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BEHAVIOUR OF THE Bp8 MOUSE ASCITES SARCOMA AFTER PARTIAL BODY IRRADIATION

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Whole body irradiation has generally been used in investigations on the post-irradiation kinetics of in situ growing ascites tumours (KIM & EVANS 1964, GÖHDE 1973, GÖHDE et coll. 1975, TRIBUKAIT 1975, FRINDEL et coll. 1970, CAO et coll. 1982 a, b). Since whole body irradiation in the higher dose range interferes with the survival, the time available for observation of the behaviour of the tumour is limited in these experiments. Thus, in the recent reports focused on the quantitative changes of Bp8 ascites sarcoma cells in the various parts of the cell cycle and the proliferation kinetics after whole body irradiation the observation time has been limited to 14 days after inoculation of the ascites tumour, i.e. the approximate survival period of the unirradiated animals (CAO et coll. 1982 a, b).

In the present investigation, the animals bearing ascites tumours were exposed to partial body abdominal irradiation. Due to the protection of part of the bone marrow stem cells and the radiation effects on the tumour cells, the survival of the animals was markedly prolonged. This made it possible to elucidate even the later growth behaviour of the tumour after irradiation.

Material and Methods

Details of the methods used have been described previously (CAO et coll. 1982c). Briefly, 10-day-old undiluted ascites fluid of the Bp8 mouse ascites sarcoma with a fixed number of cells (18×10^6) was inoculated intraperitoneally into each of 4 to 8 male NMRI mice, about 3 months of age and weighing about 25 gram.

On the fourth day after inoculation, different groups of mice were exposed to 2.5, 5.0 and 8.0 Gy (250 kV, 15 mA, 0.5 mm Cu added filtration, SSD 50 cm, exposure rate 1.3 Gy per min). The mice were placed into a 50 ml plastic centrifuge tube (Falcon 2070 F) closed by a cap with a plastic cylinder to fix the position of the mice. The tubes were perforated with a large number of holes, allowing the animals to breath in a normal way. The head and a major part of the chest were covered by 3 mm thick lead shield.

The ascites volume was measured by using an isotope dilution technique. Based on the cell con-

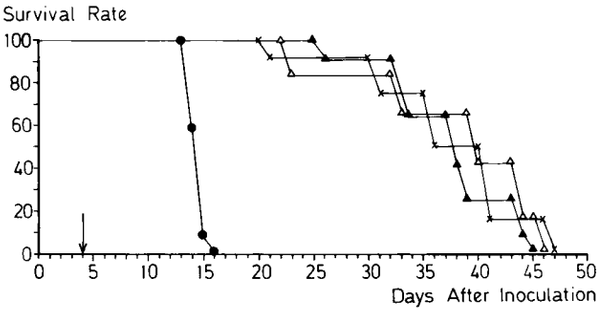

Fig. 1. Mouse survival rate (in per cent) following partial body irradiation (→) with 2.5 (△), 5 (x) and 8 (▲), Gy; controls (●). Each group comprised 12 mice. Inoculation of 18×10^6 Bp8 ascites sarcoma cells on day 0, irradiation on day 4.

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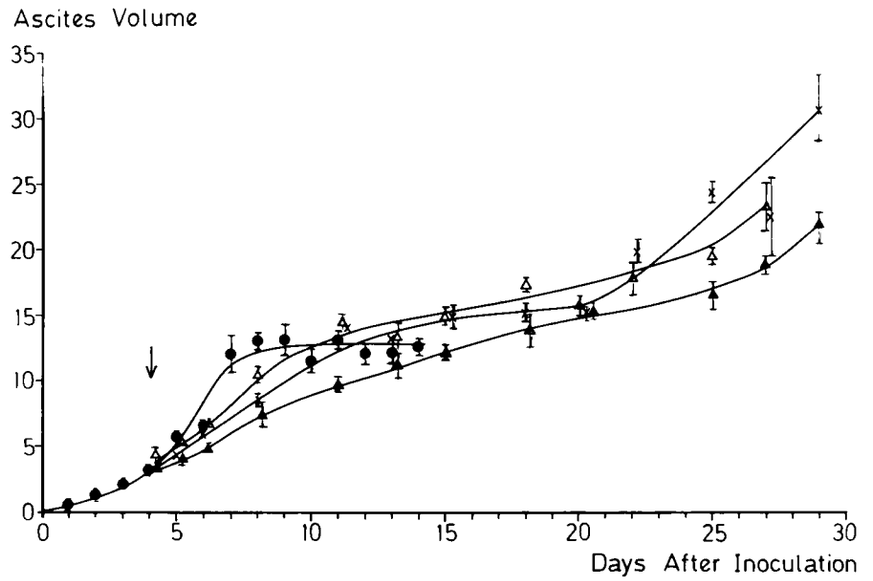

