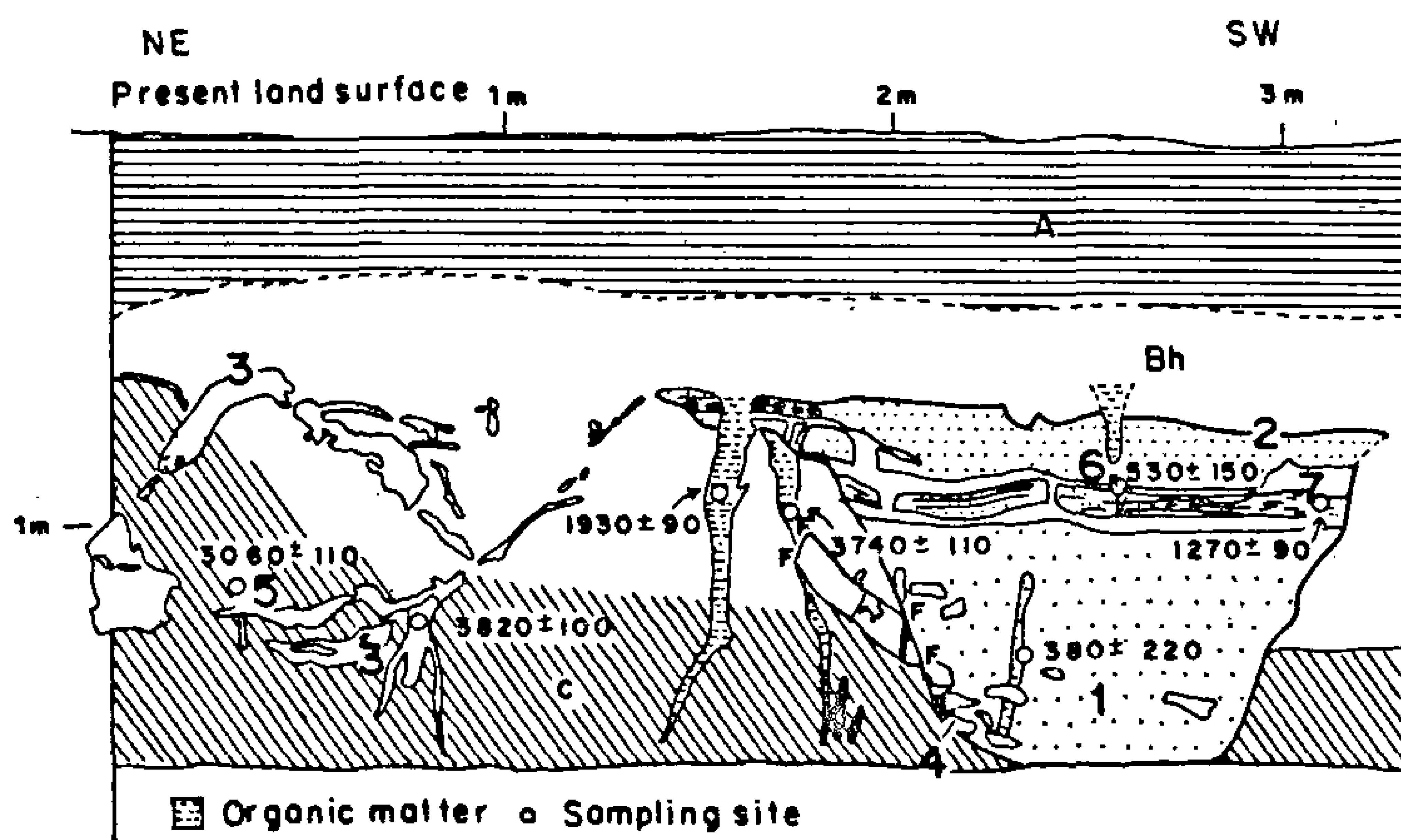

Figure 2. Cross-sectional trench at a site where seven samples were dated by ^{14}C analysis. The preserve crater (1) has the same internal grading and bedding (2) and slumped clasts of Bh material (4). A preserved conduit or sandblow (3) is northeast of crater. Faulting (F) associated with formation of the crater offsets roots dated at 3740 ± 110 years before present. Dates obtained from roots crosscutting the infilled crater yielded ages of 530 ± 150 years (6), 380 ± 220 years, and 1270 ± 90 years (7). Dates from the infilled crater indicate that it was emplaced after 3740 ± 110 but before 1270 ± 90 years BP (adapted from Talwani and Cox[9]).

