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Dominant lethal mutations induced by ^{14}C in mice[1]

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Summary. The mutagenic potential of 1.0 μCi ^{14}C was evaluated in Swiss albino male mice by the dominant lethal assay. A significant increase in post-implantation loss was seen, the maximum being in the 3rd week after treatment.

^{14}C is a naturally occurring radionuclide that is produced in a nuclear reaction between cosmic ray neutrons and the nitrogen atoms of the air. It is also a by-product of nuclear fission. ^{14}C is widely used as a tracer in different biochemical studies and in the diagnosis of some human disorders[2-4]. Earlier investigations on the genetic effects of ^{14}C were confined to onion[5,6], *E. coli*[7], fish eggs[8], Chinese hamster cells[9] and *Drosophila*[10-13]. Its mutagenic potential has not been studied in mice. Hence the present investigation was taken up.

Materials and methods. 25 male mice of the Swiss albino strain weighing 20-25 g (8 weeks old) were injected i.p. with 1.0 μCi/0.5 ml of of ^{14}C in the form of glucose-C-14 (sp. act. 215 mCi/mM supplied by Isotope Group, Bhabha Atomic Research Centre, Bombay). The control mice received 0.5 ml of physiological saline (0.9%). Immediately after treatment each male was caged with 2 virgin females (8 weeks old) which were replaced at weekly intervals for 6 consecutive weeks. Females were autopsied on 17th day of gestation and the uterus was checked for live and dead implantations. Dominant lethals were estimated in terms of pre-, post- and total-implantation losses[14].

Results. The results on the data on uterine contents are presented in the table. Their significance was tested using the chi-square and t-tests. There was a significant ($p < 0.05$) increase in the dead implantations per female in the ^{14}C-treated group during the 1st, 3rd and 6th weeks of mating. However, there was no significant ($p > 0.05$) change in the live and total embryos when compared to controls. The difference in pregnant females also remained statistically insignificant ($p > 0.05$) between control and treated groups.

Discussion and conclusions. The test for dominant lethal mutations is one of the few methods available for evaluating the in vivo mutagenic potential of chemicals and other environmental pollutants in the germ cells of mouse and rat. The genetic basis of dominant lethality can be due to structural and numerical chromosomal aberrations and point mutations. It is evident from the results, that 1.0 μCi of ^{14}C induces damage in post-meiotic and in pre-meiotic stages of spermatogenesis, causing a significant increase in post-implantation loss.

Chromosomal aberrations produced by C-14 were first reported by McQuade et al.[5] in onion root tips. In their subsequent study they found that ^{14}C is more effective than

Results on the uterine contents in treated and control mice

	Mating week	Pregnant females	Total implants	Dead implants	Live implants	Pre-implantation loss Total implants / Female	Post-implantation loss Dead implants / Female	%	Total loss Live implants / Female
^{14}C-treated (1.0 μCi)	1	39	343	37	306	8.79	0.95*	10.79*	7.85
	2	37	355	20	335	9.59	0.54	5.63	9.05
	3	41	353	58	295	8.60	1.41*	19.18*	7.20
	4	42	381	30	351	9.07	0.71	7.87	8.35
	5	39	316	25	291	8.10	0.64	7.91	7.46
	6	35	310	29	281	8.86	0.83*	9.35*	8.02
Control (saline)	1	40	336	16	320	8.40	0.40	4.76	8.00
	2	38	342	18	324	9.00	0.47	5.26	8.53
	3	40	347	21	326	8.65	0.53	6.05	8.15
	4	37	311	16	295	8.40	0.43	5.14	7.97
	5	35	339	15	324	9.69	0.43	4.42	9.26
	6	39	306	14	292	7.85	0.36	4.58	7.49

* Significant in comparison to controls at 0.05 level.

^{3}H in inducing the cytogenetic effects. Kuzin et al.[15] showed that the ^{14}C incorporated in plant seedlings was 10 times more effective in producing chromosomal aberrations than that of an equal dose of external gamma irradiation. ^{14}C was also found to induce a very high frequency of sex-linked recessive lethal mutations in *Drosophila*[10-13]. The mutagenic potential of ^{14}C is both due to the emission of beta particles and the transmutation of ^{14}C into ^{14}N[6,15].