Fig. 2. Ascites volume (in ml) of the Bp8 mouse ascites sarcoma after partial body irradiation (→) with 2.5 (△), 5 (x) and 8 (▲), Gy; controls (●). Each mouse was irradiated on day 4 after the inoculation of 18×10^6 cells injected intraperitoneally. Mean values ±1 SEM of 4 to 8 mice.

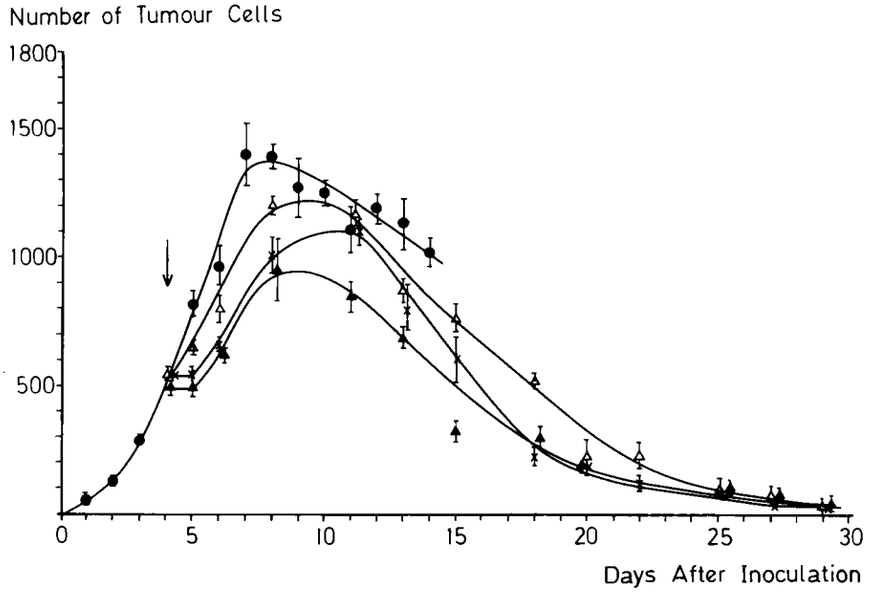

Fig. 3. number of Bp8 mouse ascites sarcoma cells ($\times10^6$) after partial body irradiation (→) with 2.5 (△), 5 (x) and 8 (▲), Gy; controls (●). Each mouse was irradiated on day 4 after the inoculation of 18×10^6 cells injected intraperitoneally. Mean values ±1 SEM of 4 to 8 mice.

centration and the ascites volume, the total number of cells was estimated. The mitotic index was determined from 1 000 cells. The percentage of dead cells was calculated by dye exclusion of 5 per cent Lissamine Green B. The mean relative cell volume was calculated from the packed cell volume and the cell concentration. The cellular DNA content was measured by flow cytometry, from which both the distribution of the tumour cells in the various parts of the cell cycle and the proportion of normal cells were calculated.

The flow rate of the cells through the cell cycle up to 96 hours following irradiation was calculated on the basis of sequential analysis of the total number of cells and the proportion of cells in G_1, S-phase, G_2 and M (CAO et coll. 1982 b).