Purnea, Darbhanga, Muzzafarpur and Champaran districts[12]. Similarly extensive sand cratering is reported in the 1897 great Assam earthquake[13]. The liquefaction of the soils was reported at fewer and areally smaller localities for the 1905 great Kangra earthquake around Roorkee. Ambala and Gujranwala districts[14]. Thus, a possibility appears that the experience of palaeoseismicity studies in the cases of Charleston and New Madrid earthquakes could be used to establish further constraints on the repeat times of great earthquakes in the Himalaya.

Some of the localities in the alluvial belt of the Ganga foredeep that have been reported to have suffered soil liquefaction are shown in Figure 1. It is suggested that palaeoseismic investigations of such localities be taken up urgently. Selected sites should be trenched and explored for liquefaction signatures that may have preserved datable organic material from past earthquake events. The known liquefaction sites could be studied for characteristic geomorphic features. In addition, seismic noise studies can be quickly conducted at these sites using portable accelerographs to characterize the noise spectrum and the shallow velocity structure. These characteristic features together will serve to identify further sites in the regions of Ganga foredeep in front of the seismic gaps for similar studies.

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Cholera toxin, zonula occludens toxin and accessary cholera enterotoxin gene-negative *Vibrio cholerae* non-O1 strains produce the new cholera toxin

Vibrio cholerae non-O1 have been reported to produce cholera toxin (CT), although certain strains did not do so[1]. In recent years, several other extracellular products such as a heat stable toxin (NAG-ST), a thermostable direct haemolysin, El Tor-like haemolysin, a shiga-like toxin, haemagglutinin and zot[2] produced by *V. cholerae* non-O1, have been reported to play some role in causing disease. However, none of these virulence factors alone was considered

as the cause of enteropathogenicity. Moreover, the recent observation of Kurazono *et al.*[3] that the majority strains of *V. cholerae* non-O1 of clinical and environmental origin lack the virulence genes cassette comprising CT, zonula occludens toxin (zot) and accessary cholera enterotoxin (ace), indicates that these strains may possess another unidentified virulence gene encoding an yet unrecognized secretogen.

Earlier we demonstrated that CT^- or CT^+ strains of *V. cholerae* O1, biotype El Tor or classical, serotype Ogawa or Inaba of clinical or environmental origin or genetically engineered in the laboratory produce a new cholera toxin (NCT)[4–6], and the disease cholera may be caused either by CT or NCT or both. However, the production of NCT by *V. cholerae* non-O1 has not yet been reported, although these organisms cause diarrhoea in humans and a secretory response in experimental animals. In this study, an attempt was therefore, made to examine the production of NCT by *V. cholerae* non-O1, isolated from the River Ganga in Varanasi, India. These strains of *V. cholerae* non-O1 were *ctx*, *zot* and *ace* negative as tested using specific DNA probes[3] and were, therefore, devoid of the core dynamic region of *V. cholerae* O1[7] (G.B. Nair, pers. commun.).

