Eur J Cancer Clin Oncol, Vol. 22, No. 5, pp. 549–556, 1986.
Printed in Great Britain.

0277–5379/86$3.00+0.00

Growth Kinetics and *in Vivo* Radiosensitivity in Nude Mice of Two Subpopulations Derived from a Single Human Small Cell Carcinoma of the Lung*

MOGENS SPANG-THOMSEN,†‡ MAURIZIA CLERICI,† SVEND A. ENGELHOLM,†§ and LARS L. VINDELØV¶

†*University Institute of Pathological Anatomy, University of Copenhagen, 11, Frederik V's Vej, DK-2100 Copenhagen, Denmark;* §*Department of Oncology, Copenhagen University Hospital, Herlev Ringvej, DK-2730 Herlev, Denmark and* ¶*Department of Internal Medicine, Finsen Institute, 49, Strandboulevarden, DK-2100 Copenhagen, Denmark*

Abstract—*The growth kinetics and the* in vivo *radiosensitivity of two human small cell carcinomas of the lung (SCCL) grown in nude mice were investigated. The tumors, CPH SCCL 54A and 54B, were derived by* in vitro *cloning of a single SCCL and were subsequently serially grown in nude mice. The growth curves were described according to a transformed Gompertz function, and the cell kinetics were examined by flow cytometric DNA analysis (FCM) and by the technique of labelled mitoses. The effect of single-dose irradiation was estimated by the specific growth delay calculated from the growth curves, and by the cell cycle distribution changes monitored by FCM. The results showed that the tumors differed in the* in vivo *radiosensitivity despite similarities in the growth kinetics. The results support the concept that difference in sensitivity among tumor subpopulations is an important reason for therapeutic failures.*

INTRODUCTION

HETEROGENEITY of tumors has been ascribed to inappropriate differentiation processes and to genetic instability during the clonal evolutionary processes leading to fully developed malignancies [1]. A consequence of these hypotheses is the presence of subpopulations in tumors with different sensitivity to antineoplastic treatment [2–5]. Accordingly, the treatment of human tumors of identical histological type results in a great variation of the treatment response.

Since the cellular sensitivity to irradiation and to cell cycle phase-specific drugs is dependent on the position in the cell cycle [6, 7], a detailed characterization of the cell kinetics and especially of the treatment-induced changes is important for the understanding of the response patterns of tumors. However, due to ethical and practical problems the cell kinetics of human tumors are only sparsely investigated [8].

In immune-suppressed and in thymus aplastic nude mice [9, 10] it is possible to establish serially transplantable human malignant tumors, and, since investigations have shown that many important tumor characteristics are preserved even after numerous serial transplantations [11, 12], the results obtained in these model systems may be of relevance to similar tumors in patients.

This study reports the effect of single-dose irradiation on the growth kinetics of two subpopulations derived from a single human small cell carcinoma of the lung (SCCL). The tumor growth kinetics were examined by gompertzian growth curves [13], the technique of per cent labelled mitoses (PLM) [14], and by flow cytometric DNA analysis (FCM) [15]. The *in vivo* radiosensitivity was evaluated by calculation of the treatment-induced specific growth delay (SGD) [16], and FCM was performed on tumor tissue obtained by sequential fine-needle aspiration to monitor the effect on the cellular level [17].

The results showed that the tumors differed in radiosensitivity despite close similarities in growth kinetics. This supports the concept that difference in sensitivity among tumor subpopulations may be important for therapeutic failures.

MATERIALS AND METHODS

Tumors and transplantation

The tumors, CPH SCCL 54A and 54B (T54A and T54B) were obtained as described elsewhere

Accepted 24 October 1985.
*This study was supported by grants from the Foundation of Hafnia-Haand i Haand, the foundation of K. and V. Skovgaard, and the Danish Cancer Society.
‡To whom correspondence should be addressed at: University Institute of Pathological Anatomy, University of Copenhagen, 11, Frederik V's Vej, DK-2100 Copenhagen, Denmark.

