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EFFECTS OF ^{60}CO GAMMA RADIATION ON *Biomphalaria glabrata* (SAY, 1818) EMBRYO. II. MALFORMATIONS

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ABSTRACT

The morphogenetic effects of ionizing radiation were investigated in *Biomphalaria glabrata* embryos irradiated in the cleavage, blastula, gastrula, young trochophore and trochophore stages with 5 to 25 Gy doses of ^{60}CO gamma radiation. The number of malformed embryos rapidly increased with increasing radiation dose, reaching a maximum between 5th to 8th day after irradiation in all stages analyzed. Susceptibility to malformation induction was higher the younger the age of the irradiated embryo. However, for the cleavage stage the frequency of malformed embryos was inversely proportional to radiation dose for the same radiation dose. Several types of morphogenetic malformations were obtained, among them cephalic malformations, exogastrula, shell malformations and embryos with everted stomodeum, unspecific malformations being the most frequent. The results show that the types of malformation induced by radiation probably are not radiation-specific and do not depend on the dose applied.

INTRODUCTION

Ionizing radiation is a powerful malformation inducer in living organisms. Although many studies have been conducted on different animal species, the mechanisms leading to these anomalies are not well understood. However, the final effect is known to be preceded by a series of abnormal and complex events occuring during embryogenesis which result in a deviation from normal embryonic development (Russell and Russell, 1954; Wolsky, 1982).

The first studies on experimental mollusc embryology date back to the end of the last century (Crampton,1896).These organisms represent useful material for the study of experimental embryology since they have eggs whose different regions are destined to form the future organs soon after fertilization and before the first cleavage. Thus, many experiments have been performed using chemical or physical processes or agents such as LiCl in L. stagnalis (Verdonk, 1965), caffeine (Kawano and Simões, 1987), 2-chloroethyl trimethyl ammonium chloride, gibberellin (Kawano et al, 1982), and Laurus nobilis extract(Ré and Kawano, 1987) in B. glabrata, blastomere removal in L. stagnalis, L. (stagnicola) palustris and Bithynia tentaculata (Cather et al, 1976) and in Ilyanassa obsoleta (Clement, 1967), centrifugation(Geilenkirchen, 1964), heat shock (Geilenkirchen, 1966;Boon-Niermeijer, 1976) and UV (Labordus, 1971) in L. stagnalis. Several investigators obtained different types of morphogenetic malformations such as exogastrula, hydropia, and shell, cephalic and unspecific malformations.

However, with respect to the morphogenetic effects of ionizing radiation, the only paper available in the literature is that by Kawano (1982) who analyzed the effects of a 10 Gy dose of Cs-137 and 0.1% caffeine as a synergistic agent of radiation on an albino mutant of B. glabrata.

In the present paper we report the effects of ^{60}CO gamma radiation on the morphogenesis of B. glabrata embryos submitted to different doses of ionizing radiation at different stages of development.

MATERIAL AND METHODS

Wild-type *B. glabrata* (Mollusca: Gastropoda) embryos originating from Belo Horizonte (MG) and reared in the laboratory for several years were used in the experiments. The embryo stages investigated were the first egg cleavages, i.e. first, second and third cleavages (0 to 3.50 hours of age), blastula (6 to 15 hours), gastrula (24 to 30 hours), young trochophore (48 to 54 hours) and trochophore (72 to 78 hours) (Okazaki, 1988).

The embryos were irradiated with doses of 5, 10, 15, 20, and 25 Gy of gamma radiation (136 Gy/h) using a Gamma-cell 220 ^{60}CO source (Irradiation Unit of the Canadian Atomic Energy Commission, Ltd.). Immediately after radiation treatment, the egg masses were placed in a container with dechlorinated water, identified and maintained at 25 $\pm$ 1^{o}C. Embryos were analyzed daily under a Zeiss stereoscopic microscope up to the 15th day after irradiation. Five replications were performed for each embryo stage using approximately 100-200 embryos per radiation dose per experiment.