Live cells and one of the five isolates of *V. cholerae* non-O1 tested in ligated ileal loops of adult albino rabbits (Belgian strain), following the method of De and Chatterje[8], caused accumulation of fluid in the initial test (0.7–1.2 ml/cm of RIL). The other four isolates did so after one to four consecutive passage(s) through rabbit gut in the range of 0.5–1.1 ml/cm of RIL, and thereafter outpouring of fluid by every strain increased with each passage. Culture filtrates (CFs) of these strains prepared in AKI medium[9], only after their live cells caused fluid accumulation in RILs, when tested in the same assay showed a similar secretory response (0.5–1.2 ml/cm of RIL), although slightly less than that of toxigenic *V. cholerae* strain 569B (1.1–1.4 ml/cm of RIL) but did not cause lysis of sheep erythrocytes when tested by conventional method.

These observations indicate that the non-O1 *V. cholerae* strains that lack gene for all known toxin factors such as CT, zot and ace, except NCT, produce a secretogen. Enhancement of secretory response upon passage through the gut of a susceptible host suggests that if such strains circulate in the community, because aquatic life is the reservoir of non-O1 *V. cholerae* strains, its virulence may increase further.

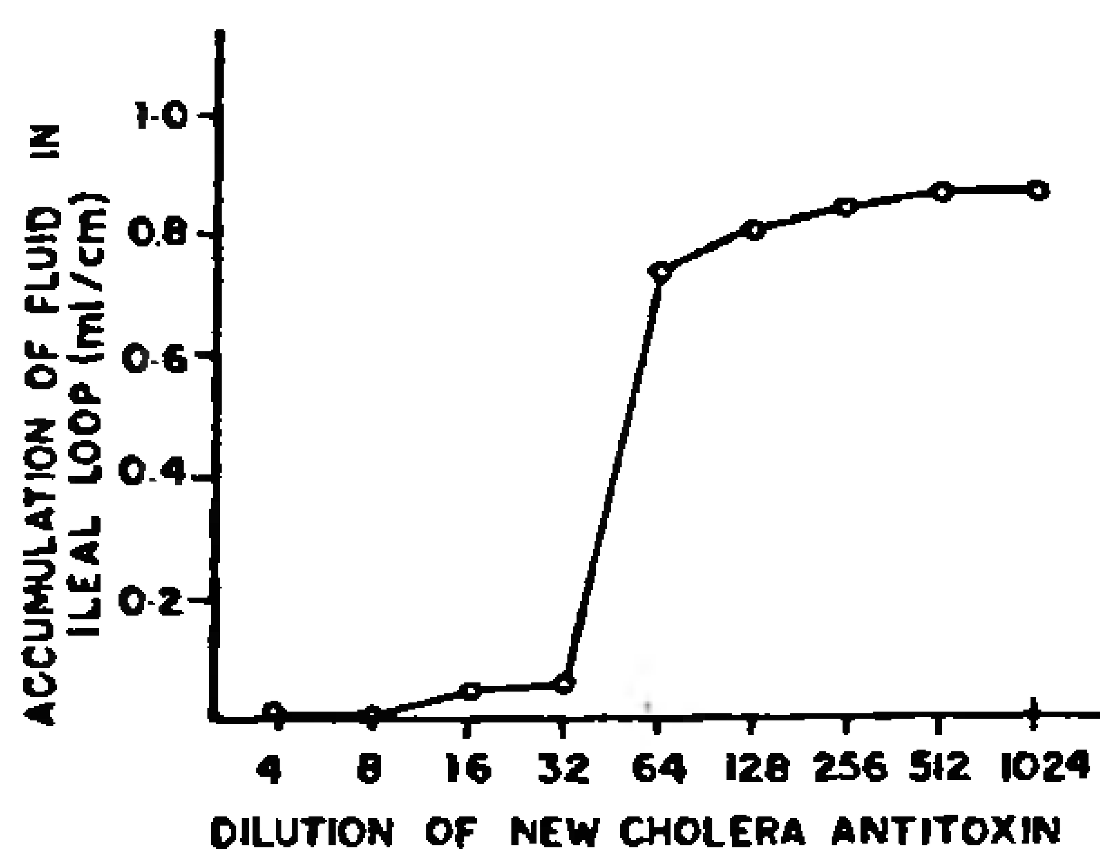

Figure 1. Neutralization of non-O1 *V. cholerae* enterotoxin by new cholera antitoxin. The results are means of filtrates of five cultures.