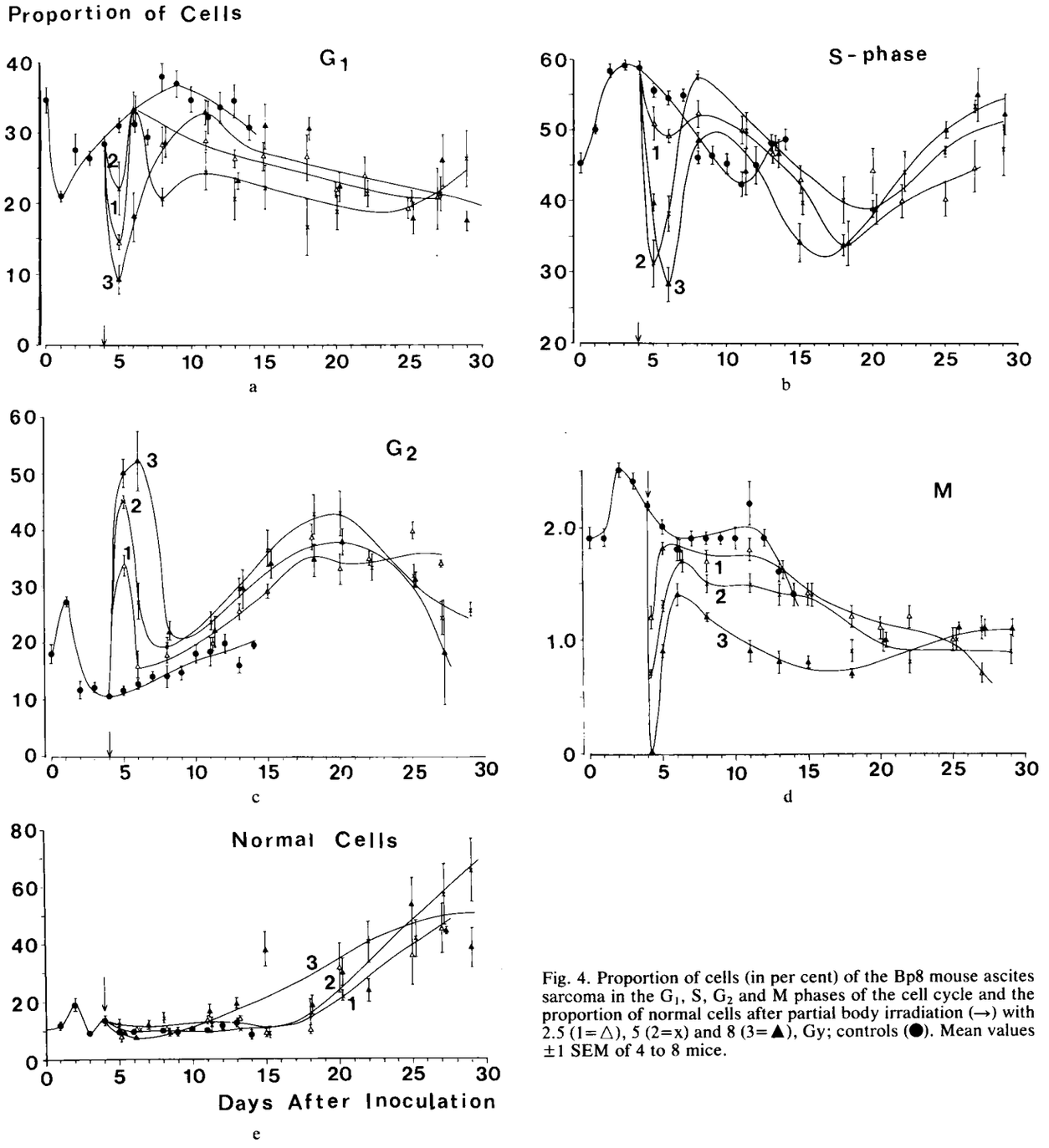

Fig. 4. Proportion of cells (in per cent) of the Bp8 mouse ascites sarcoma in the G_1, S, G_2 and M phases of the cell cycle and the proportion of normal cells after partial body irradiation (→) with 2.5 (1=△), 5 (2=x) and 8 (3=▲), Gy; controls (●). Mean values ±1 SEM of 4 to 8 mice.

For evaluation of the survival of the mice 12 animals in each group were observed daily.

Results

The survival rate of the mice bearing the Bp8 ascites sarcoma after partial body irradiation did not differ significantly between the doses of 2.5, 5 and 8 Gy with an LD_{50} of 38 to 40 days (Fig. 1). The first deaths in the non-irradiated animals occurred on day 13 and no mice survived day 16. Compared with the results from whole body irradiation with the same doses, the same survival was found only in the 2.5 Gy groups while already following 5 Gy the survival rate after whole body irradiation was slightly reduced and following 8 Gy markedly reduced (CAO et coll. 1982a).