Malformed embryos were identified using the criteria of Raven (1949), Verdonk (1965) and Geilenkirchen (1966). Embryos showing a reduction in size or delayed development were considered to be "normal" as long as they did not present any *in vivo* identifiable malformation.

For *in toto* embryo analysis, the material was prepared by the method of Camey and Verdonk (1970).

RESULTS

Figure 1A-E show the frequencies of malformed embryos obtained throughout the experiment as a function of radiation dose.

Table I shows the modes of malformed embryos frequencies obtained with various radiation doses in the different developmental phases. Mode increased with increasing radiation dose and with decreasing embryo age for the same dose. However, in the cleavage stage there was a significant fall in the modes of malformed embryo frequencies with increasing radiation dose ($Y = 0.53-0.02X$, $b = 0$, $R = 0$, $F_{1.13} = 22.89$, $P < 0.05$).

The morphogenetic malformations obtained were predominantly of the unspecific type (figure 2) in all embryo stages and at all doses investigated. However, some cephalic malformations of the monophthalmia and eye reduplication type (figure 3), as well as exogastrula, shell malformation and everted stomodeum were also detected. Figure 4 shows the appearance of normal snails.

Exogastrulas and cephalic malformations were observed in eggs irradiated in the earliest stages, while embryos with stomodeum eversion and shell malformations were observed after irradiation of the more advanced stages of young trochophore and trochophore.

DISCUSSION

The malformed embryo curves obtained for B. glabrata eggs irradiated at different developmental stages showed that, in general, the biological response of the embryos to radiation followed a definite trend which was evaluated in terms of the doses selected. At all embryo phases, the frequency of malformed embryos increased rapidly with increasing radiation dose, reaching a maximum between the 5th and 8th day after irradiation and later decreasing because of gradual embryo death. The malformations present by the embryos must surely have been so severe that most of the malformed embryos were unable to survive and died before hatching. On the other hand, the survivors that reached hatching rarely showed malformations, at least apparent ones.

In the cleavage stage, however, the rate of malformed embryos decreased with increasing radiation dose, with values of 45.9, 36.3 and 17.4% at the doses of 5, 10, and 15 Gy, respectively. A significant fall in malformed embryo frequency modes was due to the progressive increase in embryo mortality with increasing radiation doses, as a direct consequence of the lethal action of radiation during the first days after exposure (Okazaki and Kawano, in press).

The more evolved the embryo, the higher the doses needed to produce a quantitatively similar effect in relation to earlier embryos. The modes tended to accompany the increase in radiation dose: the higher the dose, the shorter the time needed to detect its effect.

The same types of morphogenetic malformations obtained with irradiation are also produced in mollusc embryos by different agents such as heat shock (Boon-Niermeijer, 1976), centrifugation (Geilenkirchen, 1964), blastomere removal (Cather et al, 1976), LiCl (Verdonk, 1965), caffeine (Kawano and Simões, 1987), 2-chloroethyl trimethyl ammonium chloride (Kawano et al, 1982), and _Laurus nobilis_ extracts (Ré and Kawano, 1987). These observations show that the morphogenetic malformations induced by radiation are not radiation specific.

Apparently, the type of morphogenetic anomaly resulting from radiation of _B. glabrata_ embryos did not depend on the dose applied. Unspecific malformations, the predominant type detected by us, were observed at all doses analyzed. However, the frequency was clearly dose dependent. Similar observations were made by Verdonk (1965) on eggs of _Limnaea stagnalis_ submitted to different LiCl concentrations (2, 2.5 and 3.5 x 10^{-5} M) during the various phases of development.

