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COMPARED EFFECTS OF RADIOSTRONTIUM AND ROENTGEN RAYS ON GERM CELLS IN MALE MICE

by

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The effect of ^{90}Sr on male gonads has been studied by several workers. TRUSOVA (1956) reported periodic disturbances of the sperm activity on long term dietary administration of ^{90}Sr in dogs with a decrease in volume of the ejaculate and an increased frequency of atypical forms of spermatozoa; the ejaculates were radioactive. These results were confirmed by BURYKINA & TRUSOVA (1963) in a series of dogs given ^{90}Sr orally for 24 to 40 months. BURYKINA (1957) described a reduction and change in the spermatogenesis in rats after treatment with ^{90}Sr. OWEN et coll. (1957) also found a very much reduced spermatogenesis in rabbits after administration of ^{90}Sr.

ÅBERG & GILLNER (1964) in an investigation on the effect of a single dose of ^{90}Sr in rams demonstrated oligospermia, atypical forms of spermatozoa and a considerable incorporation of ^{90}Sr into the single spermatozoa. HENRICSON et coll. (1962) found in mice that the type B-spermatogonium does not seem to be so severely affected as the type A-spermatogonium. It has also been

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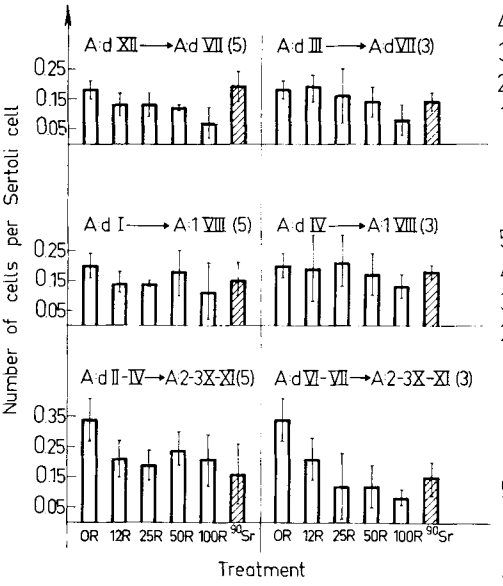

Fig. 1. Mean number and 95 % conf. int. of spermatogonia type A per Sertoli cell per tubular cross-section. Symbols to the left of the arrow indicate cell type and tubular stage irradiated, symbols to the right of the arrow indicate those scored.
A:d — Spermatogonium type A dormant
A:1 — Spermatogonium type A, first generation
A:2—3 — Spermatogonium type A, second and third generation
Roman figures indicate tubular stages
Figures (3) and (5) indicate the respective days after treatment; see also Table.

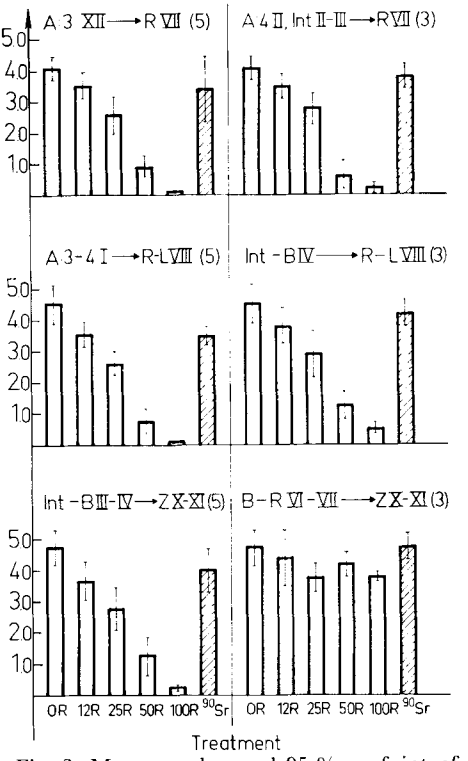

Fig. 2. Mean number and 95 % conf. int. of primary spermatocytes per Sertoli cell per tubular cross-section. Same symbols as in fig. 1. (See also Table.) Further symbols:
Int. — Spermatogonia intermediate type
B — Spermatogonia, type B
R — Resting primary spermatocytes
L — Leptotene
Z — Zygotene

observed that in embryos from ^{90}Sr-treated male mice there is a considerable variation in the chromosome numbers (Henricson & Nilsson 1964), which may be of interest in connection with the findings by Lüning et coll. (1963) of increased intra-uterine deaths of embryos with fathers treated with the same dose of ^{90}Sr as used by the present authors.

