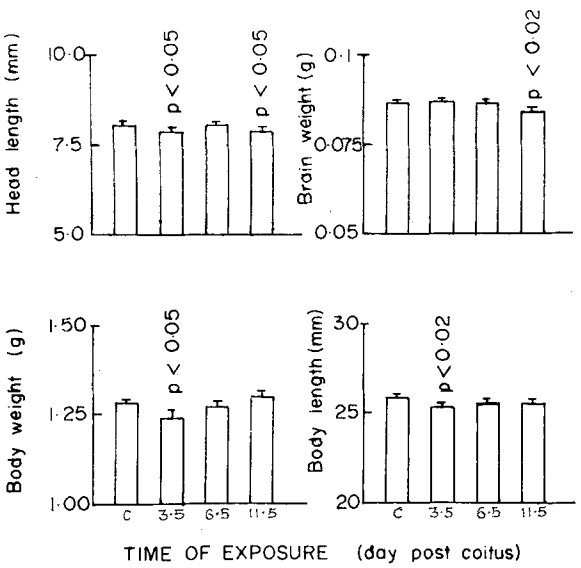
Results

Ketamine anesthesia did not have any effect on the fetuses. The data from the anesthetized animals were identical to those from unanesthetized controls.

Exposure on day 3.5 post coitus (d.p.c.) resulted in an increased incidence of prenatal mortality, mainly due to a significant increase in the preimplantation death, as compared to other stages (table). Examination of live fetuses on day 18 showed a significant reduction in the fetal length, weight and head size in this group, but brain weight did not show any change from the control. The frequency of retarded fetuses increased from 1.39% (4 out of 287) in the control to 4.67% (5 out of 107) in the exposed group, which was statistically significant (table). When the animals were exposed at 6.5 d.p.c., there was a significant increase in the number of retarded fetuses as compared to the control. However, body length, body weight, head and brain size did not vary from that of the control. A statistically non-significant increase in the incidence of microphthalmia was observed in this group (table).

Exposure on 11.5 d.p.c. did not produce any change in the general body growth or in the number of retarded fetuses, but the head size and brain weight were significantly reduced compared to those of the sham-exposed fetuses (table and fig.). Following the growth to later stages, this group of animals showed a significantly lower brain weight-body weight ratio at 6 weeks ($p < 0.05$) and 1 year ($p < 0.02$) of age as compared to the control (unpubl. obs.).

Prenatal mortality in the fetuses exposed during early or late organogenesis were comparable to the incidence in the control. The ratio of male to female offspring remained more or less the same in all the groups.



Morphological changes in 18-day mouse fetuses exposed to 9 mGy of 70 kVp X-rays at different stages in intrauterine development. C, Sham-irradiated control.

Observations on 18-day fetuses exposed to a low dose of X-rays at different stages of intrauterine development

Observations Exposure groups	C	3.5 d.p.c.	6.5 d.p.c.	11.5 d.p.c.
No. of mothers	36	15	18	15
Total No. of implants	304	117	169	126
Total mortality (%)	5.59 (17)	8.55 (10)	5.92 (10)	3.17 (4)
Preimplantation death (%)	0	3.4 (4)[c]	0	0
Growth retarded fetuses (%)	1.39 (4)	4.67 (5)[a]	4.40 (7)[a]	3.28 (4)
Microphthalmia (%)	3.83 (11)	3.74 (4)	7.55 (12)	4.92 (6)
Sex ratio male/female	0.99	1.10	0.93	0.93
Body weight (g) (mean ± SE)	1.28 ± 0.0077	1.24 ± 0.012[a]	1.27 ± 0.011	1.30 ± 0.013
Body length (mm) (mean ± SE)	25.85 ± 0.106	25.39 ± 0.152[b]	25.67 ± 0.156	25.59 ± 0.156
Head length (mm) (mean ± SE)	8.10 ± 0.0390	7.96 ± 0.052[a]	8.16 ± 0.054	7.97 ± 0.0518[a]
Brain weight (g) (mean ± SE)	0.087 ± 0.00098	0.088 ± 0.00081	0.087 ± 0.00079	0.084 ± 0.001[b]

d.p.c., day post coitus; C, sham-irradiated control. Figures in the parentheses indicate the actual number. [a–c] Difference from control; [a] $p < 0.05$, [b] $p < 0.02$, [c] $p < 0.001$.

Discussion

These results indicate that a single dose of low energy X-rays during intrauterine development of mice can induce fetal death and abnormalities. The type of anomaly and the frequency of incidence depended on the developmental stage at which the irradiation was done.