On the other hand, a certain association between type of morphogenetic malformation and stage of development submitted to irradiation was noted, except for unspecific malformations, which were observed at all stages. The precursor cells responsible for the formation of the entire cephalic structure of the _B. glabrata_ embryo, except the mouth, practically originate from the cellular descendants of the first micromere quartet (1a-1d) at the third cleavage stage (Camey and Verdonk, 1970). Several investigators have suggested that the inductive process important for gastrulation probably occurs also in the first cleavage stages (Geilenkirchen, 1967; Biggelaar, 1971 a, b). Thus, the action of physical or chemical agents during these developmental stages may cause a deviation in cephalic pattern or a suppression of gastrulation, with the consequent occurrence of anomalies of the cephalic malformation or exogastrula type.

The cephalic malformations of the monophthalmia and eye reduplication types detected in the present study suggest a possible interference of ionizing radiation with the chronology of division of the cephalic plates (future regions of eye and tentacles) which were probably in a phase of intense proliferation. Since the apical plate and the cerebral vesicle were probably already formed (Camey and Verdonk, 1970), the cephalic malformations of the cyclocephalic series were not observed. Ionizing radiation is known to cause a delay in division at any phase of the cell cycle, although G_2 seems to be the most sensitive phase (Denekamp and Rojas, 1989).

On the other hand, in B. glabrata embryos, structures such as the stomodeum and shell gland are formed at more advanced stages (young trochophore and trochophore). Thus, we suggest that ionizing radiation somehow affected the cells that are active in the morphogenesis of these organs, thus resulting in embryos with shell malformations and stomodeum evagination.

These data lead us to assume that, in the case of unspecific malformations, several cell lines were probably affected by radiation. Thus, embryos exposed to radiation may present irregular development, with more affected parts developing less and other areas growing and developing in a normal manner. This does not imply in the exclusion of other factors, but the morphogenetic significance of the chronology of cell division deserves special attention, since this event is a prerequisite for the establishment of a normal relationship between cells and tissues, which is obviously of great relevance for the developing organism (Boon-Niermeijer, 1975).

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RESUMO

Foram analisados efeitos morfogenéticos da radiação ionizante em embriões de B. glabrata, irradiados nos estádios de clivagem, blástula, gástrula, trocófora jovem e trocófora com doses de 5 a 25 Gy de Co-60. O número de embriões malformados aumentou rapidamente com o aumento da dose de radiação, atingindo o máximo entre o 5º e o 8º dia após a irradiação, em todos os estádios embrionários analisados. A susceptibilidade à indução de malformações foi maior quanto menor a idade do embrião irradiado, contudo, para o estádio de clivagem, a frequência de embriões malformados foi inversamente proporcional à dose de radiação. Vários tipos de malformações morfogenéticas foram obtidas, entre as quais, malformação cefálica, exogástrula, malformação da concha e embrião com eversão do estomodéo, sendo a malformação inespecífica a mais frequente. Os resultados obtidos mostraram que os tipos de malformações induzidas pela radiação provavelmente não são específicas dela e que não dependem da dose aplicada.

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FIGURE LEGENDS

Figure 1 (A-E) - Malformed embryos curves obtained from *B. glabrata* after CO-60 gamma irradiation at different development stages. ●—● 0 Gy; o—o 5 Gy; Δ—Δ 10 Gy; □—□ 15 Gy; x—x 20 Gy; θ—θ 25 Gy.

