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Cytogenetic effects of ^{60}Co gamma radiation on *Biomphalaria glabrata* (Say, 1818) embryos

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SUMMARY — *Biomphalaria glabrata* embryos irradiated with ^{60}Co gamma radiation at doses of 5, 10 and 15 Gy during the blastula stage were analyzed cytogenetically 24 hours after treatment. For embryos exposed to the 10 Gy dose, the chromosomal study was repeated 48 hours after irradiation. Several types of structural chromosome aberrations were detected, the most common being dicentrics and acentric fragments. Dicentric data fitted the linear model better, while the acentric fragments fitted the quadratic model. A significant decrease in the frequency of metaphases with aberrations was detected 48 hours after irradiation (12%) when compared with 24 hours (38.9%), indicating a probable elimination of cells with chromosome aberrations. The results also suggest a possible chromosome loss in cells irradiated with the 15 Gy dose, 24 hours after treatment. A fall in mitotic index with increasing radiation dose was observed, showing a certain parallelism between the frequency of chromosome aberrations and the degree of mitotic depression 24 hours after irradiation. An association of radioinduced chromosome aberrations with the morphogenetic effects of radiation is suggested to have occurred in embryos irradiated in the blastula phase.

INTRODUCTION

Ionizing radiation is known to be effective in producing chromosome aberrations in eukaryote cells. Several studies have shown that chromosome aberrations play an essential role in plant and animal cell death (KIHLMAN 1977), in the inhibition of germinating ability (EVANS 1968), in the reduction of root growth (READ 1961), and in the loss of colony-forming ability of cells *in vitro* (SCOTT and ZAMPETTI-BOSSELER 1985).

In the same way as certain human diseases are associated with chromosome breaks (THERMAN 1986), an intimate association between chromosome aberrations and developmental anomalies has been observed by several investigators in different animal species (RUGH 1969; MCGREGOR and NEWCOMBE 1972; WOLSKY 1982), including man (UNITED NATIONS 1986).

The induction of chromosome aberrations by radiation is probably one of

the main perceptible biological manifestations of damage to proliferative cells. Thus, chromosome analysis can provide useful information related, for example, to the prediction of the susceptibility of irradiated systems to radiation-induced genetic damage.

Studies on the snail *Biomphalaria glabrata,* an important vector of schistosomiasis, have focussed on several aspects, but only two papers have been published on the cytogenetic effects of ionizing radiation (NARANG 1974; NARANG and NARANG 1974).

Previous radiosensitivity studies conducted on *Biomphalaria glabrata* eggs have shown a relatively high frequency of dead, malformed and unhatched embryos after exposure to doses of 5 to 25 Gy of gamma radiation (OKAZAKI and KAWANO, in press, *a,b,c*). Embryo age at the time of irradiation and dose applied were fundamental factors in terms of the response of the organism to radiation.

The present paper reports the cytogenetic analysis of *B. glabrata* embryos submitted to different doses of gamma radiation and the relationship between dose and frequency of chromosome aberrations.

MATERIALS AND METHODS

Wild-type *Biomphalaria glabrata* (Say, 1818) (Mollusca: Gastropoda) embryos were from a colony originally obtained from Belo Horizonte (MG) and reared in the laboratory over the past 12 years. Embryos in the blastula stage (6-15 hours after the first egg cleavage at 25° C, according to CAMEY and VERDONK 1970) were selected because this is the phase in which a large number of cell divisions occurs, with consequent high sensitivity to radiation and the occurrence of a larger number of alterations detectable by cytogenetic analysis. Embryos were irradiated with doses of 5, 10 and 15 Gy (136 Gy/h) using a ^{60}Co Gamma-Cell 220 source and processed for cytogenetic analysis 24 hours after treatment, when most of them were in the gastrula stage (24 to 36 hours, at 25° C). For embryos submitted to 10 Gy, cytological preparations were also made 48 hours after irradiation, during the young trochophore stage (48 to 54 hours, at 25° C). Untreated embryos in the same developmental stages as the irradiated ones were used as controls. Approximately 50 embryos were used for each cytological preparation. Metaphase chromosomes were obtained by the cell suspension technique of KAWANO *et al.* (1987).

A total of 450 to 550 metaphases were selected at random for each radiation dose and examined 24 and 48 hours after exposure. Chromosomes were classified by the criterion of LEVAN *et al.* (1964) based on centromeric position and arm ratio.

The metaphase preparations were classified according to two basic criteria: a) number of centromeres, and b) presence of structural chromosome or chromatid aberrations. Metaphases containing $2n$-1 and $2n$-2 centromeres were included in the analyses, and those with less than $2n$-2 centromeres were discarded. Structural aberrations were classified according to BUCKTON and EVANS (1973) and to the International Atomic Energy Agency (IAEA 1986).

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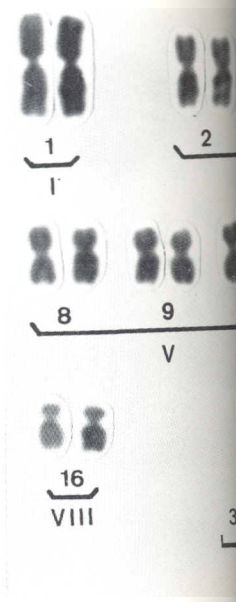

Fig. 1. — Normal karyo
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Keeping in mind the possibility that the cells may have undergone more than one mitotic division after treatment, the rearrangement aberrations and acentric fragments detected in the same metaphase figure were counted separately. The mitotic index was determined 24 hours after irradiation by analyzing approximately 2000 nuclei selected at random from each group (irradiated and control).

The data were adjusted by the least squares method.

RESULTS

Fig. 1 shows the normal karyotype of a *B. glabrata* embryo with $2n = 36$ chromosomes classified into 12 metacentric pairs (groups I, II, V, VII and IX), 4 submetacentric pairs (groups III and VIII) and 2 subtelocentric pairs (groups IV and VI). Even though the normal chromosome number for the *B. glabrata* karyotype is 36, a few polyploid metaphases were detected both in control embryo preparations processed in the young trochophore stage (Fig. 2) and in irradiated embryos analyzed 48 hours after exposure (Fig. 3).

