



*Original Contribution*

**GENETIC EFFECTS IN SOMATIC AND GERM CELLS IN RABBITS  
FOLLOWING EXTERNAL GAMMA RADIATION**

**II. Chromosome aberrations in peripheral blood lymphocytes**

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**ABSTRACT**

The aim of the present study was to evaluate the effect of gamma irradiation on the hereditary structures of peripheral blood lymphocytes and to quantify this in a dose-effect study. We used adult male rabbits subjected to total gamma irradiation at a dose range of 0.5 – 3.0 Gy. Twenty-four (24) hours later, we began the process of culturing and determination of chromosome aberration frequency using peripheral blood lymphocytes from the blood of these animals.

The analysis of results showed a dose-dependent increase in chromosome aberration frequency. The highest percentage of aberrant cells ( $29.3 \pm 2.02$ ) was observed following irradiation at 3.0 Gy.

The regression analysis of results showed that the dose-effect relationship (yield of dicentric, fragments and percentage of abnormal cells) satisfied the function,  $y = \alpha D^n$ .

**Key words:** cytogenetics, radiation mutagenesis, rabbits

**INTRODUCTION**

Many forms of ionised radiation have contributed to potent genetic and cytotoxic events in evolution. Mutations produced by ionised radiation can be divided into two types: somatic cell mutations (1, 2) and germ cell mutations (3, 4, 5).

These mutations can be identified in humans and other animals using methods that recognise mutagenic effects at various levels, including the tissues. A combination of these helps primarily in evaluating genotoxicity at the point of origin and then predicting further risks in the entire population.

Monitoring of genotoxicity and mutagenicity can be done using chromosome aberration study in somatic cells (6), micronuclear tests for bone marrow cells and peripheral blood lymphocytes (7), specific morphological and biochemical locus-tests (8), electrophoresis (9), tests detecting the

percentage of abnormal spermatozoa as an indirect evidence of mutagenicity and/or cancerogenicity (10), etc.

Various mammalian species are used as models for genetic risk evaluations following some of these procedures: different doses of ionised radiation, including dose densities, whole-body or local irradiation; assessment of substances with probably radioprotective effect, etc.

Peripheral blood lymphocytes are preferred as cell model for cytogenetic analysis because of their relatively long life span and easy access without adverse effects on the host.

The present study aims at performing a more extensive study on the effect of gamma irradiation, using a dose range of 0.5–3.0 Gy, on hereditary structures in lymphocytes and germ cells of rabbits in order to establish the correlation between a given dose and dose density on the incidences of chromosome aberration in both somatic and germ cells. This communication presents the results of various doses and the dose-effect correlation on the radiation sensitivity of the hereditary structures of peripheral blood lymphocytes in

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rabbits after a whole-body gamma irradiation.

## MATERIALS AND METHODS

Mature New Zealand male rabbits, aged 4.5 months, were subjected to total irradiation at dose range of 0.5-3.0 Gy, dose density of 18 cGy/min, using a  $^{60}\text{Co}$  gamma equipment (Rokus). Four experimental groups ( $n > 3$ ) were formed:

- Group I: rabbits irradiated at 0.5 Gy;
- Group II: rabbits irradiated at 1.5 Gy;
- Group III: rabbits irradiated at 2.5 Gy;
- Group IV: rabbits irradiated at 3.0 Gy.

All animals were of equal body weight (4.0-4.5 kg) and were housed in individual cages in uniform conditions prior to and during the experiment.

The exposure dose was calculated according to the geometrical parameters of the source, its power and the source-object distance (11).

Blood for cytogenetic analysis was drawn from the auricular vein 24 hours prior to and 24 h following the irradiation procedure.

Chromosome preparations were made by a short-time culture of peripheral blood lymphocytes using the micromethod of Hungerford (12). For culture, 0.5 ml peripheral heparinised blood was added to sterile 20 ml flasks containing 7 ml nutrient medium for cell culture and RPMI-1640 supplemented with L-glutamine and HEPES buffer, 3 ml heat-inactivated normal calf serum, 0.2 ml resubstituted phytohaemagglutinin; 100 E/ml penicillin and 50 mg/ml gentamycin. The flasks were closed in a sterile box and incubated for 72 h in a thermally-controlled oven at 37°C. After 70 hr, 0.5 g/ml colchicine was added to each flask. Then the classic method for processing short-term lymphocyte cultures was used and it comprises briefly the following: 10 min processing with hypotonic solution of 0.075 M KCl and at least four fixation procedures with methanol/glacial acetic acid, 3:1 v/v. The obtained lymphocyte suspensions were dropped onto cold slides. The slides were stained with Giemsa.

The scoring was done with 100 metaphase cells from each individual suspension. Data were statistically processed.

## RESULTS AND DISCUSSION

The incidence of chromosome aberration in peripheral blood lymphocytes of rabbits irradiated with various doses of gamma ray is

presented on **Table 1**.

The analysis of results shows a dose-dependent increase in the incidence of induced aberrations within the 0.5-3.0 Gy range. After irradiation at 0.5 Gy, the incidence of dicentrics ( $1.6 \pm 0.3\%$ ) is not significantly higher than that of acentrics ( $1.0 \pm 0.5\%$ ). The metaphase study on specimens from 0.5 Gy rabbits does not exhibit ring-shaped chromosomes.