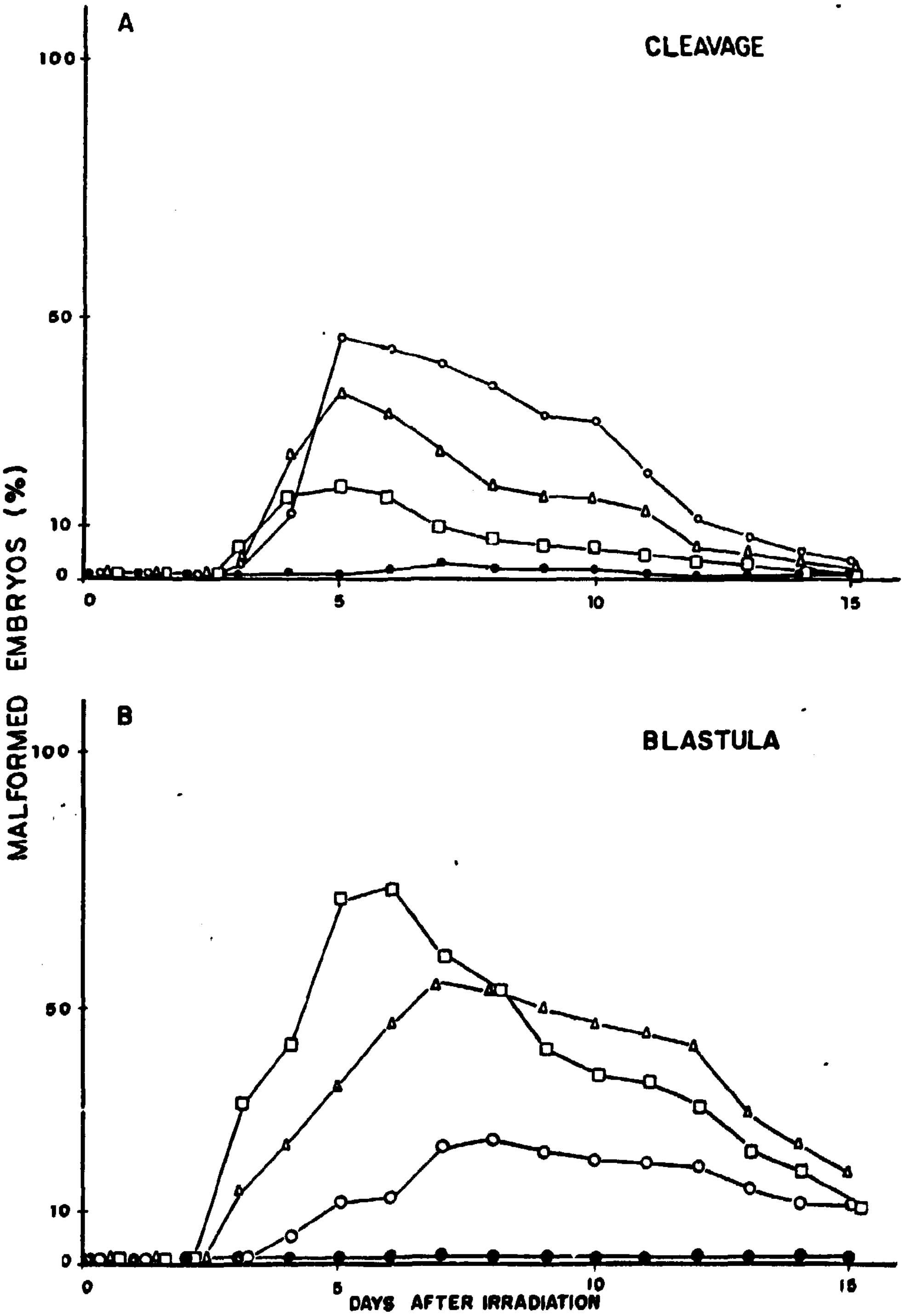
Figure 2 - Egg mass irradiated with 10 Gy at the blastula - stage, at the 6th day after treatment showing several unspecific malformed embryos. The arrow shows a dead embryo. X 48.

Figure 3 - The outlined drawings of head malformations obtained from *B. glabrata* embryos irradiated at the blastula stage with 10 Gy of gamma radiation and fixed at the 5th day after exposure, at the veliger stage. a) normal embryo; b) monophthalmia dextra; c) monophthalmia sinistra; d) reduplication sinistra; e) reduplication dextra; f) reduplication dextra - and sinistra. M = mouth; S = shell; F = foot.

Figure 4 - Control egg mass of *B. glabrata* at 7 days of age showin young snails. X 48.

TABLE I - Modes of malformed embryos frequencies obtained by different radiation doses at the different development stages.