Fig. 4 shows several types of structural chromosome aberrations detected

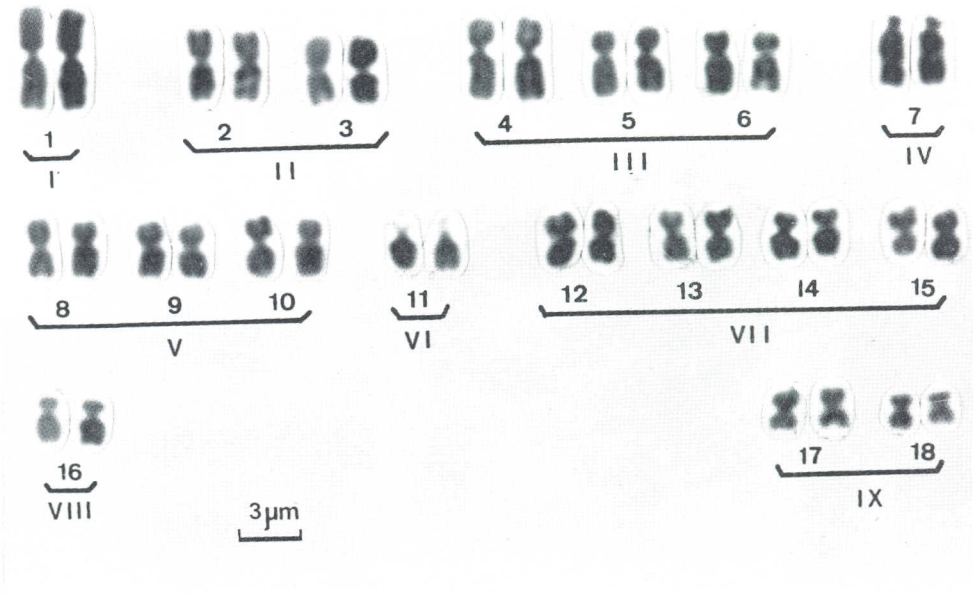


Fig. 1. — Normal karyotype of *Biomphalaria glabrata* embryo containing 18 chromosome pairs classified into 12 metacentric (groups I, II, V, VII and IX), 4 submetacentric (groups III and VIII) and 2 subtelocentric pairs (groups IV and VI).

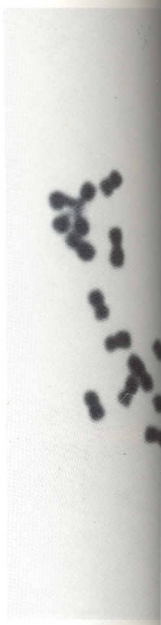
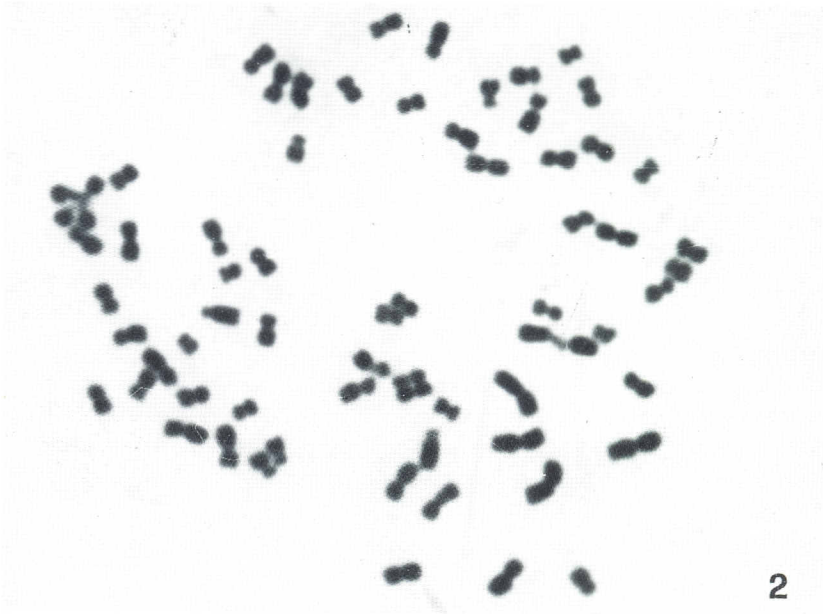


Fig. 2. — Normal polyploid metaphase obtained from *Biomphalaria glabrata* young trochophore. × 1600.
Fig. 3. — Polyploid metaphase with several dicentrics and tricentrics chromosomes obtained from embryos irradiated at the blastula stage with 10 Gy and processed 48 hours after treatment. × 2560.



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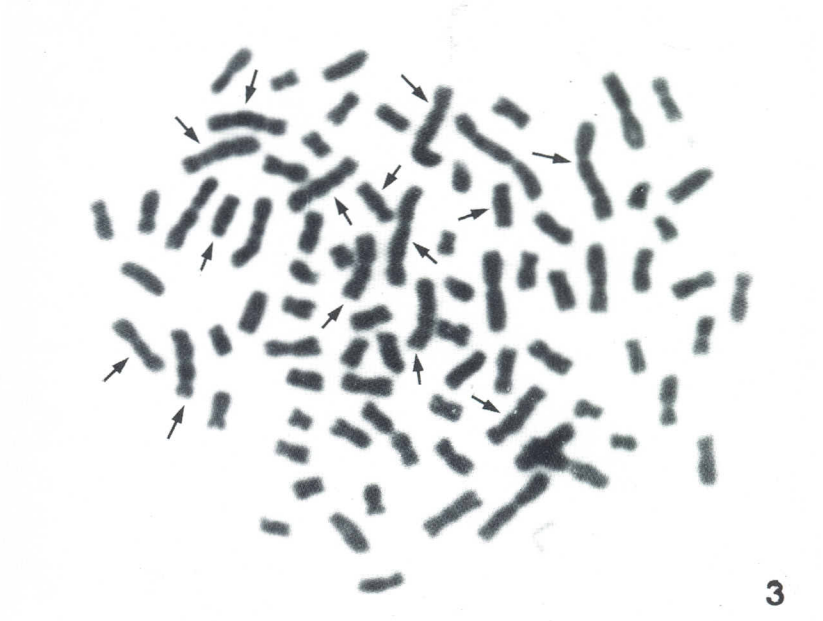

