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SURVIVAL OF MOUSE TYPE B SPERMATOGONIA
FOR THE STUDY OF THE BIOLOGICAL EFFECTIVENESS
OF VERY HIGH ENERGY NEUTRONS

by

M. Bianchi^{*} and M. Quintiliani
Istituto Superiore di Sanità
Rome

and

J. Baarli and A.H. Sullivan
CERN
Geneva

* Present address: CERN Geneva

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INTRODUCTION

The effect of high-energy radiation on biological systems cannot necessarily be extrapolated from the known effects of conventional radiation. This is on account of the spallation interactions that can occur at energies above about 20 MeV, which result in the liberation of highly ionizing nuclear fragments that deposit their energy close to the site of interaction. In order to study the biological efficiency of this type of interaction it is necessary to use either a high-energy neutron beam, or slowed-down negative pions⁽¹⁾. Such beams are at present of low intensity as they are secondary beams produced in a cyclotron. The biological tests that can be made are therefore limited on account of the low intensity, and furthermore, in order to make comparisons with conventional radiations it is preferable that the biological effect studied is independent of dose rate.

This note describes our investigation of the suitability of the survival of type B spermatogonia in mice as a system for estimating the biological effectiveness of high-energy neutrons, and describes some preliminary measurements made in a nominal 400 MeV neutron beam from the CERN 600 MeV synchro-cyclotron.

BIOLOGICAL STUDIES

Mice of the C₃H/RL/Cas inbred strain, 9 to 12 weeks of age and weighing 21 to 24 g were used for these studies. Investigations were first made of the effect of dose rate by exposing the mice to ⁶⁰Co gammas and X-rays. The mice were contained in individual perforated plastic tubes such that they were unable to turn around. They were irradiated in groups of 3 from above at dose rates from 0.1 to 4.5 rad/min. The dose received was estimated using a victoreen ionization chamber.

The experimental animals were killed by cervical dislocation 46 hours after the start of irradiation. The testes were then fixed in Orth fluid, embedded in paraffin and cut in 5 μ sections. The sections were stained using periodic acid fuchsin sulfurous acid technique as described by Hotchkiss⁽²⁾ and counterstained with acid haematoxylin. Seminiferous tubules were classified on the basis of the acrosoma development of spermatids as described by Leblond and Clermont⁽³⁾ and Oakberg⁽⁴⁾. Survival of type B spermatogonia was evaluated by scoring the number of resting primary spermatocytes (RPS) in circular cross-sections of tubules at stage 7.

The results of the investigation of dose-rate dependence for X-rays and ^{60}Co gamma radiation are shown in Table 1, which also includes the calculated standard deviation for the RPS per tubule. The control group for the experimental series at the lowest dose rate also includes animals restrained in the irradiation tubes for 6 hours. No difference in the number of RPS per tubule was observed in the animals under stress.

The experimental investigation shows a high degree of reproducibility, dose-rate independence and further, no difference between the 220 kV X-rays and the ^{60}Co gamma radiation. The dose-rate independence over a range from 0.1 to 4.5 rad/min of type B spermatogonia survival compares well with a similar observation made by Oakberg on type A cells⁽⁵⁾. The whole set of data can be fitted to a survival curve which on a semilog plot shows a straight line with a small initial shoulder, characterized by D_0 (37%) = 19.4 rad and

an extrapolation number of 1.4. This is in good agreement with corresponding values calculated from data published by Oakberg^(6,7).

Further sets of mice were exposed in the 400 MeV neutron beam in order to compare the biological effect with that from X and gamma rays. The dosimetry studies made in this beam will be described elsewhere. The neutrons had an average energy of about 120 MeV and a gamma contamination of less than 8% of the absorbed dose. The dose rate in the beam as a function of depth in water is shown in Fig. 1. The mice were exposed directly in the beam and behind 20 g/cm² of polythene where the maximum dose build-up occurs. Owing to the initial rapid change in dose rate with depth, the dose received was estimated from calibrated LiF thermoluminescent detectors placed at the position of the testes in polythene phantoms that were simultaneously exposed with the mice⁽⁸⁾.

For the neutron-irradiated animals a separate scoring of each testis was made to see whether there could be a systematic difference between survivals as a result of being irradiated from the side and owing to the rapid dose build-up. No difference was found that exceeded the experimental errors and the results for the two testes were therefore used together. The results are given in Table 2, where also the dose rate and total dose are indicated.

When plotted semilogarithmically these data do not seem to fit the usual type of survival curves, therefore no attempts has been made at this stage of the study to establish a relation between survival and dose for the neutron-irradiated animals.