[18] by cloning of an *in vitro* cell line established from a previously untreated human SCCL. The tumor was histologically subclassified as the intermediate type (WHO).

After cloning and subsequent growth *in vitro* the tumor cells were transferred to nude mice of BALC/c background for further serial transplantation. The mice were kept under sterile conditions in laminar air flow clean benches. The room temperature was 25 ± 2°C and the relative humidity was 55 ± 5%. Sterile food and water were given *ad libitum*.

Serial transplantation was performed under general anaesthesia with propanidid (Epontol®) by inserting tumor blocks of about 2 mm subcutaneously into both flanks of the mice.

Growth data

From about 2 weeks after transplantation the tumors were measured in two dimensions, three times a week using a sliding gauge [13], and the tumor area, A = the product of the two measurements, was calculated. The growth data were described according to the Gompertz equation:

$$A(t) = A(\mathrm{O}) \exp \{ (1 - \exp(-\alpha t))\alpha/\beta \}. \quad (1)$$

The growth function used was a transformation of equation (1) [13]:

$$\ln \{ \ln A(\max) - \ln A(t) \} = \ln\beta/\alpha - \alpha t. \quad (2)$$

The A-values were used to construct normalized mean growth curves [13] according to equation (2). This function depicts the growth as a straight line with negative slope (α), when the tumor size $\ln \{ \ln A\ (\max) - \ln A(t) \}$ is plotted against time. $A(\max)$ is the theoretical maximum area and $A(t)$ is the tumor area at time t. The $\ln A(\max)$ was graphically [13] estimated to be 7 for both tumors.

Tumor volume was calculated from tumor area as described elsewhere [13].

Irradiation and growth delay

One or both tumors in each animal were treated with a single dose X-irradiation under general anaesthesia and under sterile conditions as described previously [19], using a Stabilipan (Siemens) therapeutic unit, which yields 4.58 Gy/min at 300 kV, 12 mA, and with a Thoreus I filter. The doses stated below are calculated doses at the center of 10-mm-diameter tumors.

The T54A tumors were treated 30 days after transplantation. A total of 48 tumors in passage 28 in nude mice were randomized to a treatment dose of 0, 3, 5 and 10 Gy, the groups comprising 20, 9, 10 and 9 tumors, respectively. Three tumors were excluded as the mice died before the end of the observation period.

The T54B tumors were treated 22 days after transplantation. A total of 42 tumors in passage 19 in nude mice were randomized to the treatment of 0, 3, 5 and 10 Gy, the groups comprising 15, 10, 8 and 9 tumors, respectively. Six tumors were excluded as the mice died before the end of the observation period.

The effect of irradiation was evaluated from the mean growth curves by calculation of the treatment-induced growth delay (*TGD*), defined as the time to grow to twice the mean treated volume [19]. The *TGD*-values were used to calculate the specific growth delay (*SGD*) [16].

$$SGD = \frac{TGD - CGD}{CGD},$$

where *CGD* is the growth delay of untreated control tumors.

Flow cytometric DNA analysis (FCM)

The irradiated tumors were examined for treatment-induced changes in the cell cycle distribution. Tumor tissue for analysis was obtained by fine-needle aspiration at intervals after the treatment. In order to obtain reference values the first aspirations were performed prior to the irradiation. The individual tumors were aspirated at intervals of at least 2 days.

In a separate experiment 15 untreated T54A tumors in passage 26 in nude mice and 15 untreated T54B tumors in passage 23 were aspirated weekly to investigate the possible cell cycle distribution changes during the growth of the tumors. A total of 49 (T54A) and 42 (T54B) samples were analyzed, and the results were stratified according to the tumor volume at the time of sampling.

Preparation of samples, storage at −80°C, and staining by propidium iodide were performed as described elsewhere [20–22]. The flow cytometer used was a FACS III (Becton Dickinson, Sunnyvale, CA). The DNA index of the tumors [23], defined as the ratio of the DNA content of the tumor G_1 cells to that of human diploid cells, was determined by use of chicken and trout red blood cells as internal standards [22]. The cell cycle distribution of the tumor cells and the fraction of diploid mouse stromal cells were determined by statistical analysis of the DNA histogram [24].