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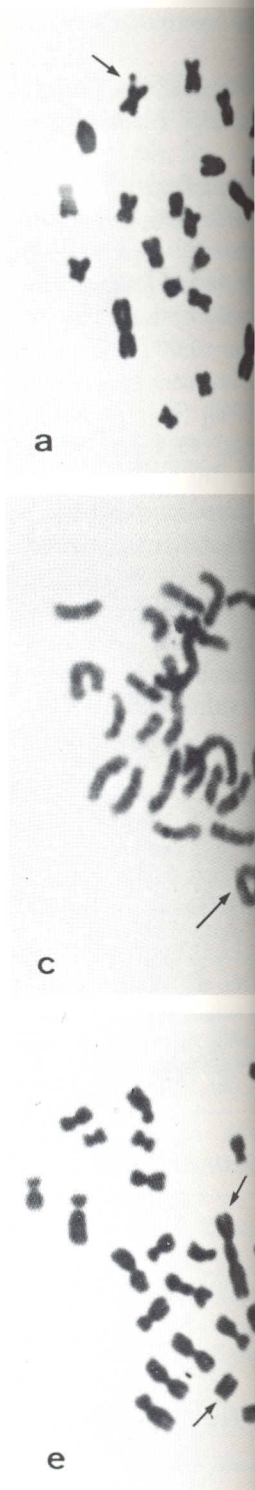
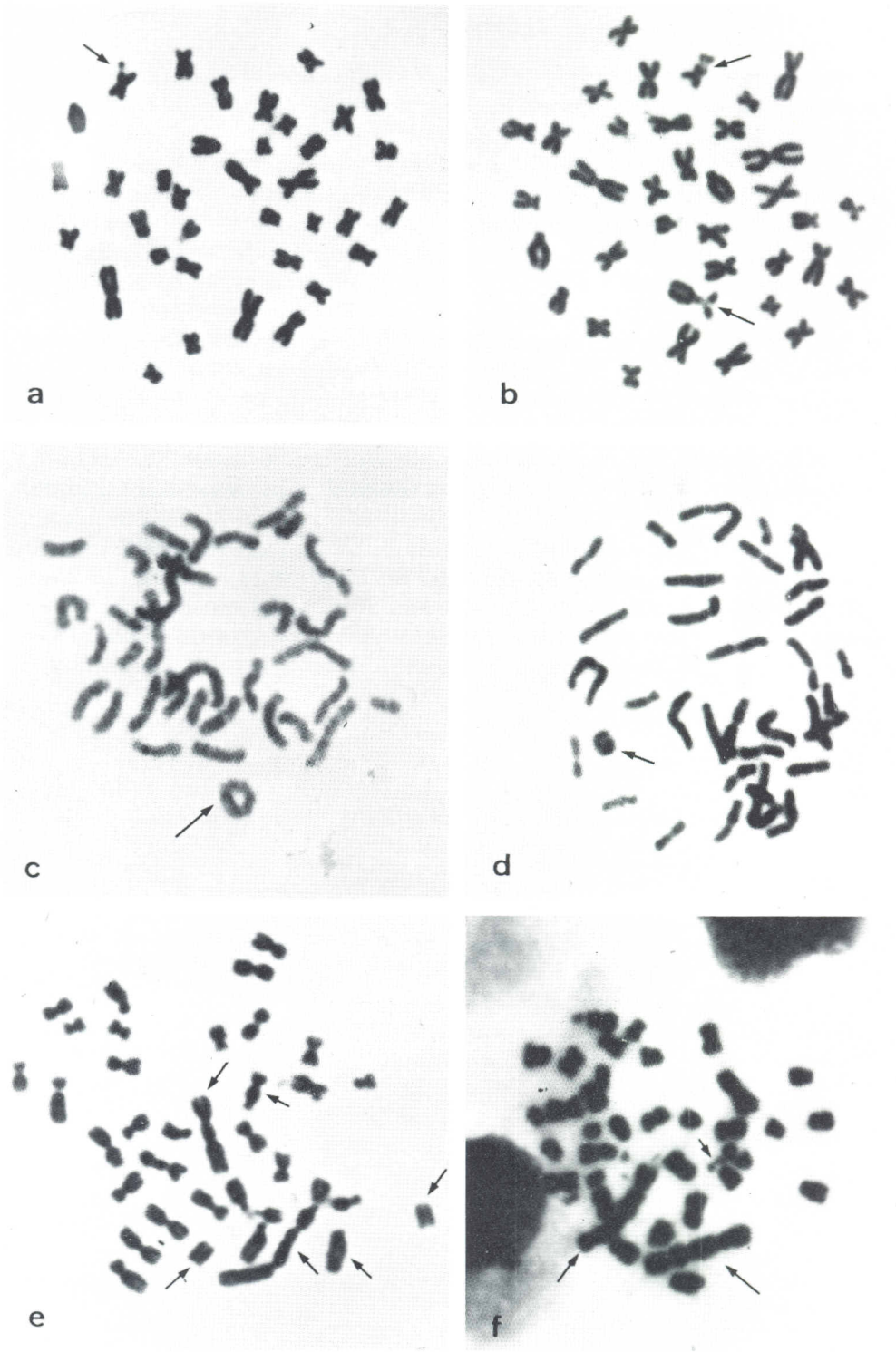


Fig. 4. — Different types of structural chromosomal aberrations obtained from *Biomphalaria glabrata* embryos: a) chromatid gap; b) chromosome gaps; c) centric ring; d) acentric ring; e) dicentrics and acentric fragments; f) tricentrics and minutes.