As a consequence of these findings there seems to be a great need of more precise information about the ^{90}Sr effect on the seminiferous cells. The aim of this work was also to evaluate, if possible, the radiation dose to the testicular tissue from an intravenously injected dose of ^{90}Sr by comparing its tissue-

destruction effect with that of different doses of roentgen irradiation under standardized conditions. As the last-mentioned procedure is a total body irradiation, a comparison with the β-ray effect of ^{90}Sr on pure physical grounds is very complicated.

Material and Methods. CBA male mice were used as test animals. Fifteen animals were injected intravenously with 0.7 μCi ^{90}Sr per gram bodyweight at the age of 75 days. Four groups of 10 mice each received 12, 25, 50 and 100 R of total body roentgen irradiation (Müller MG 300, 260 kV, 10 mA, focal distance 40 cm, inherent filtration 4 mm Al, additional filter 0.5 mm Cu, dose rate 85 R/min). Ten of the strontium-treated animals, and 5 from each of the roentgen-treated groups, were killed after 72 hours. Five strontium-treated and the remaining 5 mice in the roentgen-treated groups were killed after 120 hours. A control group of 10 mice were killed at 75 + 3 days of age. The testicles were removed immediately and fixed in Stieves fluid. The left testicle was embedded and sectioned at 4 μ and stained according to Hotchkiss' PAS-method. Ten circular tubular cross-sections were selected at random from each of the stages VII, VIII and X-XI (Oakberg 1956) in the cycle of the seminiferous epithelium of the mouse. The number of Sertoli cells, spermatogonia type A, resting primary spermatocytes, or those in meiotic prophase (leptotene, zygotene) were counted in the selected tubular cross-sections. We have chosen to refer the number of germ cells to the number of Sertoli cells in the cross-sections even if this procedure is of greater importance at longer intervals between treatment and evaluation (Oakberg 1959). There is always a variation in tubular diameter but the influence of this when counting a limited number of cross-sections can be reduced by reference to the Sertoli cells.

Results

The present study covers the effects of ^{90}Sr and roentgen irradiation on several phases in the development from dormant A-spermatogonia to intermediate and B-spermatogonia. The different development phases differ greatly in their sensitivity to irradiation (Figs 1 and 2), apparently a reflection of the critical stages the cells have to pass through in their development (see Table). Cells irradiated in tubule stages XII and III and counted as dormant A-spermatogonia in tubule stage VII after 5 or 3 days (Fig. 1, first row) were much less affected than A-spermatogonia which had passed through mitosis. A reduction in the number of A-spermatogonia in stage VII occurred after three days with only 100 R. Five days after irradiation there was a depletion also after exposure to 50 R. The ^{90}Sr effect was weaker and was apparent only after three days.

Table

Cell types and tubular stages involved in the investigation

Irradiation		Stages of cell death	Interval in days between treatment and scoring	Scoring	
Cell type	Stage			Cell type	Stage
A d	XII		5	A d	VII
A d	I		5	AI	VIII
A d	II—III		3	A d	VII
A d	III—IV	VIII, X	5	A d + II—III	X—XI
A d	IV	(VIII)	3	AI	VIII
A d	VI—VIII	VIII, X	3	A d + II—III	X—XI
AIII	XII	XII, II, III, VI	5	Rest.	VII
AIII—IV	I	II, III, VI	5	Rest.-Lept.	VIII
AIV-Int.	II—III	III, VI	3	Rest.	VII
Int.-B	III—IV	III, VI	5	Zyg.	X—XI
Int.-B	IV	VI	3	Rest.-Lept.	VIII
B-Rest.	VI—VII	VI	3	Zyg.	X—XI

In tubule stage VIII, the cells which were dormant A-spermatogonia in tubule stage I and IV at the time of irradiation, were counted as first generation A-spermatogonia (Fig. 1, second row). The appearance of the tubules varied somewhat and a cell depletion could be traced only for the 100 R group but not for the ^{90}Sr groups.