DISCUSSION

A preliminary evaluation of the relative biological efficiency of the 400 MeV neutron beam using an estimated value for the ED_{50} dose and comparing it to the X and ^{60}Co gamma results give values of 3.7 for the pure beam and 2.7 at maximum build-up. The lower value at maximum build-up is no doubt due to the long-range secondary charged particles produced in the nuclear cascade.

At the moment it is not felt that a full discussion and an appropriate comparison with other work using the same system should be made. The present investigation has shown, however, that the system chosen is well suited both with respect to radiosensitivity and reproducibility to allow a study of the biological effectiveness of nuclear interaction processes from high-energy radiation.

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Table 1

Type B spermatogonia survival after X and ^{60}Co γ irradiation

Total dose (rad)	220 keV X rays				^{60}Co γ rays					
	4.5 rad/min		2.7 rad/min		4.5 rad/min		0.3 rad/min		0.1 rad/min	
	Tubules scored	RPS* per tubule	Tubules scored	RPS* per tubule	Tubules scored	RPS* per tubule	Tubules scored	RPS* per tubule	Tubules scored	RPS* per tubule
0	243	33.5 $\pm$ 4.2	243	33.3 $\pm$ 4.3	248	30.5 $\pm$ 4.0	243	30.6 $\pm$ 4.5	324	31.3 $\pm$ 3.9
4.6	182	27.3 $\pm$ 3.2	169	30.7 $\pm$ 4.6	165	28.4 $\pm$ 4.0	180	27.6 $\pm$ 4.0	180	27.1 $\pm$ 3.0
9.1	192	23.3 $\pm$ 3.8	186	27.9 $\pm$ 3.5	187	25.9 $\pm$ 4.2	193	24.7 $\pm$ 4.5	190	23.9 $\pm$ 4.1
14	182	19.3 $\pm$ 3.9	204	20.5 $\pm$ 4.0	188	19.3 $\pm$ 4.2	182	17.9 $\pm$ 5.5	182	19.1 $\pm$ 3.4
18	204	14.9 $\pm$ 3.8	216	13.0 $\pm$ 4.8	198	15.7 $\pm$ 4.6	220	14.6 $\pm$ 4.7	194	12.9 $\pm$ 4.1
27			199	11.7 $\pm$ 5.3						
36	268	7.3 $\pm$ 3.3			196	8.1 $\pm$ 3.4	260	7.7 $\pm$ 4.6	268	7.3 $\pm$ 3.8
46			213	4.7 $\pm$ 3.1						
64			251	1.6 $\pm$ 1.4						
82			223	0.7 $\pm$ 0.9						
100			269	0.3 $\pm$ 0.7						

* RPS denotes resting primary spermatocytes.

Table 2

Type B spermatogonia survival after neutron irradiation

Total dose (rad)	400 MeV neutron beam Dose rate 0.036 rad/min		Total dose (rad)	Attenuated 400 MeV neutron beam Dose rate 0.067 rad/min	
	Tubules scored	RPS [*] per tubule		Tubules scored	RPS [*] per tubule
0	243	31.8 $\pm$ 3.8	0	243	31.8 $\pm$ 3.8
1.8	232	26.3 $\pm$ 3.0	2.9	240	22.2 $\pm$ 3.0
3.2	275	18.1 $\pm$ 3.3	5.8	291	17.0 $\pm$ 2.8
6.5	305	13.7 $\pm$ 3.3	11.0	301	12.3 $\pm$ 2.9
13.0	276	8.1 $\pm$ 3.6	24.0	314	7.9 $\pm$ 3.1

* RPS denotes resting primary spermatocytes.

REFERENCES

1. A.H. Sullivan and J. Baarli, Phys. Med. Biol. 13, 435 (1968).
2. R.D. Hotchkiss, Arch. Biochem. 16, 136 (1948).
3. C.P. Leblond and Y. Clermont, Ann. N.Y. Acad. Science 55, 548 (1952).
4. E.F. Oakberg, Amer. J. Anat. 99, 391 (1956).
5. E.F. Oakberg and E. Clark, Cellular and Comparative Physiology, Suppl. I, Vol 58, 3, 173 (1961).
6. E.F. Oakberg, Strahlentherapie 51 (suppl.), 103 (1962).
7. E.F. Oakberg, J. Exp. Zool. 134, 343 (1957).
8. J. Baarli, A.H. Sullivan, G. Bühner, M. Quintiliani and M. Bianchi, Proc. First European Congress on Radio-protection, Menton, October 1968 (in press).

Fig. 1