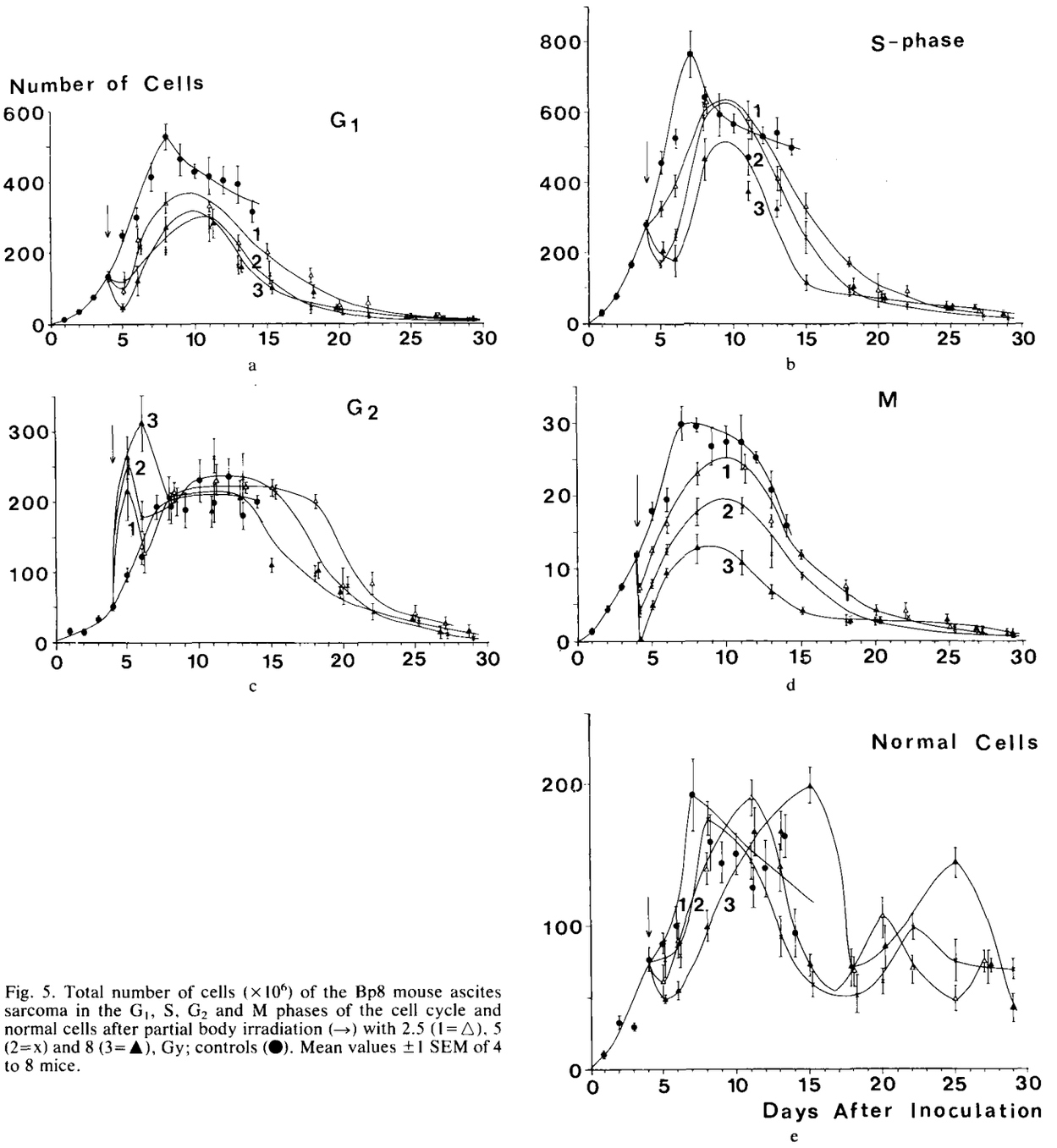

Fig. 5. Total number of cells ($\times 10^6$) of the Bp8 mouse ascites sarcoma in the G_1, S, G_2 and M phases of the cell cycle and normal cells after partial body irradiation (→) with 2.5 (1=△), 5 (2=x) and 8 (3=▲), Gy; controls (●). Mean values ±1 SEM of 4 to 8 mice.

The ascites volume (Fig. 2) showed a dose-dependent delay in the increase and reached the control level of about 12 ml around days 10, 12 and 15 for the doses of 2.5, 5 and 8 Gy, respectively. Up to day 20 the volume increased to around 15 ml at a fairly constant rate. Thereafter the increase was accelerated and at 30 days final values of up to 35 ml were found, i.e. more than the initial body weight.