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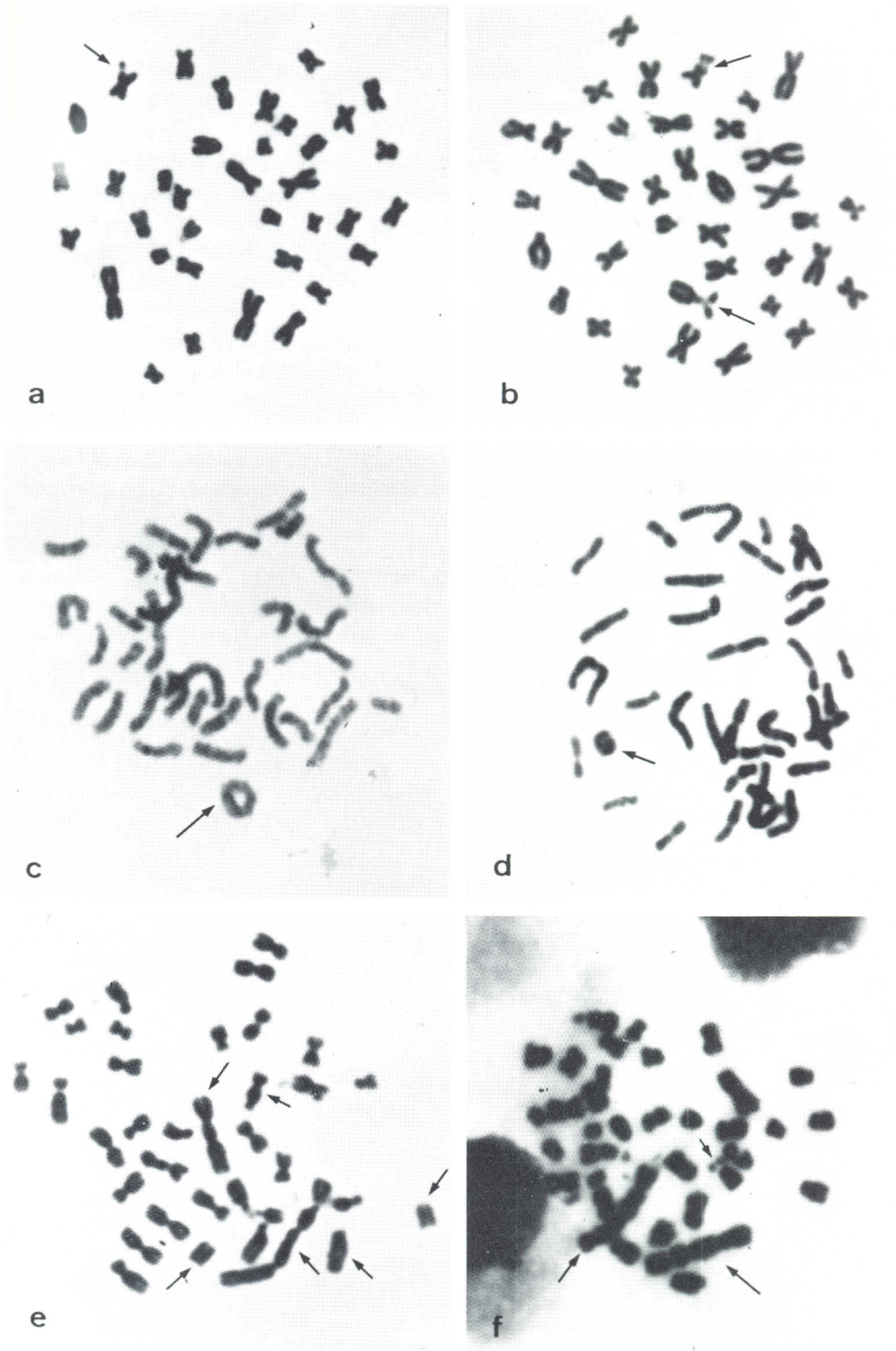

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in embryo cells irradiated with different radiation doses, 24 and 48 hours after exposure. The main types of aberrations were dicentrics, rings, breaks, gaps and acentric fragments.

Table 1 shows the frequencies of several types of radio induced chromosome aberrations detected in embryo cells irradiated with doses of 5,10 and 15 Gy during the blastula stage and fixed 24 hours after treatment. It can be seen that the frequency of structural chromosome aberrations increased perceptibly with increasing radiation dose. The linear function showed the best fit for dicentric frequency ($Y = 1.3X$; R-SQ (ADJ) = 0.992; P-value = 0.0003) (Fig. 5) and the quadratic function showed the best fit for acentric fragment frequency ($Y = 0.8X + 0.2X^2$; R-SQ (ADJ) = 0.999; P-value = 0.0008) (Fig. 6).

The frequencies of chromosome aberrations detected 48 hours after exposure to 10 Gy of gamma radiation are shown in Table 2. The percentage of cells with chromosome aberrations and the number of aberrations/cell were approximately 3 and 4 times lower, respectively, than in preparations analyzed after 24 hours.

Table 3 shows the distribution of cells with modal or hypomodal chromosome or centromere number in the irradiated and control groups, 24 hours after treatment. Note that the modal and hypomodal numbers were very similar in the control group and in the groups irradiated with 5 and 10 Gy. However, the frequency of metaphases with 36 chromosomes obtained with

TABLE 1 - *Frequencies of radioinduced chromosome aberrations obtained from «Biomphalaria glabrata» embryos irradiated at the blastula stage with doses of 5, 10 and 15 Gy of gamma radiation, 24 hours after exposure.*

Dose (Gy)	Number of metaphases	Aberration frequencies										No. of metaphases with aberrations (%)	Total No. of aberrations (number of aberration per cell)
		Chromosomes							Chromatids				
		D	T	CR	AR	AF	B	G	AF	B	G		
0	450	1	0	0	0	0	0	3	1	4	3	10 (2.22)	12 (0.03)
5	500	34	1	2	1	51	2	7	20	1	4	93 (18.60)	123 (0.25)
10	550	81	2	2	4	147	33	40	30	6	10	214 (38.91)	355 (0.64)
15	450	82	5	5	8	258	3	17	38	5	12	224 (49.78)	433 (0.96)

D = Dicentric; T = Tricentric; CR = Centric Ring; AR = Acentric Ring; AF = Acentric Fragment; B = Break; G = Gap.

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TABLE 2 - *Frequencies of radi irradiated at the b exposure.*

Dose (Gy)	Number of metaphases	D
0	450	0
10	500	19

D = Dicentric; T = Tric
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TABLE 3 - *Frequencies of me from «B. glabrata» blastula stage and*

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15	4

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224 (49.78)	433 (0.96)

ntric Ring; AF = Acentric

the 15 Gy dose was lower than in the remaining groups. The chi-square homogeneity test showed no significant difference among the first 2 doses ($P = 0.3180$), but a significant difference for the 15 Gy dose ($P = 0.0001$).

The frequencies of cells with a modal and hypomodal chromosome number observed 48 hours after irradiation with 10 Gy are presented in Table 4. There was no significant difference in modal chromosome number between the control and irradiated groups 48 hours after irradiation with 10 Gy (chi-square: $t = 2.27$; d.f. $= 2$; $P > 0.05$).