Per cent labelled mitoses (PLM)

In a separate experiment untreated T54A and T54B tumors in passage 26 and 23, respectively, were measured for determination of the transformed Gompertz growth curves and were subsequently examined by the technique of PLM as

previously described [14]. Briefly, 29 days after transplantation the mice were given 40 μCi ^{3}H-labelled thymidine i.p. (specific activity 6.7 Ci/mM, New England Nuclear). The tumors were excised at intervals and autoradiographs were prepared using the dipping technique (Kodak K-2 emulsion). After 6 weeks of exposure the PLM (labelled/total mitoses × 100) were calculated from at least 200 labelled and unlabelled mitoses scored in representative areas of the tumors.

A total of 42 (T54A) and 42 (T54B) tumors were transplanted for this examination. Five were excluded because the tumors did not grow and four because the autoradiographs were unsuccessful.

The PLM data were analyzed by a computer program [25] calculating the duration of the cell cycle phases (T_{G_1}, T_{G_2} and T_S), the median cell generation time (T_C), and the frequency distribution of T_C.

Derived parameters

The growth curve parameters were used to calculate the tumor volume doubling time [26]:

$$T_D = -\frac{1}{\alpha} \ln \{ 1 + \ln 2 / \ln \frac{V(t)}{V(\max)} \} .$$

The potential doubling time (T_{pot}) and the cell loss factor (ϕ) were calculated from [27, 28]:

$$T_{pot} = \lambda \frac{T_S}{F_S}$$

$$\phi = 1 - \frac{T_{pot}}{T_D} .$$

In the calculation of T_{pot}, F_S is the S phase fraction obtained by FCM.

The growth fraction (GF) was calculated from T_C and T_{pot} according to [28]:

$$\ln a = \frac{T_C}{T_{pot}} \ln 2$$

and

$$GF = a - 1.$$

RESULTS

The PLM data of the tumors and the best-fit computed curves appear from Fig. 1.

The curve of tumor T54A shows 'fade' in the second peak [8], i.e. the points are below the curve. Thus, the resultant median T_C-value of 34.0 hr for T54A (Table 1) is not very reliable.

The PLM data of T54B show a greater spread in the cell cycle time compared to that of T54A, mainly due to a greater spread in the G_1 duration time (Table 1). This is visualized in Fig. 2, which shows the frequency distributions of the cell generation time.

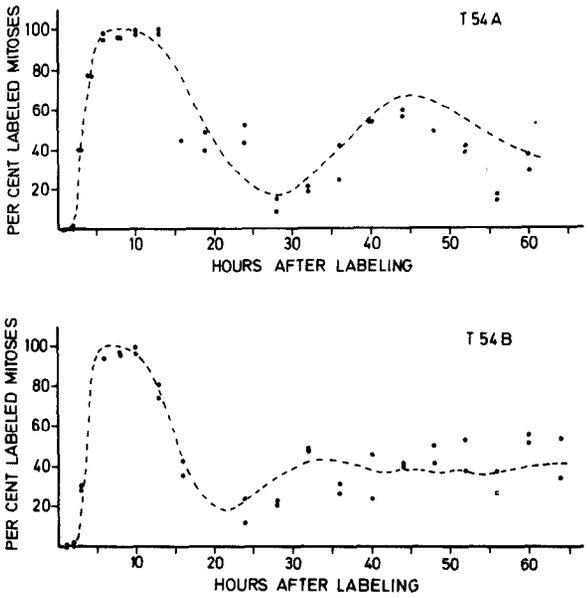

Fig. 1. Fractions of labelled mitoses after pulse labelling with ^{3}H-labelled thymidine of the T54A and T54B human small cell carcinomas of the lung. The tumors were labelled 29 days *after transplantation following recording of the growth for determination of the mean growth curves.*

The computed cell cycle duration times are summarized in Table 1 together with the calculated growth parameters. It appears that the growth characteristics of the tumors were rather similar in the growth curves as well as in the cell cycle kinetics. This is in agreement with the results obtained by the repeated FCM (Table 2). The cell cycle distributions showed no systematic changes during the growth of untreated tumors (data not shown). Therefore, all the results were pooled in the calculation of the mean values listed in Table 2.