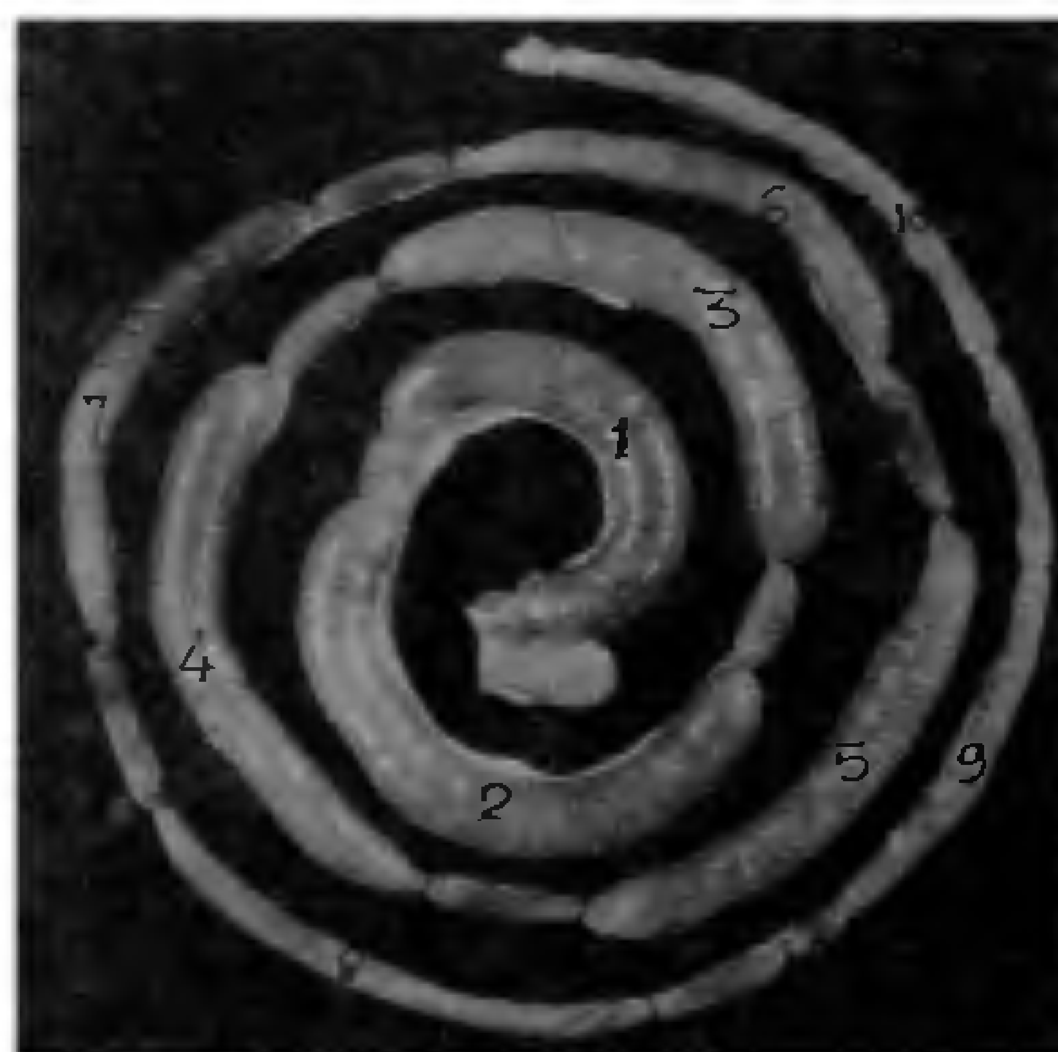

Figure 2. Neutralization of non-O1 *V. cholerae* enterotoxin by new cholera antitoxin. Loop 1: Positive control, 0.15 ml of culture filtrate (CF, optimal loop-reacting dose) mixed with 0.85 ml of phosphate buffered saline (PBS, 0.02 M, pH 7.4); Loop 2–9: 0.15 ml of CFs in 0.35 ml of PBS with 0.5 ml of 1 in 512, 1 in 256, 1 in 128, 1 in 64, 1 in 32, 1 in 16, 1 in 8 and 1 in 4 dilutions of new cholera antitoxin. Loop 10: negative control, 1.0 ml of PBS.