TABLE 2 - *Frequencies of radiation-induced chromosome aberrations detected on «B. glabrata» embryos irradiated at the blastula stage with 10 Gy of gamma radiation, processed 48 hours after exposure.*

Dose (Gy)	Number of metaphases	Aberration frequencies										No. of metaphases with aberrations (%)	Total No. of aberrations (number of aberration per cell)
		Chromosomes							Chromatids				
		D	T	CR	AR	AF	B	G	AF	B	G		
0	450	0	0	1	0	2	3	2	0	0	4	11 (2.44)	12 (0.03)
10	500	19	1	3	0	29	2	9	12	0	3	60 (12.0)	78 (0.16)

D = Dicentric; T = Tricentric; CR = Centric Ring; AR = Acentric Ring; AF = Acentric Fragment; B = Break; G = Gap.

TABLE 3 - *Frequencies of metaphases with modal and hypomodal centromeres (or chromosome) obtained from «B. glabrata» embryos submitted to doses of 5, 10 and 15 Gy of gamma radiation at the blastula stage and processed 24 hours after treatment.*

Dose (Gy)	Number of metaphases	Frequencies of chromosome or centromeres number (%)		
		36	35	34
0	450	388 (86.22)	42 (9.33)	20 (4.44)
5	500	422 (84.40)	50 (10.0)	28 (5.60)
10	550	485 (88.18)	48 (8.73)	17 (3.09)
15	450	317 (70.44)	90 (20.0)	43 (9.56)

TABLE 4 - *Frequencies of metaphases with modal and hypomodal centromeres (or chromosome) detected on «B. glabrata» embryos irradiated at the blastula stage with 10 Gy and processed 48 hours after exposure.*

Dose (Gy)	Number of metaphases	Frequencies of chromosome or centromeres number (%)		
		36	35	34
0	450	364 (80.89)	52 (11.56)	34 (7.56)
10	500	422 (88.40)	50 (10.0)	28 (5.60)

TABLE 5 - *Mitotic index of «B. glabrata» embryo cells irradiated with doses of 5, 10 and 15 Gy of gamma radiation, obtained 24 hours after exposure.*

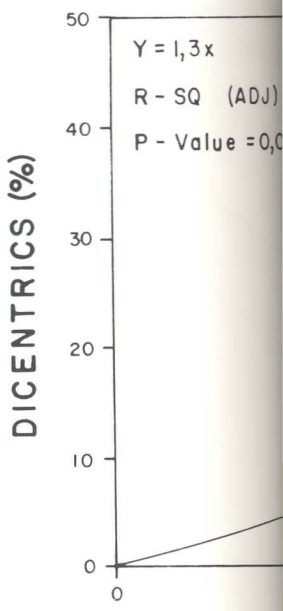
Dose (Gy)	Number of analyzed cells			M.I. (%)
	Total	Interphase	Division	
0	2169	1872	297	13.70
5	2013	1876	137	6.80
10	1981	1865	116	5.90
15	2063	2000	63	3.10

Since the metaphase figures in cytological preparations from irradiated embryos were relatively few when compared with the control, we determined the mitotic index of embryo cells submitted to doses of 5, 10 and 15 Gy of gamma radiation, 24 hours after exposure (Table 5). Mitotic index decreased with increasing radiation dose (Fig. 7).

DISCUSSION

Analysis of metaphases from normal *B. glabrata* embryos showed a chromosome number of $2n = 36$, with 12 metacentric, 4 submetacentric and 2 subtelocentric pairs, confirming data reported in previous studies by our group (KAWANO *et al.* 1987).

It should be pointed out that polyploid metaphases were detected in cytological preparations from both control and irradiated embryos treated during the young trochophore stage. Polyploid cells were not detected in the gastrula stage.



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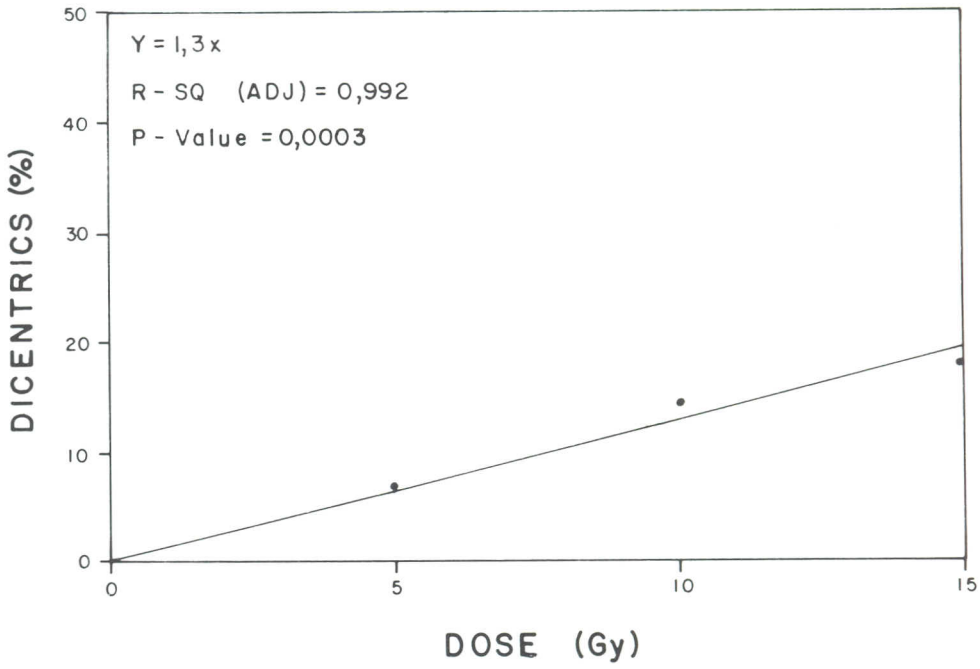


Fig. 5. — Frequency of dicentric chromosomes in *Biomphalaria glabrata* embryos irradiated with several doses of Co-60 gamma radiation, 24 hours after exposure.

A possible origin of polyploid cells was proposed by SCHREIBER (1966) and SCHREIBER and CAMEY (1966), who, on the basis of the amount of DNA detected in blastomeres, suggested that they may originate from endomeres that appear at the final blastula stage. During gastrulation, these endomeres may undergo three cycles of endoreduplication producing 16 c cells which, according to FALLIERI *et al.* (1969), may give origin to the embryonic digestive gland in the trochophore stage. Since the ootestis and digestive gland of adult snails essentially consist of polyploid cells (TUAN *et al.* 1984), one may assume that these cells play an important role in the formation of these organs. An observation favoring this assumption is the presence of polyploid metaphases with several structural aberrations (Fig. 3) observed by us in preparations from embryos irradiated in the blastula stage and processed in the young trochophore stage. These observations disagree with those reported by NARANG (1974) who considered the presence of polyploid cells in the ootestis of irradiated snails and in F_1 embryos to be solely an effect of radiation.