The transformed Gompertz growth curves of the two tumors were described by the same theoretical maximum size (lnA(max) = 7) and by the slopes,

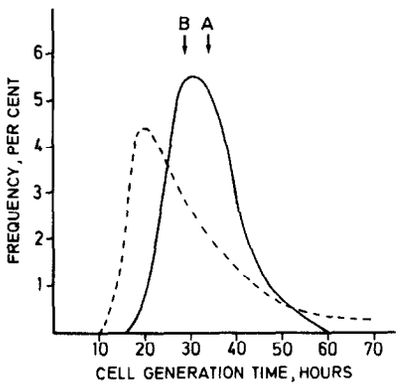

Fig. 2. Distribution of the cell generation time of the T54A (full line) and the T54B (dotted line) human small cell carcinomas of the lung. The distributions are consistent with the labelled mitoses data of Fig. 1. The computed median generation times are indicated by the arrows.

Table 1. Proliferation characteristics of the T54A and T54B human small carcinomas of the lung grown in nude mice. The tumors were derived by in vitro *cloning from the same original human tumor. The median cell generation time (T_C), the duration times of the G_1, S, and G_2 phases of the cell cycle and the standard deviation of the means* (S.D.) *were determined by computer analysis of the data obtained by the technique of labelled mitoses. The slope of the transformed Gompertz growth curve* (α), *the tumor volume doubling the time (T_D) the potential doubling time (T_{pot}), the cell loss factor* (ϕ), *and the growth fraction (GF) were calculated as described in the Materials and methods section. All time values are in hr.*

	No.	Mean vol. (mm^3)	T_C	T_{G_1}			T_S			T_{G_2}			α 10^3	T_D	T_{pot}	ϕ %	GF %
				Mean	S.D.	Median	Mean	S.D.	Median	Mean	S.D.	Median					
T54A	38	257	34.0	15.0	6.7	13.7	16.6	5.9	15.7	3.6	1.0	3.5	38.9	120	100	1.3	26.6
T54B	37	238	28.9	20.5	31.7	11.2	12.3	3.8	11.8	3.7	0.7	3.6	35.9	127	69	46.6	34.0

Table 2. The mean per cent cells in the cell cycle phases ± S.D. determined by flow cytometric DNA analysis of the T54A and T54B human small cell carcinomas of the lung. The cellular DNA content of the tumor cells (DNA index) was determined in two samples from each of the tumors.

	T54A	T54B
No of analyses	49	42
G_1	87.2 ± 3.8	84.7 ± 3.0
S	11.8 ± 3.9	13.1 ± 2.7
G_2 + M	1.0 ± 0.5	2.2 ± 0.9
DNA index	2.25	1.32

$\alpha = 0.0389$ and $\alpha = 0.0359$, respectively (Table 1). Statistical analysis of the α-values by Wilcoxons tests for two samples showed no significant difference between the growth of the tumors ($u = 1.43$, $P > 0.05$).

The experimental growth data were well fitted by the growth equation used. The coefficients of correlation were $r = 0.993$ and $r = 0.998$ for tumor T54A and T54B, respectively.

The radiation effect on the growth curves of the tumors appears from Figs. 3 and 4. The treatment induced a dose-dependent growth delay (TGD) in both tumors, and the calculated SGD-values show that the response of T54A was almost twice that of T54B (Fig. 5).

Despite the evidence in both tumors of a substantial cell kill (SGD), the irradiation caused no tumor shrinkage even after the highest dose applied. This is illustrated for the most sensitive tumor (T54A) in Fig. 6, top diagram. It is seen from the course of the volume growth curves that no significant differences appeared during the initial 10–15 days after the treatment.

