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MAMMALIAN SPERMATOGENESIS AS A NEW SYSTEM FOR BIOLOGIC DOSIMETRY OF IONIZING IRRADIATION

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The term biologic dosimetry is mostly understood in the sense proposed by BENDER & GOOCH (1962) as estimating the radiation dose an individual has been exposed to by an analysis of the radiation induced chromosome injury in lymphocytes of the peripheral blood. The lymphocytes have to be stimulated to proliferate in vitro and are arrested at metaphase by the technique proposed by MOORHEAD et coll. (1960) or any modification of it. This technique and the subsequent evaluation of the chromosome arrangements are very time consuming. One day's work is needed to analyse 100 to 200 metaphases of one sample; in the low dose range (below 0.3 Gy) even 1 500 to 2 000 metaphases have to be counted which takes about 10 days (STEPHAN 1982). Thus, the necessity of counting a large number of cells limits the value of this model of biologic dosimetry in the low dose range. Methods designed for use in radiation accidents where physical dosimeters are not available must be sufficiently rapid and sensitive to yield results within the time limits required for successful remedial action and genetic counselling for exposed individuals. Acceptable time limits will partially depend on the number of individuals exposed. Thus, the technique of cytogenetic dosimetry cannot be used for accidental radiation exposures of large scale (e.g. accidents in nuclear power plants) because facilities for analysing large quantities of blood samples do not exist.

Other models of biologic dosimetry of similar or even greater sensitivity should be available. A method is now proposed using the in vivo system of spermatogenesis in combination with an automated cytophotometric technique, the flow cytometry.

Material and Methods

Male NMRI mice, aged 9 to 12 weeks, were irradiated with 200 kV roentgen rays, $1.29 \cdot 10^{-2}$ C/kg/min, 0.5 mm Cu. The doses ranged from 0.1 to 2.5 Gy. Three mice per dose and time point (2–70 days after the irradiation) were killed; at doses of 0.5 Gy and below 5 or 10 mice were analysed. The data of the irradiated mice and the 18 sham irradiated control mice were pooled. Testes were prepared using pepsin-HCl for cell separation and ethidium-bromide/mithramycin for DNA staining according to the method described previously (ZANTE et coll. 1976, 1977, HACKER et coll. 1981). Flow cytometry was performed with a pulse cytophotometer developed by GÖHDE et coll. (1979), which had a special device to count the number of cells in a certain volume of suspension (0.2 ml) in order to calculate the total number of the different cell types in the testis.

Results

The composition of a testis DNA histogram, the mutagenic radiation effects (increase of the coefficient of variation and induction of diploid sperm)

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and the cytotoxic radiation effects as measured by a reduction of elongated spermatids and sperm, respectively, have been described previously (HACKER et coll.). The most sensitive criterion of radiation action in the low dose range is, however, the reduction of the number of DNA synthesizing cells (spermatogonia and preleptotene spermatocytes). Fig. 1 shows the time dependent reduction of the number of S-phase cells following an irradiation with 2.5 Gy 2 to 70 days after the irradiation. This reduction shows a maximum 2 to 7 days after the exposure. A similar timing of the reduction of S-phase cells is found following radiation with other doses as well. Fig. 2 illustrates the dose response relationship 2 days after irradiation: The D_{50} value of this curve is about 0.25 Gy. Even a dose of 0.1 Gy leads to a significant ($p<0.05$) reduction of S-phase cells. There is no shoulder in this dose response curve (for more details cf. HACKER 1981).

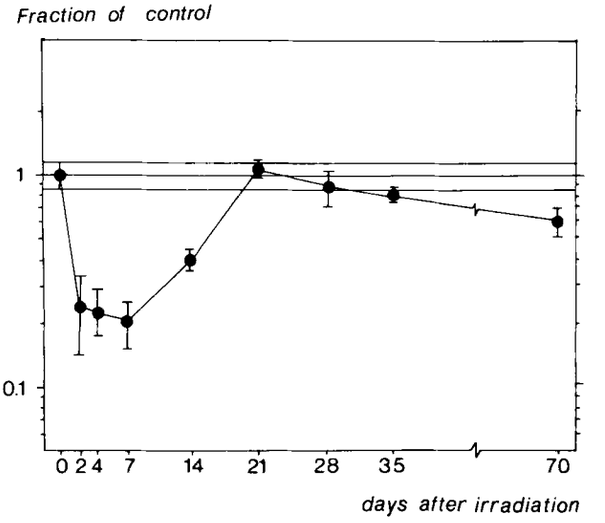

Fig. 1. Time dependent changes of the number of DNA synthesizing cells 2 to 70 days after an irradiation with 2.5 Gy. The arithmetic means and 95 per cent confidence limits are indicated. The straight lines show the mean control values with the confidence limits.