Among the various types of structural aberrations observed in *B. glabrata* embryos, dicentrics and acentric fragments were the most common. The

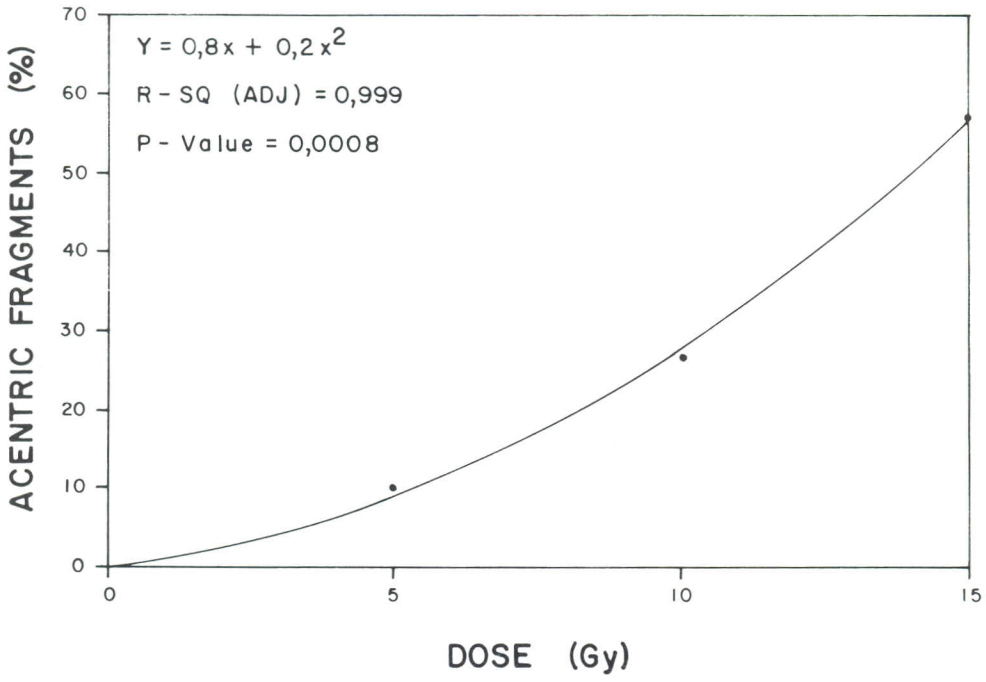
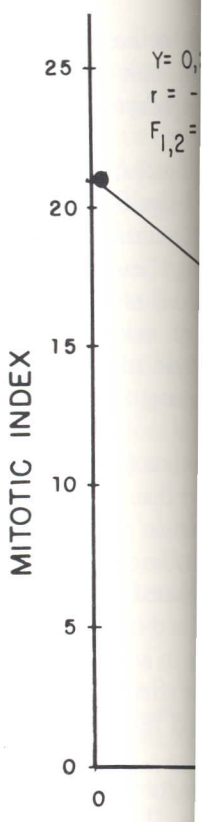


Fig. 6. — Frequency of acentric fragments in *Biomphalaria glabrata* embryos irradiated with several doses of Co-60 gamma radiation, 24 hours after exposure.

dicentric data fitted the linear model better and the acentric fragments fitted the quadratic model.

The fact that irradiated embryos presented an elevated frequency of acentric fragments in relation to dicentrics leads us to raise a few hypotheses, one of which concerns the manner of formation of these aberrations. Both terminal and interstitial deletions result from breaks that occurred in the same chromosome and may be produced before or after DNA synthesis. In contrast, dicentrics result from two breaks involving different chromosomes and are produced before DNA duplication. In this case, for a dicentric to be formed the two breaks would need to be produced within a certain period of time, so that the first will not udergo rejoining before the second is produced, and in such a manner that they will interact and exchanges will occur between the broken ends. In addition, the two breaks produced would need to be close enough to one another for the rearrangement to occur owing to an error of repair (IAEA 1986). The possibility that acentric fragments originate from rearrangement aberrations should also be considered (STEPHAN 1986).

To determine the effect of time after treatment with irradiation on the



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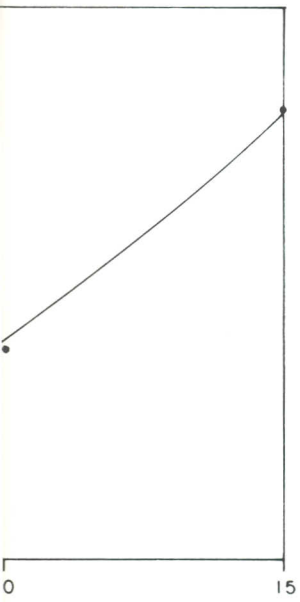

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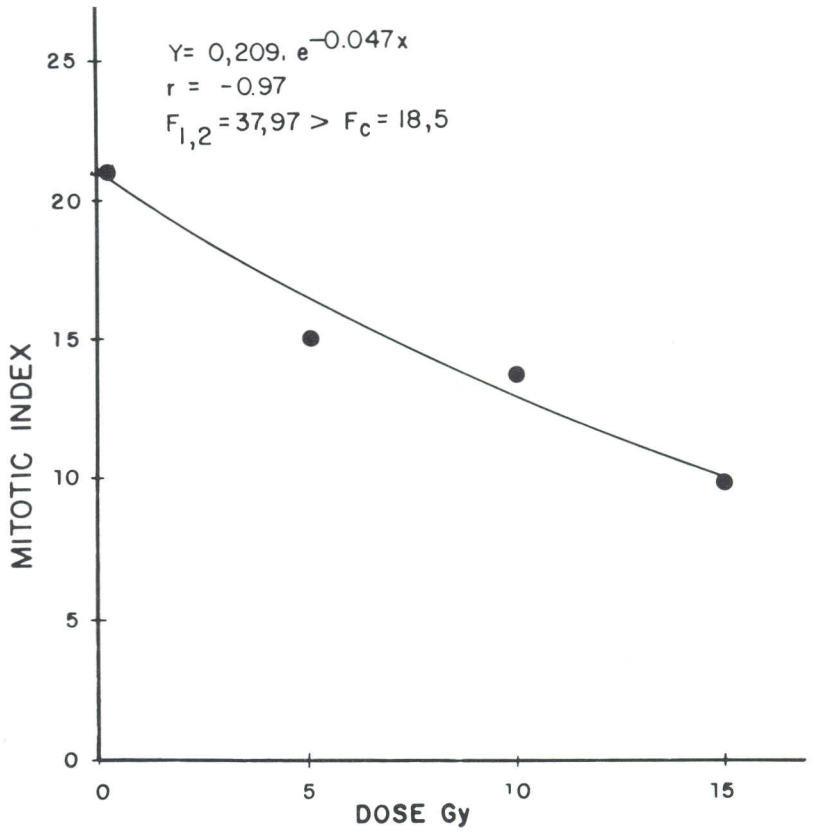