In addition to the volume changes of T54A, the variation in the fraction of diploid cells are de-

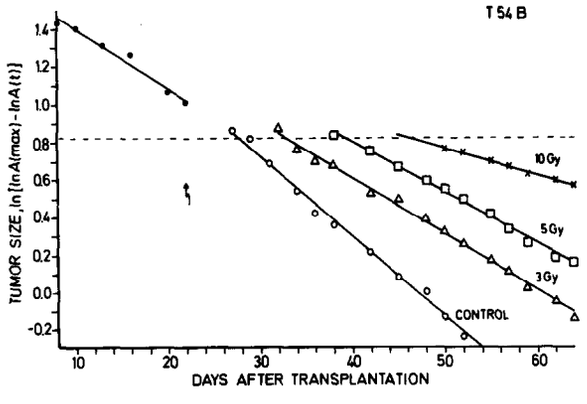

Fig. 3. Normalized mean transformed Gompertz growth curves of the T54A human small cell carcinoma of the lung following single-dose irradiation 30 days (*arrow*) *after transplantation. The curves were used to calculate the time (growth delay) to reach twice the mean treated tumor volume (dotted line).*

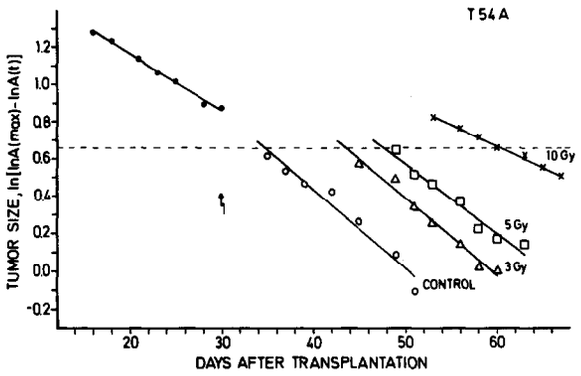

Fig. 4. Normalized mean transformed Gompertz growth curves of the T54B human small cell carcinoma of the lung following single-dose irradiation 22 days (*arrow*) *after transplantation. The curves were used to calculate the time (growth delay) to reach twice the mean treated tumor volume (dotted line).*

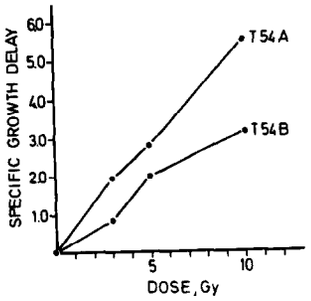

Fig. 5. Specific growth delay of the T54A and T54B human small cell carcinomas of the lung following single-dose irradiation.

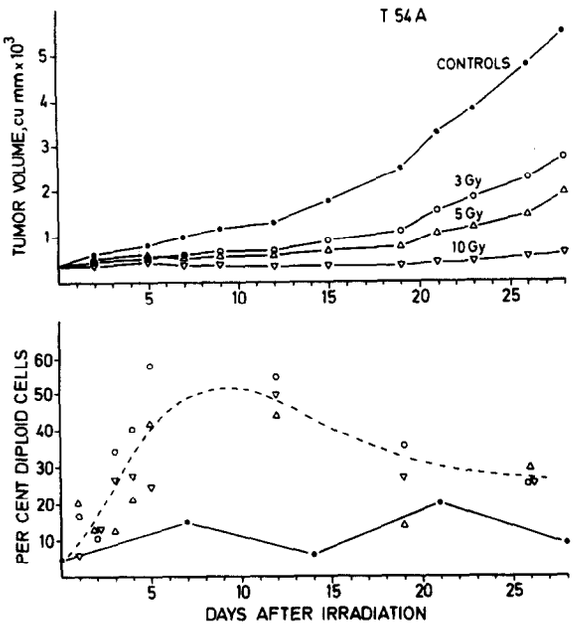

Fig. 6. Mean volume growth curves (top) and mean per cent diploid cells (bottom) of the T54A human small cell carcinoma of the lung following single-dose irradiation. The tumor volume data were calculated from the growth data of Fig. 1. The fraction of diploid cells were computed from flow cytometric DNA histograms. Each point reprsents one to five untreated control tumors (●), or two to five tumors treated with 3 Gy (○), 5 Gy (△), and 10 Gy (▽). The dotted line (bottom diagram) were drawn by the eye.

picted in Fig. 6 (bottom diagram). It appears that the diploid cells rapidly increased to a maximum of about 50% on day 12 after the treatment, and that the level remained elevated throughout the observation period. The changes in the diploid fraction were independent of the radiation dose.