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Sequential analysis of Giemsa banded chromosomes in *Vicia faba*

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Summary. *Vicia faba* (broad bean) root-tip chromosomes were subjected to the SSC-Giemsa and trypsin-Giemsa regimes. Phase-lucent cross-bands seen after fixation/SSC treatment subsequently stain positively with Giemsa. Sequential analysis of the trypsin-Giemsa regime shows, in contrast, that bands are manifest through selective removal of material from interband regions.

Several investigators have shown that Giemsa banding involves a removal or rearrangement of chromosomal material[2-4]. Most evidence indicates that this is a removal, or conformational change, of nucleoprotein. The question of the biochemical basis of Giemsa banding is still the subject of much research and is partially equated with the questions of the difference between euchromatin and heterochromatin. This study is a sequential cytological analysis of *Vicia faba* chromosomes subjected to the SSC-Giemsa and trypsin-Giemsa regimes. Band formation by the former method is primarily determined at the SSC step by the selective removal of chromosomal material. Giemsa bands are seen at specifically exposed sites by subsequent staining with Giemsa. On the other hand trypsin-Giemsa bands represent stained chromosomal regions resistant to trypsin digestion.

Materials and methods. *Vicia faba* var. Coles Early Dwarf beans were germinated in vermiculite. Excised lateral meristems from 10-day-old seedlings were pretreated with 0.05% colchicine for 3.5 h, fixed in freshly prepared 1/3 glacial acetic acid/methanol overnight, softened in 10% aqueous pectinase for 5 h at 37 °C, and then squash preparations were made in 45% acetic acid. a) Slides were immersed in 2 × SSC (pH 7.0) at 65 °C for 20 h, rinsed in 3 changes of deionized water, air dried, then placed in Giemsa stain (2 ml of Gurr's improved R66 diluted with 2 ml of 0.1 M Sørensen's buffer, pH 6.9) for 2 h. b) Slides were twice washed in 100% ethanol, dipped briefly into 1 N saline, then treated with a buffered trypsin solution in an ice bath for 1–7 min. The buffer composition was 1.6 g NaCl, 0.04 KCl, 0.4 KH_2PO_4, 0.23 g Na_2HPO_4, 0.02 g $CaCl_2$, 0.02 g $MgCl_2 \cdot 6\ H_2O$, 0.5 g trypsin (from beef pancreas · BDH), dissolved in deionized water and made up to 200 ml[5]. The slides were then washed in 70%, 95% and 100% ethanol, air dried, then stained with Giemsa as in a) for 10 min.

Results. Directly after fixation, c-metaphase chromosomes show no differentiation of light or dense regions along the chromosome arms. After fixation/SSC treatment most chromosomes show 2 positive dots at the centromere, 1 on each sister chromatid (fig. 1). Different techniques applied to other organisms also reveal these C-dots[6]. Phase-lucent cross-bands appear in certain positions along many chromosome arms. For example, the large M chromosome in figure 2a shows 1 phase-lucent band on the M1 arm close to the centromere and 2 similar bands on the M2 arm. The phase-lucent bands evident after SSC treatment are stained positively by Giemsa (fig. 2b). The centromeric regions, presumably the centromeric dots, can stain positively with Giemsa (fig. 3) but this is not a consistent feature.

Chromosomes stained after 1–2 min trypsin treatment appear swollen and slightly understained with suggestions of bands (fig. 4). After 4–7 min trypsin treatment there is a decrease in chromosome stainability with a corresponding differentiation of bands (fig. 5 and 6). These bands show very little increase in stain intensity. Concomitant with decrease in chromosome stainability is the appearance of an amorphous 'ghost' around the boundary of each chromosome which is not seen around chromosomes which retain their stain. Ghosting is weak or absent in banded regions (sub-telocentric S chromosome in fig. 7). Monitoring of the above procedure with phase contrast microscopy endorses these results.

In advanced stages of trypsin digestion (> 7 min) individual chromosomes, along with their bands, are faint and barely discernible.

Discussion. The SSC-Giemsa technique reveals bands by the removal of material from banded regions by SSC and these regions subsequently stain positively with Giemsa. The exposure of specific chromosomal sites leads to selective banding possibly by the linking of the thiazine group of Giemsa to free DNA phosphate groups. Some investiga-