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MAMMALIAN SPERMATOGENESIS AS A BIOLOGIC DOSIMETER FOR RADIATION

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Spermatogenesis is an interesting in vivo test system for mutagenic agents like ionizing radiation (HACKER et coll. 1980). In spermatogenesis mitotic, meiotic and differentiation processes occur at the same time.

The different spermatogenic cells show different sensitivities against the mutagenic and cytotoxic actions of ionizing radiation (OAKBERG & DI MINNO 1960). Those actions can be monitored by flow cytometry (HACKER et coll.). A high proliferative activity (typical for spermatogonia) is normally combined with a high sensitivity against radiation-induced cell death (BERGONIÉ & TRIBONDEAU 1906). A high sensitivity is of importance for a valuable dosimeter.

Material and Methods

NMRI male mice aged 9 to 12 weeks at the time of irradiation were used. They were irradiated at 200 kV employing a 0.5 mm Cu filter and with a dose rate of 0.5 Gy/min. During the irradiation the mice were rotated around their longitudinal axis with a constant velocity (9.5 rotations per min) in a carrousel in order to get a homogeneous dose distribution. Their body axis was perpendicular to the beam axis. The anterior part of the body was shielded with lead (0.2 cm thick). Doses ranged from 0.1 to 15 Gy.

In the low dose range (0.1 and 0.5 Gy) for each dose and each time point 5 mice were used, after 21 and 28 days even 10 mice were analysed. In the higher dose range (1.0 Gy and above) 3 mice were used for each dose and time point.

Control mice were sham-irradiated. Two, 7, 14, 28 and 35 days after this treatment 3 to 8 control mice were killed and analysed. The data of the mice treated in the same manner were pooled.

Flow cytometry. Each testis was removed from fat and connective tissue, weighed, minced with surgical blades, and stirred for 10 min in 1.5 ml of a pepsin solution (Pepsin, Serva, 130 Anson-units/mg, pH 1.8) at 20°C (ZANTE et coll. 1976), filtered and fixed by adding 3.5 ml of 96 per cent ethanol.

Before use, the cell suspension was stirred, 0.4 ml taken out of the suspension and mixed with 0.6 ml of pepsin solution; 5 min later 0.2 ml of the pepsin-cell suspension was taken out and stained with 2.8 ml of a dye solution containing 5 μg/ml ethidium bromide, 12.5 μg/ml mithramycin and 1.5 mg/$MgCl_2$ in tris buffer (pH 7.4; ZANTE et coll. 1976, 1977). Flow cytometry was performed with a self-developed pulse cytophotometer (GÖHDE et coll. 1979a), which had a special device to count the number of cells in a certain volume of suspension (0.2 ml).

The total cell counts per volume were needed for calculation of the total number of the different cell types in the testis.

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Results

The DNA histogram of testis cells of a normal untreated mouse (top) and a scheme of the DNA amount changes during spermatogenesis (bottom) are presented in Fig. 1.

Germ-cells and non-germ-cells exist in the testis. Nearly all DNA values of the non-germ-cells (Leydig cells, Sertoli cells, macrophages) are stored in the 2c peak.

In the S-phase region the DNA values of spermatogonia and preleptotene spermatocytes are registered. The 4c region contains the DNA values of primary spermatocytes mainly in pachytene, and, to a smaller proportion, (G_2+M)-spermatogonia.

The 1c region represents round spermatids (probably step 1–8 of spermiogenesis; MEISTRICH et coll. 1978a), which stain proportional to their DNA content. Elongated spermatids (probably step 9 or 10–16 of spermiogenesis) only show 60 to 80 per cent of the DNA fluorescence because of their highly condensed chromatin (OAKBERG & DI MINNO, MONESI 1964, 1965). The dense chromatin can be decondensated by a special enzymatic treatment with papain (ZANTE et coll. 1977). The different stainability of round and elongated spermatids was used to discriminate between these two stages of spermiogenesis.

Changes of the coefficients of variation after irradiation. The coefficient of variation (CV; which is calculated by a computer as the SD divided by the mean) in a DNA histogram is a measure of biologic variation, the staining conditions (the dye concentration and the presence of ions) and to a lower degree of optical and electronic influences. The staining procedure is the same for all the cells of one sample. The instrumentational (electronic and optical) variation is below 0.1 per cent of CV (GÖHDE et coll. 1979a). Thus, all variations in a special sample between the peaks of a DNA histogram must be due to biologic variation (the state of the chromatin, the presence of cells with less or more DNA than normal (aneuploidies) and so on.

The coefficients of variation of the four peaks (Fig. 1) in a testis DNA histogram are decreasing from the left to the right part. After irradiation characteristic changes in the CV were seen.

MEISTRICH et coll. (1978b) demonstrated that CV broadening may reflect abnormal meiotic segregation. Mutagenic drug induced increase of the CV of the DNA histogram of cells having passed mitosis was shown by GÖHDE et coll. (1979b).