The diploid fraction of cells was the same in unperturbed tumor T54A and T54B. The calculated means ± standard deviation (S.D.) were 10.9 ± 7.9% (T54A) and 10.0 ± 12.7% (T54B).

The posttherapeutic variation in tumor volume and in diploid fraction was in principle the same in T54B as that in T54A (Fig. 6).

The treatment effect on the tumor cell cycle distribution was monitored by sequential FCM. The cell cycle perturbations were small in both tumors and comprised, as exemplified in Fig. 7, an accumulation of cells in the G_2 + M phase of the cell cycle followed by an increase in the S phase fraction. In some of the dose groups (Fig. 7) the data indicate two G_2 + M peaks. The G_1 phase changes were inverse to those of the G_2 + M phase.

In both tumors the cell cycle fractions redistributed to the pretreatment levels 12–20 days after the irradiation.

The tumor cell distribution changes were more pronounced in the T54B, whereas the amount of necrosis was greater in the histograms of the T54A (not shown).

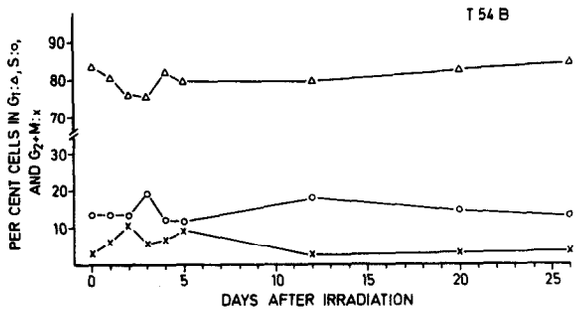

Fig. 7. Mean cell cycle changes determined by flow cytometric DNA analysis of the T54B human small cell carcinoma of the lung following 5 Gy single-dose irradiation. Each point represents the mean of three to six different tumors.

DISCUSSION

This study showed that two subpopulations derived from the same human SCCL exhibited a great difference in the *in vivo* radiosensitivity, and the close similarities in the growth characteristics suggest that this difference may be due to inherent difference(s) in the cellular sensitivity. However, the difference in sensitivity may be explained by the difference in the spread of the cell generation time of the two tumors (Fig. 2). The greater spread in T54B reflects that the spread in the G_1 duration time of this tumor is greater than that of the T54A, and thus that T54B may contain a greater cell fraction in the parts of the cell cycle in which the cells are resistant to irradiation [6].

Irrespective of the reason for the different sensitivity the results suggest that if the two tumors are representative of the heterogeneity of human tumors *in situ*, then the difference in sensitivity among subpopulations of a tumor is an important factor in therapeutic failures.

The cell kinetics of the tumors (Table 1) are comparable to the few data available from similar tumors in patients [8], and the cell cycle distributions (Table 2) are within the range found in a large series of SCCL in patients [29].

The median T_C-values of approx. 30 hr and the S phase fractions (= labelling index) of 12–13% are of the same order of magnitude as those found in other types of human tumors in patients [8] and in nude mice [14]. Thus, the proliferative parameters offer no explanation for the general experience that SCCL is a fast growing tumor type in patients. However, the fast net growth might as well be due to a relative small cell loss [30]. This would be in agreement with the calculated ϕ-values of 17.3 and 46.6. (Table 1), which are well below the values generally found in other types of human tumors [8, 14].