Fig. 7. — Relationship between mitotic index and radiation doses on *Biomphalaria glabrata* embryos exposed at the blastula stage and obtained 24 hours after treatment.

frequency of chromosome aberrations, embryos exposed to 10 Gy were also processed 48 hours after irradiation. The number of metaphases with structural aberrations was approximately three times less (12.0%) than the number obtained after 24 hours (38.9%). Similarly, the number of aberrations per cell was approximately 4 times less (0.16 aberrations/cell) than that obtained at 24 hours (0.64 aberrations/cell).

This decrease in chromosome aberration frequency with increasing time between irradiation and fixation may possibly be due to elimination of cells with chromosome aberrations. Estimates of the rate of elimination of chromosome aberrations occurring in different systems *in vitro* have shown an approximate 50% loss of dicentrics in each cell generation in organisms such as *Tradescantia* microspores (CONGER 1965), Chinese hamster cells (CARRANO 1973*b*), and human lymphocytes (SASAKI and NORMAN 1967; BAUCHINGER *et al.*

1986). The rate of elimination of acentric fragments has been estimated at approximately 70% (SASAKI and NORMAN 1967) and 20% (CARRANO and HEDDLE 1973; BAUCHINGER *et al.* 1986) in human lymphocytes, and 57% in Chinese hamster cells (CARRANO 1973*a*).

It should also be pointed out that the cytological preparations obtained from embryos exposed to the 15 Gy dose showed a significant fall in the frequency of metaphases with 36 chromosomes. The control group and the groups irradiated with 5 and 10 Gy did not differ significantly in terms of percentage of metaphases with a modal and hypomodal chromosome number 24 hours after exposure. Similarly, a comparison of modal chromosome numbers of cells examined 24 and 48 hours after irradiation with 10 Gy showed no significant difference between them (chi-square; $t = 4.74$; d.f. $= 2$; $P > 0.05$).

Even though the preparations obtained from both control and treated embryos showed incomplete metaphases, cells with more than 36 chromosomes were rarely observed. These data would appear to rule out the possibility of occurrence of mitotic nondisjunction. One of the probable causes of chromosome loss may be the delayed anaphases due to the dephased migration of one or more chromatids to one of the poles of the spindle. Another possibility is the occurrence of chromosome rearrangements that behave in an anomalous manner during anaphase, thus preventing the viability of the affected chromosome. In this manner, two hypodiploid daughter cells would result (BEIGUELMAN 1982).

On the basis of this information, the present results suggest a possible chromosome loss in cells irradiated with the 15 Gy dose. The fact that a small increase in the frequency of dicentrics was detected at the dose of 15 Gy (18.22%) compared to the dose of 10 Gy (14.73%) leads us to suspect a possible involvement of dicentric chromosomes in this phenomenon.

In cytological preparations from embryos irradiated with 5, 10 and 15 Gy, the mitotic indices were reduced by 50.4, 65.9 and 77.3%, respectively, 24 hours after exposure. There was a correlation between frequency mitotic reduction and frequency of chromosome aberrations, which increased considerably with increasing radiation doses 24 hours after treatment.

Even though the processes leading to cell death after irradiation are not well understood, many investigators (KIHLMAN 1977; SCOTT and ZAMPETTI-BOSSELER 1980) have proposed as possible causes the loss of acentric fragments leaving one or both daughter cells genetically deficient, the formation of anaphase bridges preventing cytokinesis, and the side effects resulting from bridge rupture which would cause a loss of part of the genome in one or both daughter cells.

Thus, the effects of ionizing radiation at the dose of 15 Gy on *B. glabrata* embryos are more pronounced, as demonstrated by the marked reduction in mitotic index, the relatively high frequency of structural chromosome altera-

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REFERENCES

BAUCHINGER M., SCHMID E
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tions, and a possible chromosome loss. Analysis of radiosensitivity in *B. glabrata* embryos supports these observations, showing increased susceptibility with increasing radiation dose in embryos irradiated in the blastula stage (OKAZAKI and KAWANO, in press, *a, b, c*). Both the frequency of malformed embryos (24.3, 55.5 and 73.8%) and the mortality rate (24.7, 53.6 and 76.7%) increased at the doses of 5, 10 and 15 Gy ^{60}Co gamma radiation, respectively. RUSSELL and RUSSELL (1954) raised the hypothesis that the primary intracellular damage induced by radiation in the embryo may be the occurrence of chromosome breaks that may lead to the formation of chromosome aberrations. An intimate relationship between chromosome aberrations and embryo death was documented by MATSUDA *et al.* (1983, 1985) and REICHERT *et al.* (1984) in mice. Other investigators have related chromosome aberrations to the induction of malformations in embryos of mouse (RUGH 1969), fish (MCGREGOR and NEWCOMBE 1972) and several species of amphibians, reptiles and mammals (WOLSKY 1982).

On the basis of these data, present results confirm the existence of an intimate association between radioinduced chromosome aberrations and the morphogenetic effects of radiation on *B. glabrata* embryos exposed during the blastula stage.

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REFERENCES

BAUCHINGER M., SCHMID E. and BRASELMANN H., 1986. — *Cell survival and radiation induced chromosome aberrations. II. Experimental findings in human lymphocytes analyzed in first and second post-irradiation metaphases.* Radiat. Environ. Biophys., 25: 253-260.