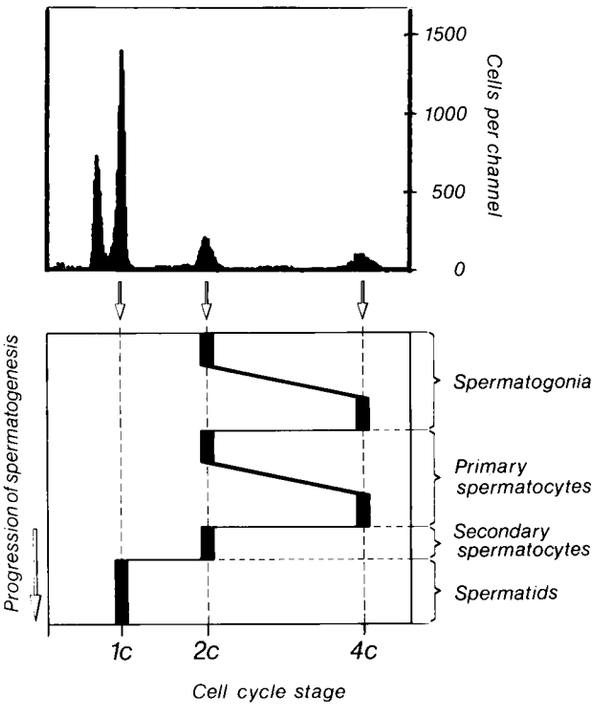

Fig. 1. Changes of DNA content during spermatogenesis as reflected in a testis DNA histogram of an untreated control mouse. Abscissa: Relative fluorescence values divided into 256 channels. Ordinate: Number of measured cells per channel. According to the progression of spermatogenesis G_1-spermatogonia, G_1-primary spermatocytes and secondary spermatocytes are stored in the 2c-peak, spermatogonia and primary spermatocytes synthesizing DNA are recorded in the S-phase region between 2c and 4c. In the 4c-peak (G_2+M)-spermatogonia and primary spermatocytes are stored, in the 1c-region the DNA values of spermatids are recorded.

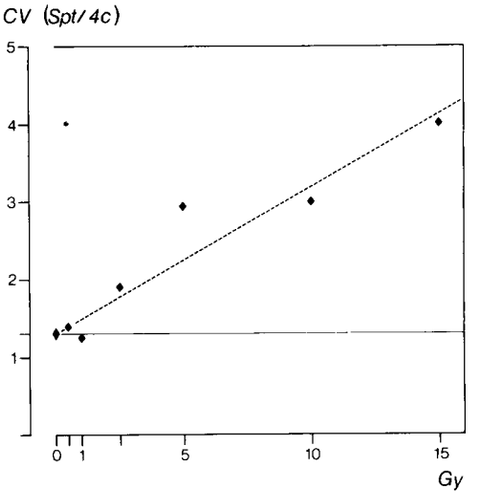

Fig. 2. Increase of the ratio of CV of peak II formed by signals of round spermatids (cf. Fig. 1) compared with CV of peak IV (cf. Fig. 1; 4c, comprising mainly primary spermatocytes) in a testis DNA histogram 7 days after irradiation with different doses. Peak II represents round spermatids that were mainly primary spermatocytes at the time of irradiation. The straight line represents the control value of 1.3.

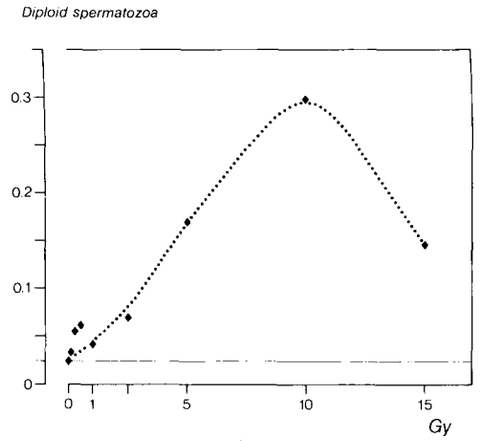

Fig. 3. Induction of diploid spermatozoa 14 days after irradiation with different doses. The cells were irradiated as primary spermatocytes. The dotted line was fitted by eye. The straight line represents the control value: the ratio of the number of cells between the 1c and the 2c peak to those in the 0.7c peak in untreated control animals was 0.024=2.4 per cent.

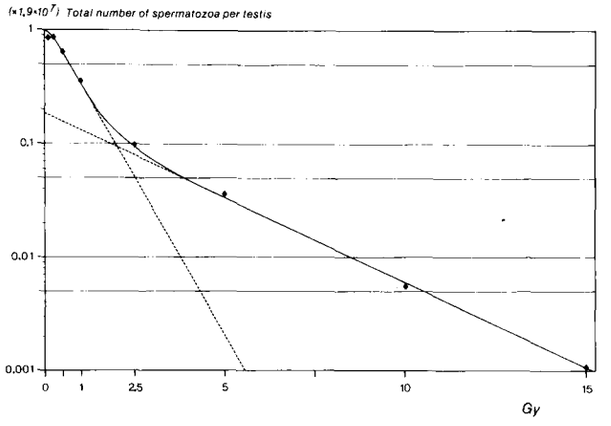

Fig. 4. Decrease of the total number of spermatozoa (cells of peak I in the DNA histogram) 28 days after irradiation with different doses. The arithmetic mean of the number of spermatozoa in each testis of untreated control mice was 1.9×10^7. The dotted lines represent the extrapolations of the survival curve fractions of the two cell subpopulations differing in their radiation sensitivity.