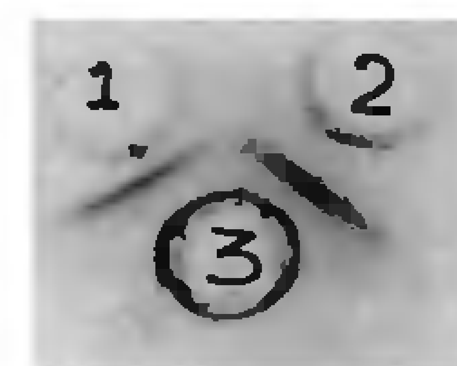

Figure 3. Immunological identity of the NCT with non-O1 *V. cholerae* (strain no. 88 SH) enterotoxin. Ouchterlony immunodiffusion analysis of concentrated CF of CT^- *V. cholerae* strain X-392 (well 1) and *V. cholerae* non-O1 strain 88 SH (well 2) against antiserum of X-392 NCT (well 3).

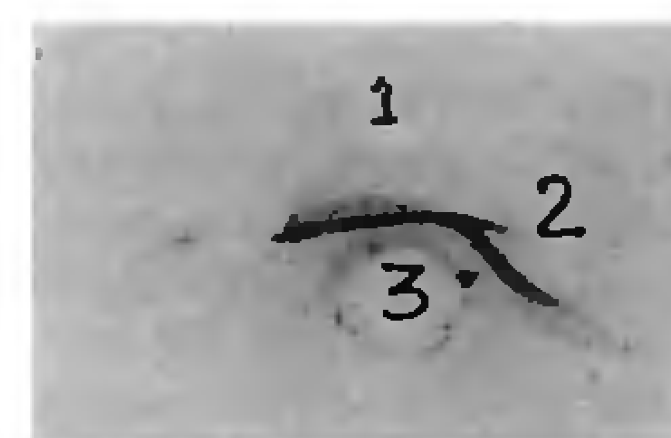

Figure 4. Immunodiffusion comparison of the NCT and *V. cholerae* non-O1 strain 86-TG2 enterotoxin. Concentrated CF of the strain X-392 (well 1) was compared with concentrated CF of the strain 86 TG2 (well 2) against antiserum of X-392 NCT (well 3).

In RIL assays, the enterotoxic activity of non-O1 *V. cholerae* strains was completely neutralized by the antiserum against purified NCT diluted in 1:32 (Figures 1 and 2) prepared with CT^- strain of *V. cholerae* X-392, which was shiga or shiga-like toxin gene-negative as tested using specific DNA probes (P. Echeverria, pers. commun.) and was non-haemolytic when tested by conventional method.

The observation that CF of one isolate showed complete identity and neutralization by anti NCT indicates that this strain produces a secretogen antigenically similar to NCT. However, the other four isolates that showed partial identity with NCT and complete neutralization of enterotoxic activity in RIL by its antiserum in the same dilution may suggest that these strains also produce NCT but differ in some weaker epitopes[10], as has been observed in CT-B subunits produced by classical and El Tor biotype strains[11]. Tamplin *et al.*[12] also observed five shared and one unshared epitope between classical and El Tor CFs as well as some variation in the extent of cross reactivity between different El Tor CT-B preparations with some of the anti-classical monoclinal antibodies. Although the subunit structure of NCT could yet be determined, its

molecular weight being as large as 61,000 Da (unpublished data) there is every likelihood that this toxin possesses some subunits, the epitopes of which may differ slightly from strain to strain. This difference, however, is minor and does not affect the neutralizing capability of the antitoxin against X-392.