The total number of tumour cells (Fig. 3) also showed a dose-dependent delay in growth. After reaching maximum values of about $1\,000\times10^6$ cells around day 10, they decreased and reached minimum values at day 30 of about 40×10^6 cells, i.e. nearly the same value as the number of cells injected.

The proportions and the total number of tumour

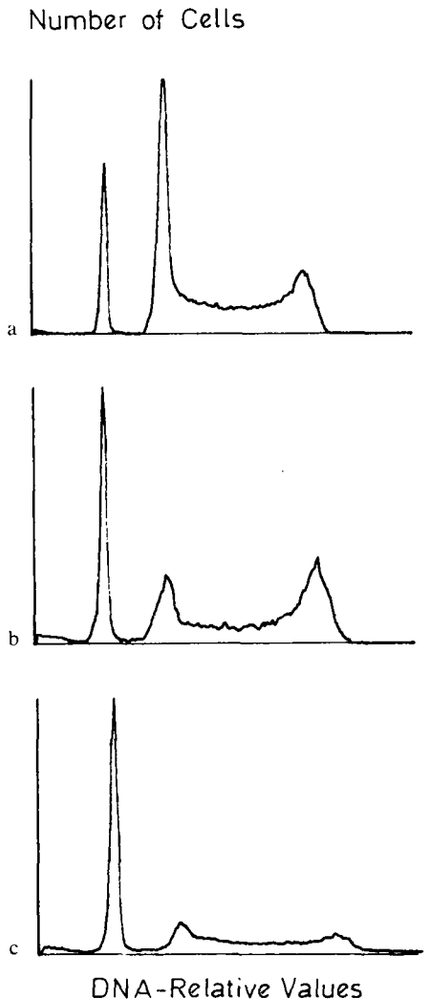

Fig. 6. Examples of cellular DNA histograms of the Bp8 mouse ascites sarcoma. a) Unirradiated cells 4 days after inoculation. b) 11 days after irradiation with 8 Gy. c) 25 days after irradiation with 8 Gy. The peak to the left in each histogram represents normal cells, the second peak G_1 tumour cells, the peak to the right G_2+M tumour cells; in between the second and the third peaks are the proportion of S-phase cells. After correction for background the proportion of cells in the various phases of the cell cycle was determined from the areas of the histograms assuming a Gaussian function of the G_1 and G_2+M maxima and attributing the remaining part of the DNA histogram to the cells of the S-phase. The proportion of normal cells in a), b) and c) is 14.6, 26.5 and 53.1 per cent, respectively. The proportion of tumour cells in G_1, S-phase and G_2+M are a) 32.5, 51.3 and 16.2 per cent. b) 19.5, 48.8 and 31.7 per cent. c) 19.8, 62.4 and 17.9 per cent. Each histogram represents 50000 measured cells.

cells in the various parts of the cell cycle as well as the proportions and the absolute values of normal cells are shown in Figs 4 and 5. These data have been obtained from the DNA cellular histograms (a few examples are given in Fig. 6) and the mitotic index.

Up to 15 to 20 days, the proportion of normal cells (Fig. 4) is around 10 per cent but it then increased to about 70 per cent on day 30. The early changes in the distribution of tumour cells with a dose-dependent accumulation of cells in G_2 24 to 48 hours after irradiation, a corresponding decrease in the proportion of cells in G_1 and S-phase, and the immediate decrease in the proportion of mitotic cells are the same as those found following whole body irradiation (CAO et coll. 1982a, b). The proportion of G_2 cells for the irradiated animals never reached that for the control animals but increased up to 20 days to new maximum values followed by decreasing values. An increase in the proportion of G_2 cells also occurred in the unirradiated controls 5 to 15 days after transplantation. Thus, the further shift towards G_2 in the irradiated cell population at later times seems to reflect the normally present tendency to an accumulation of cells in G_2 with the age of the tumour. The other characteristic change in the proportion of cells in the cell cycle was the increase in the proportion of S-phase cells beginning at around day 15. This increase was also in parallel with that normally found for the unirradiated cell population between days 10 and 15. Compared with the controls, the mitotic index decreased in a dose-dependent manner up to 10 days subsequently having dose-independent values of about one per cent. Irrespective of the irradiation dose, the proportion of dead cells during the first post-irradiation week was about 0.5 per cent and was the same as in the unirradiated controls. Thereafter it increased to a mean value of 6.0 per cent (maximum values 15%) without any correlation with the irradiation dose or the time (data not given).