EMBRYONIC STAGES	DOSE (Gy)	NUMBER OF EGG MASSES	NUMBER OF EMBRYOS	DAY AFTER IRRADIATION	MALFORMED EMBRYOS(%)
CLEAVAGE	0	28	557	7	3,5
	5	28	693	5	45,9
	10	29	701	5	36,3
	15	29	596	5	17,4
BLASTULA	0	44	1154	6	1,1
	5	41	1032	8	24,3
	10	41	1130	7	55,5
	15	41	918	6	73,8
GASTRULA	0	40	994	6	0,7
	5	41	1085	7	9,2
	10	41	1060	6	44,6
	15	41	1019	6	81,2
YOUNG TROCHOPHORE	0	35	773	1	2,3
	5	38	766	6	3,1
	10	41	858	6	26,3
	15	38	1034	7	68,7
	20	27	895	5	82,7
TROCHOPHORE	0	42	953	3 and 4	1,2
	5	42	931	1	1,2
	10	42	908	6	4,6
	15	42	1111	7	22,4
	20	24	770	6	59,0
	25	25	840	5	84,3


A
CLEAVAGE
B
BLASTULA
MALFORMED EMBRYOS (%)
100
50
10
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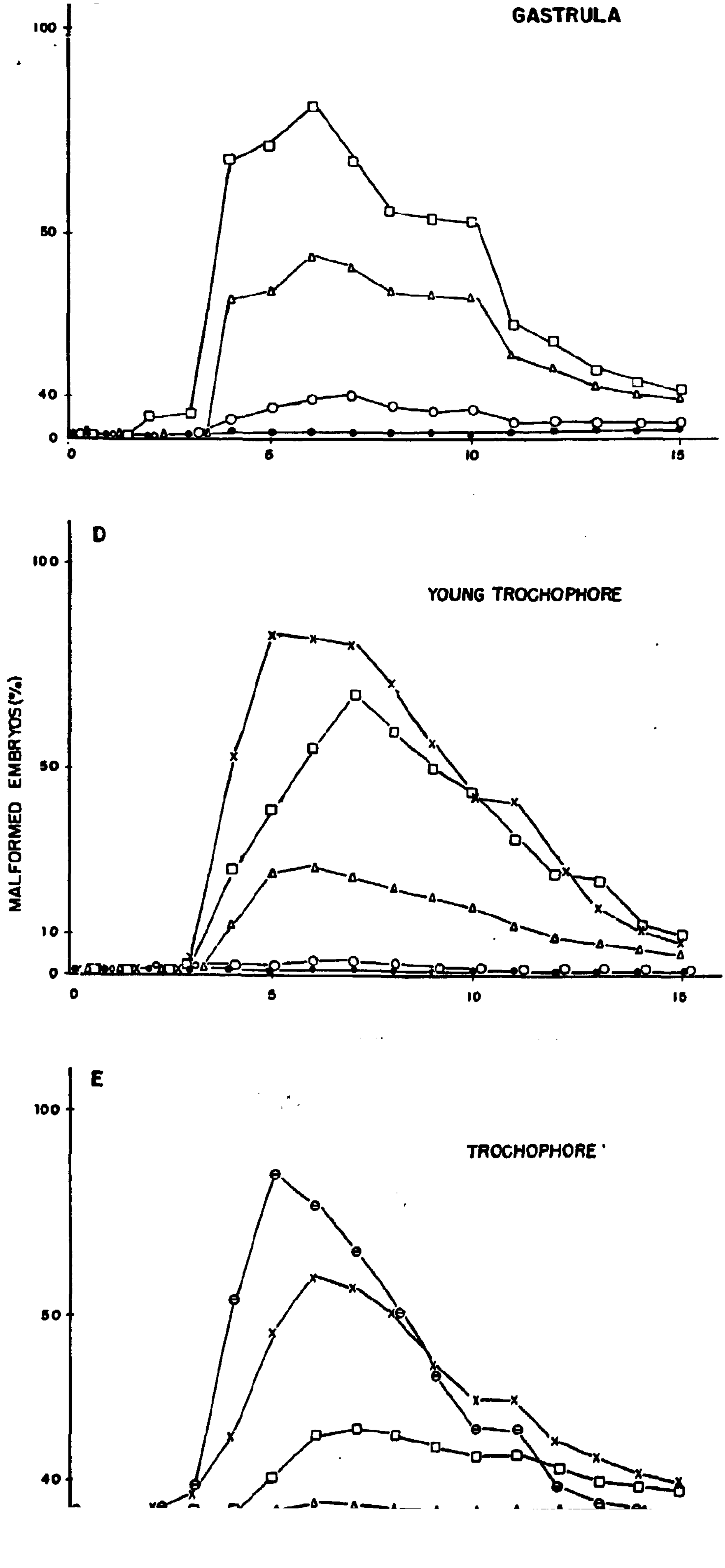
5
15
DAYS AFTER IRRADIATION