There was a significant cell reduction in the ^{90}Sr groups and most of the roentgen groups for the tubules in stage X-XI, i.e. after A-spermatogonia had passed through the period of DNA synthesis in stage VIII and X and become A-spermatogonia of the second and to some extent of the third generation (Fig. 1, third row). The cell reduction for all roentgen groups was higher three days after irradiation than after five days, the difference being particularly evident after 50 and 100 R. There were no comparable differences between the ^{90}Sr groups.

Most mice in all the roentgen groups exhibited a highly significant depletion of cells, which at the time of irradiation were third and fourth generation A-spermatogonia and five days later were resting cells and leptotene nuclei in stages VII and VIII after having passed through several periods of DNA synthesis (see Table and Fig. 2). A statistically significant cell reduction was evident for the ^{90}Sr group only in stage VIII after 5 days.

Intermediate and B-spermatogonia that three and five days after irradiation were counted as resting leptotene nuclei in stage VIII, and as zygotene nuclei

in stage X—XI, were quite sensitive to roentgen irradiation but were strongly resistant to treatment with ^{90}Sr. Irradiation with 12 R had an effect equal to or exceeding that of the ^{90}Sr dose on these cells (Fig. 2, second and third rows).

Cells that at the time of irradiation were B-spermatogonia, or resting cells in stage VI—VII, and three days later were counted as zygotene nuclei in stage X—XI, were highly resistant to irradiation with roentgen rays and not at all affected by ^{90}Sr (Fig. 2, third row).

Discussion

The CBA strain used in the experiments seems to give about the same response to roentgen irradiation as the F_1 hybride of the 101 and the C_3H strains used by OAKBERG (1957), although the particular CBA strain employed is possibly somewhat more resistant.

Our results concerning the roentgen doses seem to agree with the findings (OAKBERG 1957, and MONESI 1962) that there is a very marked difference in sensitivity between dormant spermatogonia type A and later stages of the A-type plus the intermediate and type-B-spermatogonia. The LD_{50} of late type-A-intermediate and type B- of 20—24 R roentgen irradiation given by OAKBERG (1957) corresponds to a somewhat higher value here.

The observation that dormant A-spermatogonia which have been irradiated in stages III—IV and VI—VII and scored 5 and 3 days later in stage X—XI, also react significantly to 25 R roentgen rays is of great interest. The reason for this sensitivity seems to be that the dormant-A is irradiated close to the critical stage of DNA synthesis in stage VIII. This assumption seems to agree with MONESI's (1962) findings that the critical period of cell killing is that of DNA-synthesis (interphase, early prophase) in spermatogonia type A and intermediate. The reason for the difference observed between dormant-A irradiated with 50 and 100 R roentgen rays in stages III—IV and VI—VII, respectively, is possibly that the dormant-A when irradiated in stage VI—VII are very close to the DNA synthesis preceding the first division of type A. If irradiated at stage III—IV the dormant-A might have had time to recover.

It is evident from Figs 1 and 2 that the most marked effect of ^{90}Sr occurred in the stages just discussed. The two dormant-A types received, however, the same amount of damage, which seems to indicate that in the case of ^{90}Sr no recovery takes place. ÅBERG & GILLNER (1964) have shown that ^{90}Sr is located in the spermatozoa of rams treated with ^{90}Sr. It is therefore perhaps not too much to assume an incorporation of ^{90}Sr during an early stage of spermatogonial development. If incorporated in some vital molecules ^{90}Sr continues to give its damaging effect over a fairly long time. An observation

made earlier of the present authors (HENRICSON et coll. 1962) is the insignificant effect of ^{90}Sr on the spermatogonia of type B which is contrary to the sensibility of these cells to roentgen and γ-rays (OAKBERG 1957, MONESI 1962). A possible explanation for this might be that no incorporation of ^{90}Sr takes place in this stage and that the irradiation absorbed by the cells from the injected dose of ^{90}Sr is too small to exceed the limit of cell killing.

