



Figure 1. Development of shoots from nodal segments



Figure 2. Development of multiple shoots from nodal segment shoot.

buds of *Acacia auriculiformis* were cut from the mother plant and washed thoroughly under running tap water for 1–2 h. Then they were treated with Bavistin (W/P) 0.5% for 1 h and washed thoroughly in single-distilled water 2–3 times. These branches were surface-sterilized aseptically with 0.1% mercuric chloride for 8–10 min and then thor-



Figure 3. Development of roots in *A. auriculiformis*.

oughly washed with sterilized water 4–5 times. The leaves were removed and the branches cut into small nodal segments. These segments were placed on modified Murashige and Skoog medium⁷ with 0.2% gelrite/0.8% agar supplemented with BAP (1 and 2 μ M per litre) and NAA (1 and 2 μ M per litre), and 2% sucrose; the medium was adjusted to

5.8 pH and autoclaved for 15 min at 15 lb/in² and a temperature of 120°C. Then these flasks were incubated under 16 h light and 8 h dark period at a temperature of 26 \pm 3°C. After one month the axillary buds sprouted and developed into shootlets (Figure 1). These shootlets were excised from the main explant and transferred to the multiplication medium containing MS medium with high phosphate (NaH_2PO_4 340 mg/l), supplemented with BAP (5.0 and 7.5 μ M/l) and NAA (1 and 2 μ M/l), 2% sucrose and 0.2% gelrite. Twenty days after inoculation, 5–7 small shoots per culture arose from the base (Figure 2). Research is in progress to achieve more multiple shoots by altering the cytokinin and auxin ratio. Rooting was achieved in MS medium with charcoal (0.1%) and MS medium with 0.5 mg IBA and charcoal at 0.1% (Figure 3).

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Effect of foetal exposure to low-dose X-rays on the postnatal growth of mouse

Even though the late organogenesis period in mammals is considered to be the most sensitive one to radiation-

induced developmental anomalies, a recent study from our laboratory has shown that the early foetal period, day

14.5 post-coitus (p.c.), is also susceptible to γ -ray-induced foetal growth retardation and microcephaly in mouse

Table 1 Observations on postnatal development of mice exposed to 0.05 Gy of X-rays at day 14.5 post-coitus

Observations	Treatment	Age of offspring (week)					
		0	1	2	3	4	5
Number of offsprings	C	122	121	120	119	117	115
	E	98	96	95	94	92	88
Growth-retarded offsprings(%)	C	1.64(2)	1.65(2)	2.50(3)	3.36(4)	3.42(4)	3.48(4)
	E	4.08(4)	4.17(4)	7.37(7)	10.64(10)*	11.96(11)*	11.36(10)
Body weight (mean \pm SE, g)	C	1.67 \pm 0.014	4.76 \pm 0.054	7.27 \pm 0.081	11.59 \pm 0.159	17.57 \pm 0.367	24.43 \pm 0.273
	E	1.64 \pm 0.018	4.62 \pm 0.059	7.09 \pm 0.125	10.71 \pm 0.215	15.68 \pm 0.486	22.23 \pm 0.489 ^c
Body weight (mean \pm SE, mm)	C	32.1 \pm 0.115	46.78 \pm 0.235	54.91 \pm 0.227	68.06 \pm 0.366	79.65 \pm 0.447	87.83 \pm 0.366
	E	31.88 \pm 0.135	46.19 \pm 0.317	54.79 \pm 0.417	64.68 \pm 0.670	71.62 \pm 0.789 ^c	83.95 \pm 0.949 ^{b,24}
Head length (mean \pm SE, mm)	C	8.73 \pm 0.056	13.83 \pm 0.091	15.86 \pm 0.103	20.85 \pm 0.146	22.38 \pm 0.160	12 \pm 0.115
	E	8.58 \pm 0.061	13.60 \pm 0.119	16.19 \pm 0.146	20.59 \pm 0.122	21.13 \pm 0.185 ^{c,18,8}	23.58 \pm 0.176 ^a
Head width (mean \pm SE, mm)	C	7.99 \pm 0.075	12.31 \pm 0.075	15.58 \pm 0.133	18.03 \pm 0.168	7 \pm 0.214	19.98 \pm 0.162
	E	7.99 \pm 0.096	12.11 \pm 0.146	14.86 \pm 0.239 ^b	16.83 \pm 0.204 ^c	17.71 \pm 0.257 ^c	19.23 \pm 0.243 ^a
Tail length (mean \pm SE, mm)	C	13.25 \pm 0.140	26.13 \pm 0.137	42.37 \pm 0.246	57.45 \pm 0.371	70.26 \pm 0.429	79.63 \pm 0.417
	E	13.06 \pm 0.136	25.57 \pm 0.195	40.72 \pm 0.445 ^a	54.93 \pm 0.638 ^b	68.26 \pm 0.635 ^a	74.08 \pm 0.806 ^c
							80.19 \pm 0.718 ^c

Note: Figures in parentheses are the actual numbers

*C Sham-irradiated animals, number of mothers 15.