In the 1.5 Gy group, the number of observed fragments is higher than that of dicentrics. The percentage of cells with chromosome aberrations is  $8.3 \pm 0.9\%$ . Like the 0.5 Gy group, there is no ring-shaped chromosomes in the lymphocytes of 1.5 Gy animals.

A significant increase in chromosome aberration in peripheral blood lymphocytes can be noticed after irradiation at 2.5 Gy, when the percentage of abnormal cells is  $17 \pm 2.0\%$ . The yield of dicentrics and acentrics per 100 cells is  $12 \pm 1.7\%$  and  $15.7 \pm 2.9\%$  respectively. In one metaphase plate from one animal, a ring chromosome is absent.

The observed tendency towards elevation in the percentage of abnormal cells is most apparent following irradiation at 3.0 Gy –  $29.3 \pm 2.02\%$ . The highest incidence of dicentrics ( $18 \pm 3.2$ ) and fragments ( $18.6 \pm 2.0\%$ ) in peripheral blood lymphocytes is observable in 3.0 Gy irradiated rabbits. The yield of ring-shaped chromosomes is low – only 2 rings can be observed in 300 metaphase plates.

The dose-effect relationship was determined by regression analysis of results, using the least squares method. The yield of chromosome aberrations was calculated against the number of studied metaphases. The dose-effect relationship is described by a power function from the  $y = aD^n$  type (**Table 2**).

The frequency of radiation-induced chromosome aberrations is a function of the amount of induced DNA lesions and the degree of their repair. In this regard, the types of DNA damage that result in chromosome aberrations are important and they are probably different when various doses and dose densities are used.

It must be emphasised that in the available literature, the phenomenon is detected at various dose density (13) and various post irradiation interval (14). The time for culture of peripheral blood lymphocytes was also important (15).

**Table 1.** Chromosome aberrations in peripheral blood lymphocytes of rabbits, irradiated at various dosages of gamma rays

Dose	n	Number of metaphases	Dicentrics	Fragments	Rings	Abnormal Cells
0 Gy	1	100	-	1	-	1
0 Gy	2	100	-	-	-	-
0 Gy	3	100	-	1	-	1
	$\Sigma$	300	-	2	-	2
0.5 Gy	1	100	2	2	-	2
0.5 Gy	2	100	1	-	-	1
0.5 Gy	3	100	2	1	-	3
	$\Sigma$	300	5	3	-	6
1.5 Gy	4	100	7	9	-	10
1.5 Gy	5	100	4	7	-	7
1.5 Gy	6	100	6	6	-	8
	$\Sigma$	300	17	22	-	25
2.5 Gy	7	100	15	20	-	18
2.5 Gy	8	100	12	17	1	20
2.5 Gy	9	100	9	10	-	13
	$\Sigma$	300	36	47	1	51
3.0 Gy	10	100	17	15	-	26
3.0 Gy	11	100	13	19	1	33
3.0 Gy	12	100	24	22	1	29
	$\Sigma$	300	54	56	2	81

**Table 2.** Parameters of regression evaluation of the dose-effect relationship (yield of dicentrics, fragments, % abnormal cells vs dose) following external gamma irradiation in rabbits

Chromosome aberrations	Function : $y = \alpha D^n$			
	$\alpha \pm S.E.$	$n \pm S.E.$	df	p
dicentrics	2,76 $\pm$ 1,03	1,67 $\pm$ 0,37	13	<0.05
fragments	4,21 $\pm$ 1,13	1,37 $\pm$ 0,27	13	<0.001
% abnormal cells	3,04 $\pm$ 0,98	2,02 $\pm$ 0,28	13	<0.001

The frequency of chromosome aberrations we observed was lower than that reported by others in rabbits (16, 17). The difference in aberration yield was probably due to the fact that during the 72-h culture, a certain percentage of lymphocytes underwent a second mitosis and thus, the aberrant cells were submitted to a negative selection during the first mitotic division.

On the other hand, the comparison of results obtained in vivo and in vitro experiments in rabbits shows that there was no significant difference in the level of chromosome aberrations in both cases (18). In an experiment with swine, however, a significant lower incidence of chromosome defects was observed in vivo than in vitro, a fact that was explained by the protective effect of the large body mass (19).

The data from the present study, compared to those of other authors (17), showed that the radiosensitivity of rabbits to production of dicentrics in peripheral blood lymphocytes was lower than that in humans

despite the fact that both species were characterised with identical chromosome arm number (81 in man, 81 in rabbits). Those data indicate the difficulty in the interpretation of results between species due to the various parameters of irradiation and the specifics of experimental design and methods, on one part and to the specifics of hereditary structures and repair mechanisms in the different species, on the other.

## CONCLUSION

The results of the present experiment showed that following acute gamma irradiation, the level of chromosome aberrations in peripheral blood lymphocytes in rabbits increased with the dose, at the 0.5-3.0 Gy range. The dose-effect dependence can be described by a  $y = \alpha D^n$  power function.

Our data, compared to those obtained in other species, indicated the difficulty in extrapolating results among species due to multiple factors, one of which is inter-species differences.

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