In gel-diffusion test, 10 times concentrated CF of CT^- *V. cholerae* X-392 that produces NCT[4] and *V. cholerae* non-O1 strains gave a precipitation band against anti-NCT. Only one isolate showed reaction of identity (Figure 3) and the other four showed reaction of partial identity (Figure 4).

The results of this study suggest that the strains of *V. cholerae* non-O1 can produce NCT in the absence of *ctx*, *zot* and *ace* or when these genes are deleted. They, thus possess the potential to cause diarrhoea. These observations are of importance in understanding the pathogenesis of diarrhoea caused by *V. cholerae* non-O1 strains as this toxin seems to play an important role in the causation of diarrhoea[4]. However, further study with a large number of isolates is needed to strengthen this conclusion.

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Erratum

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

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[*Curr. Sci.*, 1995, **69**, 496–498]

The table appearing on page 497 contains some mistakes. The correct table is printed below.

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	6
Number of offsprings	C	122	121	120	119	117	115	114
	E	98	96	95	94	92	88	87
Growth-retarded offsprings (%)	C	1.64 (2)	1.65 (2)	2.50 (3)	3.36 (4)	3.42 (4)	3.48 (4)	3.51 (4)
	E	4.08 (4)	4.17 (4)	7.37 (7)	10.64 (10)*	11.96 (11)*	11.36 (10)	8.04 (7)
Body weight (mean ± SE, g)	C	1.67 ± 0.014	4.76 ± 0.054	7.27 ± 0.081	11.59 ± 0.159	17.57 ± 0.367	24.43 ± 0.273	28.05 ± 0.303
	E	1.64 ± 0.018	4.62 ± 0.059	7.09 ± 0.125	10.71 ± 0.215^{b}	15.68 ± 0.486^{b}	22.23 ± 0.489^{c}	27.15 ± 0.446
Body length (mean ± SE, mm)	C	32.11 ± 0.115	46.78 ± 0.235	54.91 ± 0.227	68.06 ± 0.366	79.65 ± 0.447	87.83 ± 0.366	91.64 ± 0.417
	E	31.88 ± 0.135	46.19 ± 0.317	54.79 ± 0.417	64.68 ± 0.670^{c}	71.62 ± 0.789^{c}	83.95 ± 0.949^{b}	89.89 ± 0.814
Head length (mean ± SE, mm)	C	8.73 ± 0.056	13.83 ± 0.091	15.86 ± 0.103	20.85 ± 0.146	22.38 ± 0.160	24.12 ± 0.115	24.70 ± 0.125
	E	8.58 ± 0.061	13.60 ± 0.119	16.19 ± 0.146	20.59 ± 0.122	21.13 ± 0.185^{c}	23.58 ± 0.176^{a}	24.36 ± 0.134
Head width (mean ± SE, mm)	C	7.99 ± 0.075	12.31 ± 0.075	15.58 ± 0.133	18.03 ± 0.168	18.87 ± 0.214	19.98 ± 0.162	20.96 ± 0.167
	E	7.99 ± 0.096	12.11 ± 0.146	14.86 ± 0.239^{b}	16.83 ± 0.204^{c}	17.71 ± 0.257^{c}	19.23 ± 0.243^{a}	20.39 ± 0.223
Tail length (mean ± SE, mm)	C	13.25 ± 0.140	26.13 ± 0.137	42.37 ± 0.246	57.45 ± 0.371	70.26 ± 0.429	79.63 ± 0.417	84.21 ± 0.460
	E	13.06 ± 0.136	25.57 ± 0.195	40.72 ± 0.445^{a}	54.93 ± 0.638^{b}	68.26 ± 0.635^{a}	74.08 ± 0.806^{c}	80.19 ± 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 control (C): $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$ (Mann–Whitney test). $^{*}p < 0.05$ (Fisher's exact test).