E Exposed to 0.05 Gy X-rays, number of mothers 12

Difference from respective controls (C) ^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.05$, (Mann-Whitney test) * $p < 0.05$ (Fisher's exact test)

(unpublished observation). Therefore a study was done to find out the effects of low-dose X-rays at day 14.5 p.c. on the postnatal growth of mouse.

Virgin female and male Swiss mice (8–10 weeks old) from an inbred colony, maintained under conditions of controlled temperature ($22 \pm 3^\circ\text{C}$) and light (10 h light, 14 h dark), were mated overnight. The day the vaginal plug was observed was considered as day 0 of pregnancy. Pregnant females were exposed to 0.05 Gy of X-rays on day 14.5 of gestation, as described earlier^{1,2}. One group of animals was sham-irradiated to serve as control. The experimental and control animals were left to parturate. The offsprings were observed for changes in body weight, body length, head length, head width, tail length and post-natal mortality from day 0 (the day of birth) up to 6 weeks post-partum (p.p.). Sex ratio was determined at birth. The data were evaluated by the non-parametric Mann-Whitney U-test and Fisher's exact test, using GraphPad In-Stat software; p values < 0.05 were considered statistically significant.

A total of 220 offsprings from 27 mothers were examined. The litter size, sex ratio and postnatal mortality were not affected by irradiation. The number of growth-retarded offsprings was significantly ($p < 0.05$) higher than control at 3 and 4 weeks, but the difference became nonsignificant by 5 weeks (Table 1).

Irradiation did not affect the birth weight of the offsprings. The body weight showed significantly ($p < 0.01$, $p < 0.001$) lower than control values from 3 to 5 weeks (Table 1). A similar response was observed for body length also. The head length showed significantly ($p < 0.001$, $p < 0.05$) lower values at 4 and 5 weeks of age. However, reduction in head width was apparent from 2 to 5 weeks. All the parameters recovered by 6 weeks. The tail length was significantly ($p < 0.05$) lower than control at 2 weeks, with further reduc-

tion at later postnatal ages, with no recovery (Table 1).

Based on the early data, it was concluded that the killing of fetuses requires high doses of radiation³. However, more recent *in vitro* and *in vivo* studies have shown that relatively small doses (5–10 cGy) can cause embryonic and foetal mortality⁴. The present data did not show any significant increase in postnatal mortality.

A decrease in weight of rats and mice has been reported after exposure to higher doses of radiation^{5,6}. Studies with very low doses are few. Hande *et al*² observed that 5 cGy of X-rays given at the major organogenesis period induced a significant reduction in postnatal weight. The present study shows that such an effect can be induced by low-dose X-rays when exposed at the early foetal period also, but this effect is temporary and complete recovery is effected by the time the animals reach adulthood. There are no comparable published reports on postnatal body length and head size measurements, though our own study has shown a reduction in the head size of 18-day-old fetuses after exposure to ~1 cGy X-rays during the late organogenesis period¹. Diminished head size has been reported to be a readily detectable effect in prenatally exposed Japanese A-bomb victims⁷.

Tail growth seems to be more sensitive to exposure at this stage than the other parameters. There are no comparable studies on the effect of low-dose *in utero* exposures during early foetal period on postnatal tail measurements. However, stunting of skeletal growth has been reported by several investigators after high doses during 14–18 days p.c. in rats and mice^{8,9}.

Recently, Uma Devi *et al*¹⁰ have reported that doses below 25 cGy of γ rays produced no marked foetal mortality or growth retardation when exposed on day 11.5 p.c., which is a more radiosensitive stage than day 14.5 p.c. in mouse development. However, the

present results suggest that in the case of X-rays even doses as low as 5 cGy on day 14.5 p.c. can lead to growth retardation. Even lower doses (~1 cGy) of X-rays during the organogenesis period have been found to produce growth retardation in mouse foetus¹¹. These results strengthen our earlier assumption that at low doses X-rays may be more effective in influencing mouse development than γ -radiation¹⁰.

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