GASTRULA
D
YOUNG TROCHOPHORE
MALFORMED EMBRYOS (%)
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TROCHOPHORE
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40
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0
5
10
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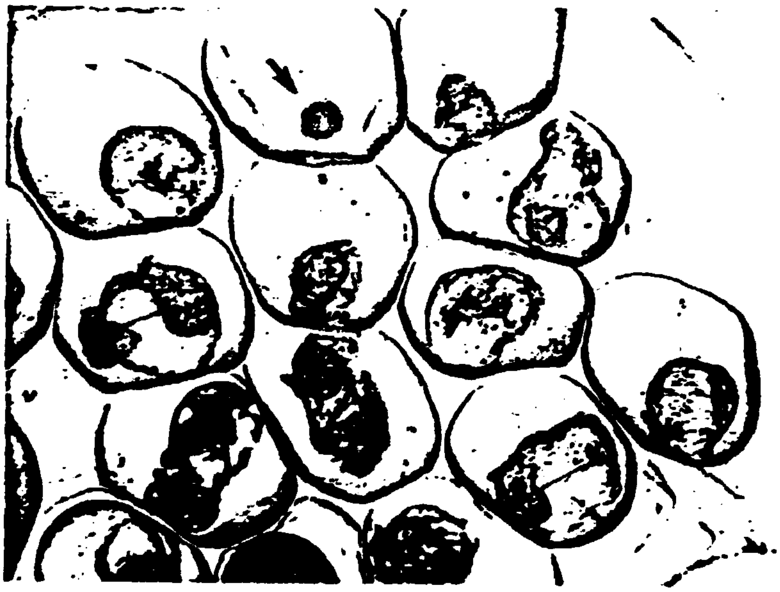