The original purpose of this study was to estimate the radiation dose absorbed by testicular tissue after a given dose of ^{90}Sr. The results suggest that three to five days after ^{90}Sr injection the irradiation dose corresponds to whole-body irradiation with 12 R or 12 to 25 R. However, no precise relation can be given without further study of the complicated functional and morphological effects induced by ^{90}Sr and which change from stage to stage in the development of the cells in the germinal epithelium.

SUMMARY

The effect of ^{90}Sr (0.7 μCi/g bodyweight) on different types of spermatogonia in mice is compared to total body irradiation of 12, 25, 50 and 100 R roentgen rays, the strontium dose used apparently corresponding to 12 R or 12 to 25 R roentgen rays. When irradiated before the DNA-synthesis of A-spermatogonia stage VIII, the strontium effect is greatest, and a comparison with the roentgen effect of most interest.

ZUSAMMENFASSUNG

Der Wirkungseffekt von ^{90}Sr (0,7 μCi/g Körpergewicht) auf die verschiedenen Arten der Spermatogenese bei Mäusen wird mit dem Effekt einer Totalbestrahlung von 12, 25, 50 und 100 R verglichen. Die Strontiumdosis scheint einer Röntgendosis von 12 bis 25 R zu entsprechen. Der Strontiumeffekt ist am grössten wenn die Spermatogonien-A, Stadium VIII, bevor der DNS-Synthese bestrahlt werden, und dann wird ein Vergleich mit dem Roentgeneffekt auch von grösstem Interesse.

RÉSUMÉ

Les auteurs ont comparé l'effet du ^{90}Sr (0,7 μCi/g de poids corporel) sur différents types de spermatogonies de la souris à l'effet de l'irradiation de tout le corps par 12, 25, 50, et 100 R de rayons roentgen, la dose de strontium administrée correspondant apparemment à 12 R ou 12 à 25 R de rayons roentgen. C'est quand il intervient avant la synthèse de l'ADN des spermatogonies-A stade VIII (de OAKBERG) que l'effet du strontium est maximum et que sa comparaison avec l'effet des rayons roentgen présente le plus d'intérêt.

REFERENCES

ÅBERG B. and GILLNER M.: Personal communication (1964).

BURYKINA L. N.: Histological changes due to the strong action of ruthenium, strontium and cesium. A.E.C.-tr. 3794 (1957), 117.

– and TRUSOVA N. E.: Changes of the spermatogenetic function in dogs with chronical injury caused by ^{90}Sr. (Translation from Russian.) Radiobiologija 3:3 (1963), 369.

HENRICSON B. and NILSSON A.: Chromosome investigations on the embryo progeny of male mice treated with ^{90}Sr. Acta radiol. Ther. Phys. Biol. 2 (1964), 315.

— KNUDSEN O. and NILSSON A.: The effect of radiostrontium on mouse testes. Acta radiol. 58 (1962), 52.

LÜNING K. G. and FRÖLÉN H.: Personal communication (1964).

— — NELSON A. and RÖNNBÄCK C.: Genetic effects of strontium-90 injected into male mice. Nature 197 (1963), 304.

MONESI V.: Relation between X-ray sensitivity and stages of the cell cycle in spermatogonia of the mouse. Radiat. Res. 17 (1962), 809.

OAKBERG E. F.: Duration of spermatogenesis in the mouse and timing of stages of the cycle of the seminiferous epithelium. Amer. J. Anat. 99 (1956), 507.

— Gamma-ray sensitivity of spermatogonia of the mouse. J. Exptl. Zool. 134 (1957), 343.

— Initial depletion and subsequent recovery of spermatogonia of the mouse after 20 r of gamma rays and 100, 300, and 600 r of X-rays. Radiat. Res. 11 (1959), 700.

OWEN M., SISSONS H. A. and VAUGHAN J.: The effect of a single injection of high dose of Sr^{90} (500—1000 μC/kg) in rabbits. Brit. J. Cancer 11 (1957), 229.

TRUSOVA N. E.: Influence of ionizing radiation on the activity of dog spermatozoa. U.N. Doc. AIAC 82 IGIR 49. Committee on Medical radiology, Ministry of health USSR. Summaries of papers presented at the conference on the remote consequences of injuries caused by action of ionizing radiation (1956). State Medical Literature Press, Moscow.