After the initial rapid changes, the total number of tumour cells in the various parts of the cell cycle (Fig. 5) reached maximum values of G_1 and S-phase cells around day 10. In contrast to the experiments with whole body irradiation, no clear dose-dependence was found and only the G_1 cells deviated significantly from the control values. The values decreased rapidly between day 10 and 15. In contrast to this behaviour the total number of G_2 cells was constant up to 15 days without any dose-dependent deviation from the control value at a plateau level of about 200×10^6 cells. The mitotic cells show a clear dose-dependence and decrease around day 10 in a similar way to the G_1 and S-phase cells. Thus, the ratio between mitotic and G_2 cells (Fig. 7) also decreased in a dose-dependent way up to day 15. It increased again later mostly due to the relative decrease of the G_2 cells.

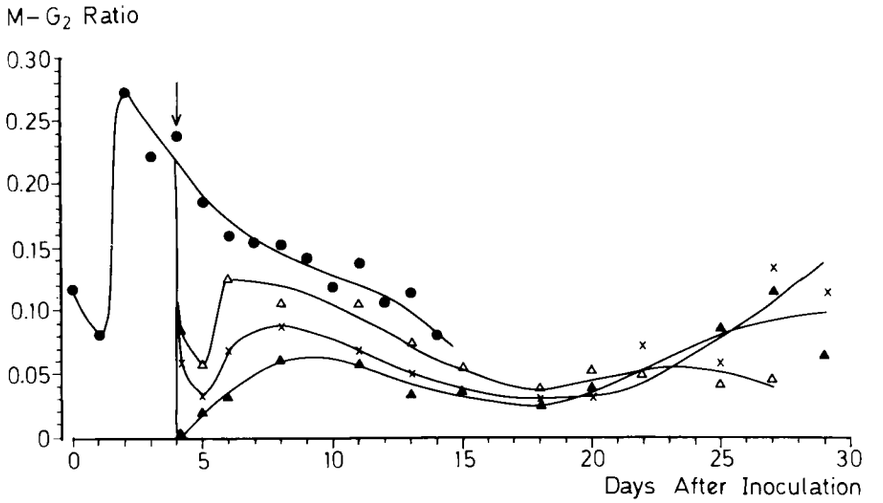

Fig. 7. Ratio (10^{-2}) between the total number of mitotic cells and G_2 cells of the Bp8 mouse ascites sarcoma after partial body irradiation (→) with 2.5 (1=△), 5 (2=x) and 8 (3=▲), Gy; controls (●).

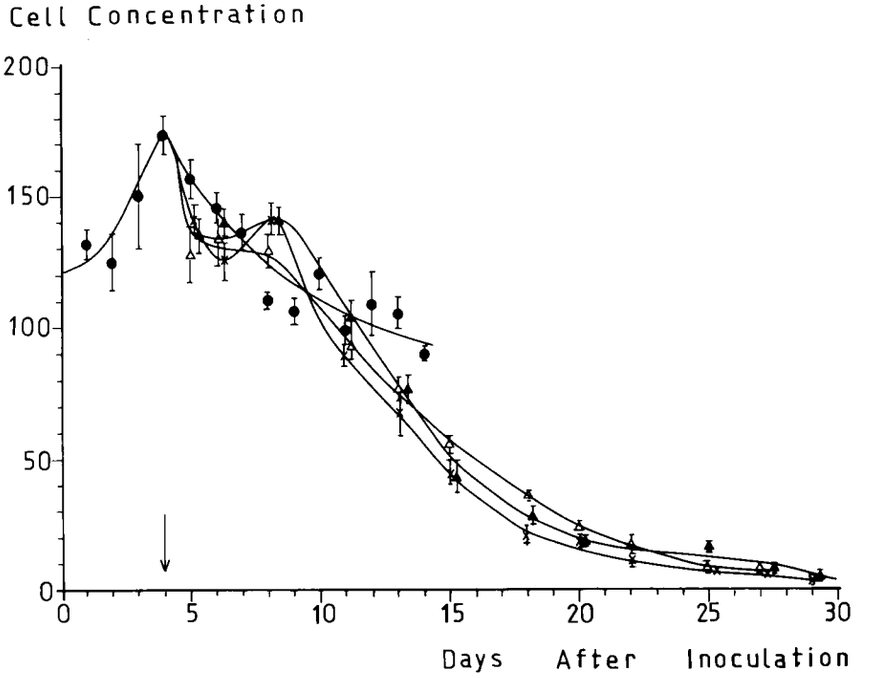

Fig. 8. Cell concentration (10^6/ml) of the Bp8 mouse ascites sarcoma after partial body irradiation (→) with 2.5 (1=△), 5 (2=x) and 8 (3=▲), Gy; controls (●). Mean values ±1 SEM of 4 to 8 mice.