Figure 2: Effects of ^{60}CO gamma radiation

... II. Malformations

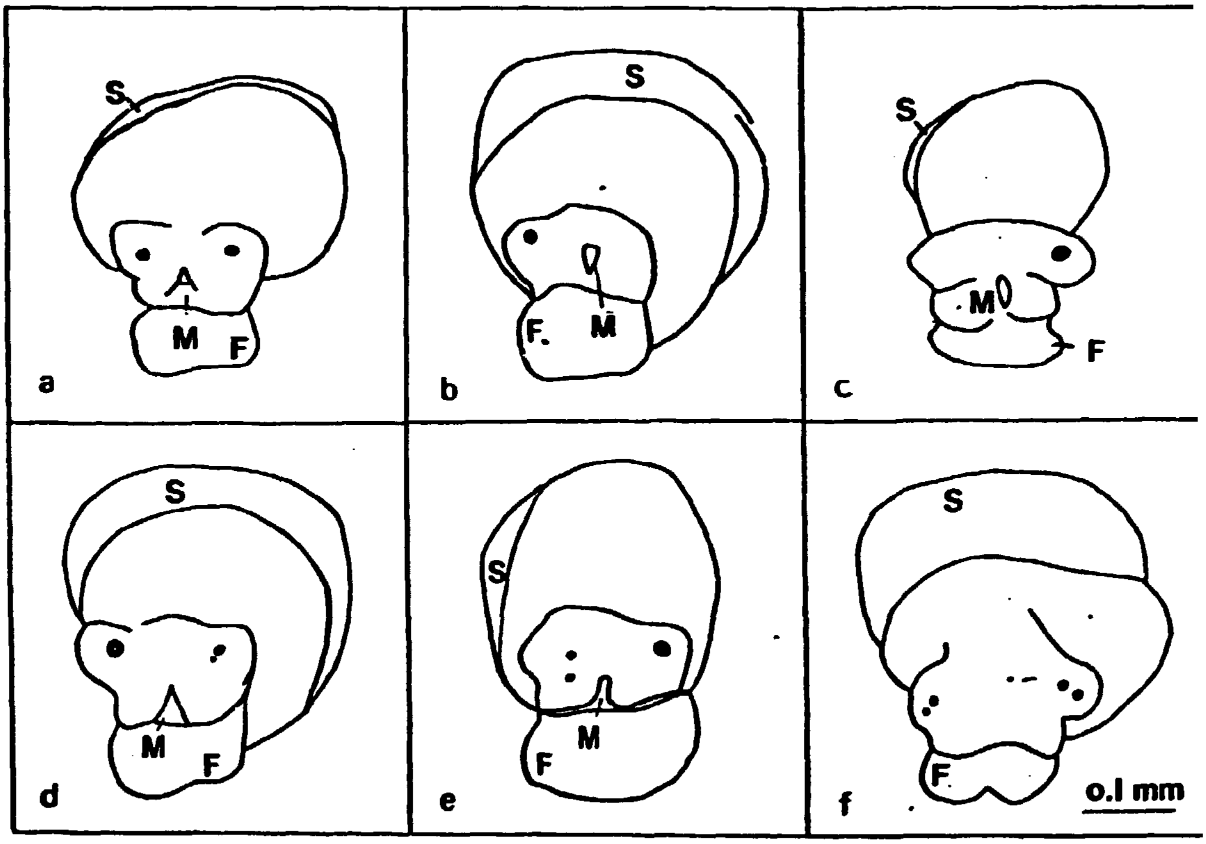

Figure 3: The effects of ^{60}CO gamma radiation on

..... II. Malformations

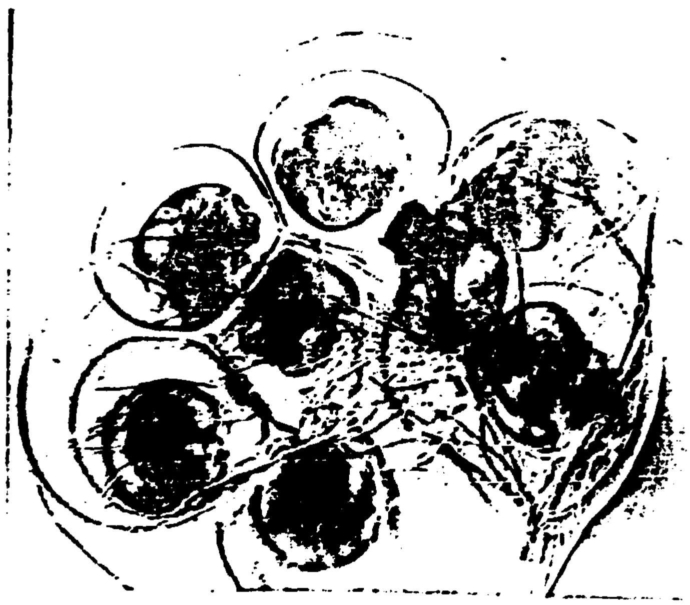

Figure 4: Effects of ... II. Malformations