The CV of peak II containing round spermatids was compared with that of peak IV in the 4c region. Peak IV, which does not broaden after irradiation was used as a standard.

The CV of peak II compared with peak IV increased 7 days after irradiation (Fig. 2). The cells now comprising peak II were mainly irradiated as primary spermatocytes. Seven days after exposure the CV increased linearly until it reached about four times the control ratio of 1.3 following irradiation with 15 Gy.

If the biologic variation is calculated by the equation given by MEISTRICH et coll. (1978b), $CV = \sqrt{CV_{1c}^2 - CV_{4c}^2}$, there is a similar increase to about the fourfold of the control value of 3.53 seven days after irradiation with 15 Gy.

Production of diploid spermatozoa. In the region between the 1c and 2c peak a small proportion of stored DNA values exist (at twice the modal channel number of the peak at the 0.7c position, representing elongated spermatids) from cells which most probably are signals of diploid spermatozoa in testis cell preparations free of cell aggregates.

The ratio of the number of cells between the 1c and 2c peak (the boundaries were chosen arbitrarily) to those in the 0.7c peak in untreated control animals was 0.024=2.4 per cent. This value is in the same order of magnitude as the frequency of diploid gametes indicated in the literature (0.025% in the northern vole, Microtus oeconomus, 0.03–2.3% in the rabbit, 0.1–0.17% in the bull, 1.53% in the gorilla; cited in the publication of TATES et coll. 1979) and 0.5 to 2.0 per cent and above in man (CAROTHERS & BEATTY 1975, PAWLOWITZKI, personal communication).

The diploid gamete frequency increased after all irradiation doses tested (Fig. 3). The shape of the curve for the production of diploid sperm shows similar appearances as typical curves of mutagenesis. The maximum frequency of diploid sperm is at 10 Gy.

Cell inactivation. Fig. 4 shows the decrease of the absolute number of spermatozoa 28 days after irradiation with different doses. At this time the spermatozoa show the maximum decrease (HACKER et coll.). They were irradiated as differentiated spermatogonia which are highly sensitive against radiation-induced cell death (OAKBERG 1955). The curve is composed of two components, which are probably formed by two different subpopulations. One subpopulation which comprises about 90 per cent of the cells obviously is more sensitive with an LD_{50} of about 0.5 Gy, the other subpopulation (10 per cent of the total cell population) has an LD_{50} of about 4.0 Gy. Most probably the more sensitive subpopulation was irradiated as differentiated spermatogonia, the less sensitive one as differentiating spermatogonia as can be concluded from the data of the time table of OAKBERG (1957). The LD_{50} values obtained in the present investigation agree very well with estimates in the literature (OAKBERG 1955), obtained with methods that were more time consuming.

Discussion

In this model especially the inactivation of highly sensitive differentiated spermatogonia delivers quantitative information on the cytotoxic action of ionizing radiation. The cells which are the most sensitive against the cytotoxic effects of radiation should be suitable as a biologic dosimeter. However, a shoulder in the dose effect curve exists, limiting the value of the method in the low dose range. Furthermore, induction of diploid sperm and CV broadening are a measure of the mutagenic action of radiation. The cells which contributed to the linear CV increase were irradiated as spermatocytes. Those CV broadenings were most marked 7 days after irradiation in peak II. If other cells were irradiated, they did not lead to such an increase of CV.

A linear increase of the CV was found with doses up to 15 Gy. The production of diploid sperm seems to be a more sensitive criterion of the mutagenic action of ionizing radiation even after irradiation with lower doses than the CV broadening. CV broadening and induction of diploid sperm deliver qualitative information on mutagenic actions of ionizing irradiation, the dose dependent inactivation of cells gives quantitative estimates of cytotoxicity. All three parameters can be used for the screening of mutagenity and cytotoxicity of drugs and ionizing irradiation (HACKER 1977, HACKER et coll.).

SUMMARY

Mouse spermatogenesis was used as an in vivo test system obviously suitable as a biologic dosimeter. Ionizing irradiation induced changes in the frequency distribution of the cellular DNA content of whole testis preparations. These changes can be analysed by flow cytometry. Such measurements deliver information on: (1) an increase of the coefficient of variation of the DNA histograms of some spermatogenic cells, (2) induction of diploid mature sperm, and (3) the dose-depending inactivation of the highly sensitive differentiated spermatogonia. In this model especially No. 3 delivers quantitative information on the cytotoxic action of ionizing irradiation and chemical noxae as well, whereas Nos 1 and 2 may be used as qualitative criteria of mutagenic action of physical and chemical agents, No. 2 obviously being more sensitive than No. 1.

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