The total number of normal cells (Fig. 5) increased after an initial delay to maximum values at day 8 to 15 and was then of the same order of magnitude as the control values. They later decreased to about half this value.

The cell concentration in the ascites (Fig. 8) decreased continuously from maximum values of about 170×10^6 cells per ml, reached at the early growth of the tumour, to 5×10^6 cells per ml at day 30. No dose-dependence was found.

The relative cell volume, which is significantly higher already one day after irradiation, increased further to maximum values between 20 to 25 days but decreased thereafter (Fig. 9).

The early changes in the flow of cells through the cell cycle after partial body irradiation, estimated

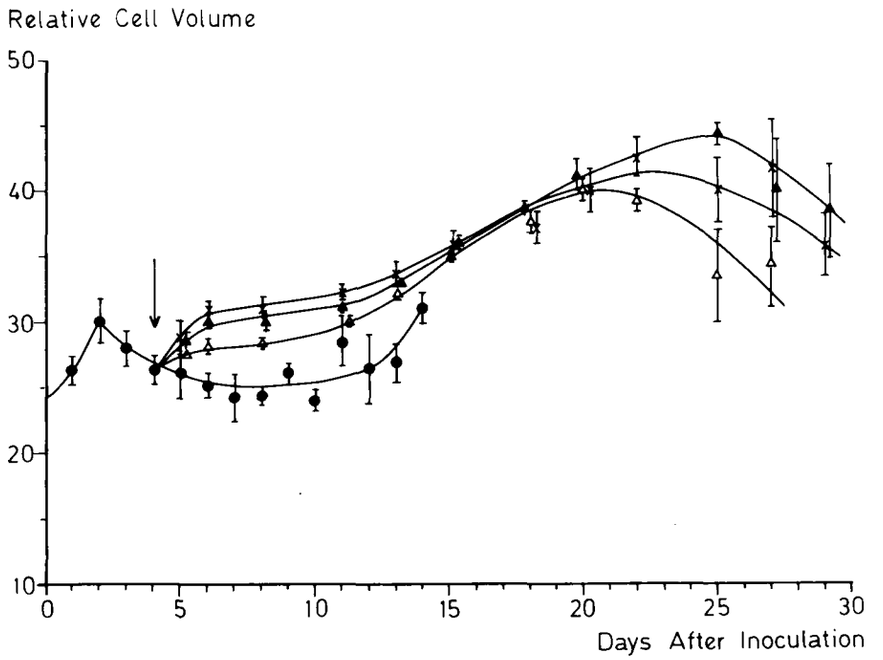

Fig. 9. Relative cell volume (10^{-6}) of the Bp8 mouse ascites sarcoma after partial body irradiation (→) with 2.5 (1=△), 5 (2=x) and 8 (3=▲), Gy; controls (●). Mean values ±1 SEM of 4 to 8 mice.

for the time intervals 0 to 6, 6 to 24, 24 to 48, 48 to 72 and 72 to 96 hours, were in good general agreement with those after whole body irradiation. Thus, the durations of G_1 and the S-phase up to 24 hours were slightly prolonged while M showed a transient very early increase in the duration. The most marked effect lasting up to 72 hours was found in the duration of G_2 which generally was responsible for the prolongation of about 50 per cent in the total duration of the cell cycle up to 72 hours (details of the data not given).