BUCKTON K.E. and EVANS H.J., 1973. — *Methods for the analysis of human chromosome aberrations.* Geneva, World Health Organization.

BEIGUELMAN B., 1982. — *Citogenética humana.* Guanabara Koogan.

CAMEY T. and VERDONK N.H., 1970. — *The early development of the snail Biomphalaria glabrata (Say) and the origin of the head organs.* Neth. J. Zool., 20: 93-121.

CARRANO A.V., 1973*a*. — *Chromosome aberrations and radiation-induced cell death. I. Transmission and survival parameters of aberrations.* Mutat. Res., 17: 341-353.

—, 1973*b*. — *Chromosome aberrations and radiation-induced cell death. II Predicted and observed cell survival.* Mutat. Res., 17: 355-366.

CARRANO A.V. and HEDDLE J.A., 1973. — *The fate of chromosome aberrations.* J. Theor. Biol. 38: 289-304.

CONGER A.D., 1965. — *The fate of metaphase aberrations.* Radiat. Bot., 5: 81-96.

EVANS H.J., 1968. — *Repair and recovery at chromosome and cellular levels: similarities and differences.* Brookhaven Symp. Biol., 20: 111-133.

FALLIERI L.A., CAMEY T., SCHREIBER M. and SCHREIBER G., 1969. — *DNA and nuclear size in early development of some invertebrates.* Genetics, Suppl., 61: 171-181.

INTERNATIONAL ATOMIC ENERGY AGENCY, 1986. — *Biological dosimetry. Chromosomal aberration analysis for dose assessment.* Technical Report Series, 260, Vienna.

KAWANO T., SIMÕES L.C.G. and ALMEIDA TOLEDO L.F., 1987. — *Nucleolar organizer region in three species of the genus Biomphalaria (Mollusca, Gastropoda).* Rev. Bras. Genet., 10: 695-707.

KIHLMAN B.A., 1977. — *Caffeine and chromosomes.* Amsterdam, Elsevier.

LEVAN A., FREDGA K. and SANDBERG A.A., 1964. — *Nomenclature for centromeric position on chromosomes.* Hereditas, 52: 201-220.

MATSUDA Y., TOBARI I. and YAMADA T., 1985. — *Studies on chromosome aberrations in the eggs of mice fertilized in vitro after irradiation. II. Chromosome aberrations induced in mature oocytes and fertilized eggs at the pronuclear stage following x-irradiation.* Mutat. Res., 151: 275-280.

MATSUDA Y., YAMADA T., TOBARI I. and OHKAWA A., 1983. — *Preliminary study on chromosomal aberrations in eggs of mice fertilized in vitro after x-irradiation.* Mutat. Res., 121: 125-130.

MCGREGOR J.F. and NEWCOMBE H.B., 1972. — *Dose-response relationships for yields of major eye malformations following low doses of radiation to trout sperm.* Radiat. Res., 49: 155-169.

NARANG N. 1974. — *Cytogenetic effects of radiation on the planorbid snail, Biomphalaria glabrata.* Caryologia, 27: 385-393.

NARANG N. and NARANG S., 1974. — *Chromosomal studies during spermatogenesis and the radiation induced chromosomal aberrations in Biomphalaria glabrata.* Rev. Bras. Pesq. Med. Biol., 7: 419-425.

OKAZAKI K. and KAWANO T., *a.* — *Effects of Co-60 gamma radiation on Biomphalaria glabrata (Say, 1818) embryo. I. Mortality* (in press).

—, *b.* — *Effects of Co-60 gamma radiation on Biomphalaria glabrata (Say, 1818) embryo.II. Malformations* (in press).

—, *c.* — *Effects of Co-60 gamma radiation on Biomphalaria glabrata (Say, 1818) embryo. III. Hatching* (in press).

READ J. 1961. — *Chromosome damage and growth reduction produced in roots of Vicia faba by x-rays and by triethylene melamine.* Int. J. Radiat. Biol., 3: 95-98.

REICHERT W., BUSELMAIER W. and VOGEL F., 1984. — *Elimination of x-ray-induced chromosomal aberrations in the progeny of female mice.* Mutat. Res., 139: 87-94.

RUGH R., 1969. — *Chairman's remarks.* In: «Radiation biology of the fetal and juveniale mammals», M.R. Sikov and D.D. Mahlum eds., Washington, D.C., USAEC.

RUSSELL L.B. and RUSSELL W.L., 1954. — *An analysis of the changing radiation response of the developing mouse embryo.* J. Cell Comp. Physol. 43 (Suppl. 1): 103-147.

SASAKI M.S. and NORMAN A., 1967. — *Selection against chromosome aberrations in human lymphocytes.* Nature, 214: 502-503.

SCHREIBER G., 1966. — *Citologia quantitativa. I.* An. Acad. Bras. Cienc., 38: 137-141.

SCHREIBER G. and CAMEY T., 1966. — *The DNA content in nculei of the blastomeres of Biomphalaria glabrata (Say) (Moll. Gaster.).* Ann. Histochim., 11: 313-318.

SCOTT D. and ZAMPETTI-BOSSELER F., 1980. — *The relationship between cell killing, chromosome aberrations, spindle defects and mitotic delay in mouse lymphoma cells of differential sensitivity to x-rays.* Int. J. Radiat. Biol., 37: 33-47.

—, 1985. — *Relationships between chromosome damage cell cycle delay and cell killing induced by bleomycin or x-rays.* Mutat. Res., 151: 83-88.

STEPHAN G., 1986. — *Cytological indicators: cytogenetic indicators.* In «Biological indicators for radiation dose assessment: international workshop on...», held in Reisensburg, 1983, pp. 106-122, Muenchen, Medizin Verlag.

THERMAN E., 1986. — *Human chromosomes: structure, behavior, effects.* 2ª ed. New York, Springer-Verlag.

TUAN R.; SIMÕES L.C.G. and KAWANO T., 1984. — *Estudo sobre poliploidia e meiose em B. glabrata.* Ciênc. Cult., 36: 843.

UNITED NATIONS, 1986. — *Genetic and somatic effects of ionizing radiation; report to the general assembly on the effects of atomic radiation.* New York, United Nations.

WOLSKY A., 1982. — *The effect of radiations on developmental processes.* In: «Radiation biology» D.J. PIZZARELLO and L.G. COLOMBETTI, eds., pp. 149-192, Boca Raton, Florida, CRC.

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