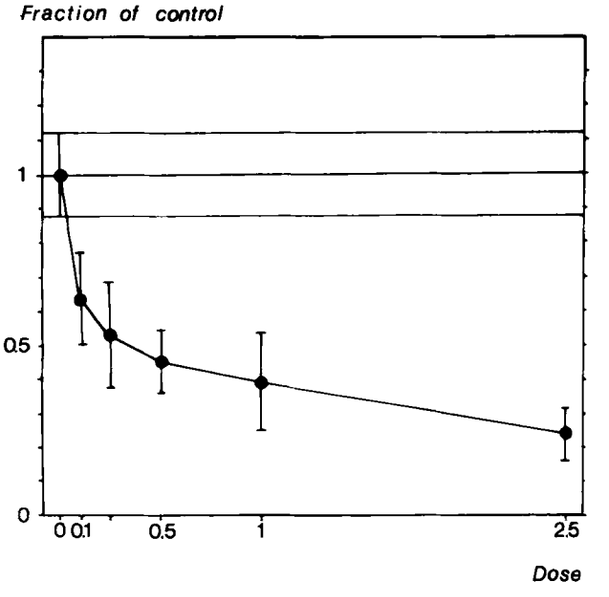

Fig. 2. Dose dependent reduction of the number of S-phase cells 2 days after irradiation with different doses (0.1–2.5 Gy). The arithmetic means and 95 per cent confidence limits are indicated. The straight lines show the mean control values with the confidence limits.

Discussion

Being able to detect a radiation exposure of only 0.1 Gy with 95 per cent confidence the model proposed is very sensitive. The usefulness of this model of biologic dosimetry in the low dose range relates especially to the absence of a shoulder in the initial part of the dose response curve. The major advantage of the system proposed is its speed and correspondingly its cheapness; the analysis of one testicular sample only takes about 15 min. Thus, in the low dose range, this technique is about 300 times less time consuming than the conventional method of biologic dosimetry, the chromosome analysis. The large number of cells analysed (about 30000 cells per histogram) minimizes the statistical error. The analysis of the incorporation changes of labelled thymidine into DNA of testicular cells has been proposed as an in vivo short-term test for the identification of chemical mutagens by FRIEDMAN & STAUB (1976), SEILER (1977) and LAMBERT & ERIKSSON (1979), and for the intercomparison of neutron beams by GERACI et coll. (1977). Labelling techniques, however, have two disadvantages: First, more time is required until results are seen, and secondly, labelling techniques cannot be applied in man.

The method now proposed might be an alternative or an additional way to detect or exclude possible accidental radiation overexposures in man. The general concept of germ-cell sensitivity of mouse can be applied to man as well (OAKBERG & LORENZ 1972). Spermatogonia which are responsible for the radiation induced S-phase reduction (cf. HACKER 1981) described seem to be even more sensitive in man than in mouse as can be concluded from the fact that an irradiation of only 0.15 Gy leads to a long-lasting

reduction of sperm in man to about 25 per cent of the control value (HELLER 1967).

The aspiration biopsy technique using a fine needle (0.6 mm) does only involve a minor discomfort to the patient (OBRANT & PERSSON 1965) and renders a sufficient number of cells for flow cytometry (THORUD et coll., cited in CLAUSEN & ÅBYHOLM 1980). This technique might also be used in special cases to evaluate radiation exposures. In case of a longer interval between irradiation and analysis, evaluation of sperm counts in the ejaculate might be applied. This system of evaluating irradiation doses and effects by means of the S-phase reduction is certainly also suitable to monitor chemical mutagens (HACKER 1977).

SUMMARY

The radiation induced reduction of the number of DNA synthesizing cells (spermatogonia) is described using the fast-working flow cytophotometer. Since there is no shoulder in the initial part of the dose response curve this model of biologic dosimetry is very sensitive. The D_{50} value is 0.25 Gy; a radiation exposure of only 0.1 Gy can be detected.

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