Discussion

The survival of the animals after partial body irradiation was significantly prolonged compared with whole body irradiation following 8 Gy and slightly prolonged following 5 Gy. The different doses of 2.5, 5 and 8 Gy partial body irradiation resulted in no differences in the survival time. Thus, under these irradiation conditions the bone marrow was apparently functioning properly, even following 8 Gy. The LD_{50} for whole body irradiation is about 7 Gy for the normal non-tumour bearing NMRI mice in this laboratory. For non-irradiated tumour-bearing mice it is reduced to 13 days (CAO et coll. 1982c). Also, since no deaths occurred up to 10 days after irradiation, the intestinal tract did not reach a critical level of injury. The cause of death of the animals is therefore not directly related to irradiation effects of normal tissues but apparently to the tumour disease. No analysis of causes of death has been done and such factors as the existence of metastasis have not not been evaluated. Some authors have pointed out that metastasis in both early or later cell growth period was obviously existent (BELLI & ANDREWS 1963, HOFER et coll. 1969, HOFER & HOFER 1971). In the present investigation, at least the marked increase in the ascites volume should be considered as a significant contributing factor. Since ascites fluid contains 2 to 3 per cent protein (KUN et coll. 1951), a considerable loss of protein during development of ascites can be anticipated.

The continuous increase in the ascites volume contrasted with the marked decrease in the tumour cells. The decrease in the total number of cells is due to a change in the balance of cell production and cell loss. In the present data both a reduced mitotic index and an increase in the proportion of dead cells were found. Since, for example, following irradiation with 8 Gy, the mitotic index is fairly constantly reduced to about one per cent and there is no reason to assume that the duration of the mitosis is shortened, while the number of tumour cells decreased, the cell loss factor in this case must have increased. The increase in cell loss of ascites tumour

with age has been reported by many authors (STEEL 1968, TANNOCK 1969, FRINDEL et coll. 1969, DOMBERNOWSKY & HARTMANN 1972, LALA 1972, SCHIFFER et coll. 1973, HARRIS et coll. 1973). In this connection it must be realized, however, that it was impossible to determine whether the mitotic cells belonged to tumour or normal cells. The decrease in the number of tumour cells in the irradiated groups parallels a decrease in tumour cells in the unirradiated mice. Repeated experiments showed that these unirradiated animals survived no longer than 17 days after inoculation. It may be assumed that this difference in survival between non-irradiated and irradiated animals is caused by a decrease in the number of tumour cells. In previous investigations using whole body irradition at a lower dose (1.75 Gy), the survival time was prolonged to the same extent as in the present one. The total number of cells, however, did not decrease in comparison with the controls up to 15 days (CAO et coll. 1982 a). Thus the tumour burden expressed as the total number of tumour cells in the ascites is not directly related to the survival.

The proportion of tumour cells in the various parts of the cell cycle after the initial irradiation-induced changes was not constant: the proportion of G_2 cells up to 20 days after inoculation increased and corresponded mainly to the decrease in the proportion of S-phase cells while the following decrease in the proportion of G_2 cells was again proportional to the increase in the S-phase cells. The increase in the proportion of S-phase cells was not followed by an increase in mitotic index which in this case would indicate a higher proliferative activity of the tumour.

The decrease in the proportion of G_2 cells might indicate the end of the irradiation-induced persistent G_2 blockage. As was found in the experiments on whole body irradiation, the total number of G_2 cells is characterized by a dose-independent constant value. This constant value, however, cannot be observed beyond day 18 at which time the total number of tumour cells decreased markedly.

The mean relative cell volume, determined from the packed cell volume of the ascites and the cell concentration, depends, to some extent as discussed previously, on both the proportion of relatively small normal cells and the proportion of relative large G_2 cells (CAO et coll. 1982 a). The increase in the proportion of normal cells and the decrease in the proportion of G_2 cells coincide with the time of the decrease in the relative cell volume 20 to 25 days after irradiation. This is one of the few parameters in which at the late time period a dose-dependence can be seen, but the question is, whether this dose-dependence is a coincidence or a cause-effect relationship.

SUMMARY

Mice bearing the Bp8 mouse ascites sarcoma were 4 days after inoculation irradiated with 2.5, 5 and 8 Gy. By shielding the head and the upper part of the thorax the mice survival, compared with whole body irradiation, was markedly prolonged and the behaviour of the ascites tumour could be evaluated up to 30 days after inoculation. While about 10 days after irradiation the total number of tumour cells started to decrease and amounted to only 4 per cent 20 days later, the ascites volume increased up to 30 ml. Using flow-cytometric cellular DNA analysis, the proportions of cells in the various parts of the cell cycle were determined. After an initial G_2 blockage, a partial blockage with an increase proportion of G_2 cells was found up to 20 days after inoculation. While the total number of G_1, S-phase and mitotic cells started to decrease on day 10, the total number of G_2 cells was independent of the dose and was constant up to day 18. This behaviour may indicate specific regulating processes linked to G_2 cells.

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