The observation that exposure at 3.5 d.p.c. increased the preimplantation mortality is in agreement with the earlier findings of Russell[10], Rugh and Grupp[11] and Ohzu[3] that preimplantation embryos are highly sensitive to radiation-induced mortality. While Russell[10, 12] reported almost no abnormalities in the survivors to term, Rugh and Grupp[11] and Ohzu[3] found a very low incidence of morphological anomalies which was not statistically significant, even though the doses used in their studies (5 cGy) were higher than in the present study. The present data showed a significant increase in retarded embryos, with reduction in body length and weight, among preimplantation embryos which had received X-rays; however, these changes were transient and complete recovery to normal was effected before 6 weeks of age (publ. elsewhere). The difference in response from those described in earlier reports[3, 11] could be due to a difference in strain sensitivity, as Michel and Fritz-Niggli[7] observed a strain difference in the resorption rates between NMRI and F/A mice after exposure to 1 cGy of 140 kVp X-rays.

Exposure at 6.5 d.p.c. resulted in a significant increase in the number of retarded fetuses, even though all the animals recovered and regained normal size during early postnatal development (unpubl. obs.). Such a transient increase in the number of retarded fetuses was also reported by Michel and Fritz-Niggli[7], after irradiation of 8-day embryos with 1 cGy of X-rays.

The main effect noticed after exposure to low doses of X-rays in the diagnostic range during late organogenesis (11.5 d.p.c.) in the present study was a significant reduction in the head and brain size, which continued through later development (unpubl. obs.) Miller[2] suggested that small head size is the simplest and most sensitive measure of radiation effect on humans which can be detected at birth. The present results show that this may apply to the mouse fetus too, especially during late organogenesis, which is the critical stage for brain damage[13].

These results indicate that there is a greater risk of early intrauterine death by exposure at the preimplantation stage, while this stage and the organogenesis period are equally sensitive to radiation-induced fetal growth retardation; head and brain growth are more susceptible at the late organogenesis period. All these effects could be induced in the mouse embryos by a whole body exposure of the pregnant mother to as low a dose as 9 mGy of diagnostic X-rays.

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1 Blot, W. J., J. Rad. Res. (suppl.) *16* (1975) 82.
2 Miller, R. W., J. Wash. Acad. Sci. *78* (2) (1988) 94.
3 Ohzu, E., Rad. Res. *26* (1965) 107.
4 UNSCEAR Report, Annex. J., p. 655. United Nations, New York 1977.
5 Kameyama, Y., Envir. Med. *26* (1982) 1.
6 Oppenheim, B. E., Griem, M. L., and Meier, P., Radiology *114* (1975) 529.
7 Michel, C., and Fritz-Niggli, H., Fortschr. Röntgenstr. *127* (3) (1977) 276.
8 Edmonds, I. R., Br. J. Radiol. *59* (1984) 165.
9 Bourke, G. J., Daly, L. E., and McGilvray, J., Interpretation and Uses of Medical Statistics, 3rd edn. Blackwell Scientific Publ., London 1985.
10 Russell, L. B., Proc. Soc. exp. Biol. Med. *95* (1957) 174.
11 Rugh, R., and Grupp, E., Exp. Cell Res. *25* (1961) 302.
12 Russell, L. B., J. exp. Zool. *114* (1950) 545.
13 Otake, M., and Schull, W. J., Br. J. Radiol. *57* (1984) 409.

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Persistence of added retinoids in organ culture media during induction of mucous metaplasia and glandular morphogenesis in hamster cheek pouches

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Summary. The retinoid concentration (determined colorimetrically) did not change significantly in retinyl acetate-supplemented (6 µg/ml) Eagle's Minimal Essential Medium containing 10% fetal calf serum when stored at − 20 or 4 °C over 7 days. After the medium was incubated at 37 °C for 48 h, 37–49% of the retinoid remained, whether or not tissue (neonatal Syrian hamster cheek pouch) was present, and irrespective of explant age. The normal retinoid level in the tissue was approximately 0.25 µg per gram. Therefore, neonatal hamster cheek pouches, incubated in medium with the addition of 6 µg of retinyl acetate per ml of medium and undergoing mucous metaplasia and some mucous gland morphogenesis, were continually being exposed to retinoid levels which, though gradually decreasing, remained well above their normal physiological level.

Key words. Retinyl acetate; retinoid concentration; culture medium; hamster; cheek pouch; organ culture; mucous metaplasia; glandular morphogenesis.