The results of the repeated FCM of untreated tumors can be used to deduce information concerning the cell cycle duration times during growth [14] without the use of the laborious and time-

consuming PLM technique. The cell cycle distributions showed no systematic changes with the growth of these tumors. This is in contrast to the finding in a heterotransplanted human malignant melanoma of an increasing G_1 fraction, which by PLM investigation was found to be caused by a prolongation of the G_1 duration time whereas the duration of the S and G_2 phases was constant with tumor growth [14]. Analogously, since the cell cycle distributions of the present SCCL tumors were independent of the tumor size, it can be argued that also the cell cycle duration times were constant during the tumor growth. This would implicate that the increase in the tumor volume doubling time (T_D), which occurs during gompertzian growth of tumors [14], is caused only by an increase in the cell loss factor, whereas the growth fraction remains constant. This means that the retardation diagram [8] of the tumors, i.e. a plot of connected values of GF and ϕ for increasing tumor sizes, would show a vertical line. Hence, in accordance with the results generally found in experimental and in other human tumors [8, 14], the cell loss is the dominant factor in the growth of these tumors.

Growth delay defined as the time of growth to reach a multiple of the tumor volume at the time of treatment is an accepted *in vivo* measure of treatment effect [31–33]. However, since the calculated value of growth delay (*TGD*) is dependent on the growth rate of untreated tumors, *TGD* is insufficient as the only parameter in the comparison of treatment effect in different tumors. Therefore it is necessary to use *SGD* or another parameter that is independent of the tumor growth rate.

The lack of shrinkage after the treatment (Fig. 6) has been described in other tumors (19, 33]. The reason for the general observation that tumors initially after irradiation show continued growth and often without a subsequent shrinkage has been ascribed to the accumulation in the G_2 phase of cells with a relatively large volume due to a radiation-induced premitotic block in the cell cycle, to treatment-induced edema, and to persistent necrosis in the tumors [19], especially in tumors with small ϕ-values [34]. Furthermore, flow cytometry has been used to demonstrate a decrease in the ratio of tumor cells to all cells by a factor of 15 after irradiation of an experimental tumor [35]. Hence, it seems that the present finding of a very pronounced accumulation of diploid cells (Fig. 6) may be a general phenomenon in irradiated tumors, and the results thus suggest that this is an important reason for the continued increase in tumor size after irradiation or, as in the present tumors, for the lack of tumor shrinkage.

The postirradiation increase in the fraction of diploid cells may be due to a radiation-induced reduction in the fraction of tumor cells and/or to an inflammatory reaction caused by the irradiation. In the present experiment the results indicate that the inflammatory reaction was the main reason, since the increase in the diploid fraction commenced rapidly after the treatment in tumors showing no shrinkage, and since the changes were independent of the dose applied (Fig. 6).

The diploid cell fraction of the individual tumors showed great variation due to a different degree of blood admixture in the fine-needle aspirations. Thus, the actual values plotted in Fig. 6 (bottom) are not very reliable, but are considered indicative of the changes induced in the treated tumors.

The cell cycle distribution changes of the treated tumors were small as exemplified for T54B following 5 Gy in Fig. 7. This is in agreement with the findings in previous studies on other heterotransplanted human tumors [19, 33], and may in part be explained by the presence of radiation-induced necrotic cells in the samples which obscure the analysis of the histogram [26, 33].

The two peaks in the $G_2 + M$ accumulation indicate that irradiation was followed by a partial synchronization of the accumulated cells. The interval between the peaks suggests a postirradiation generation time of 2–3 days, which is a prolongation compared with the median T_C found by the PLM investigation of unperturbed tumors (Table 1). However, the redistribution in all dose groups of the cell cycle fractions to pre-treatment level indicates that the treatment did not influence the regrowth cell-kinetics of the surviving tumor cells. It is therefore likely that the decreased regrowth rate of tumors treated with 10 Gy (Figs. 3 and 4) is caused by persistent radiation-induced necrosis in these tumors [26].

Acknowledgements—The authors are indebted to Dr. G. Gordon Steel for performing the computer analysis of the PLM data. We thank cand.act. A. Nielsen and Mr. I.J. Christensen for statistical assistance. The technical assistance of Mrs. C. Holstein, Mrs. V. Hornhaver, Mrs. I. Nøhr, Mrs. C. Pilgaard Andersen Bostrup, Mrs. E. Høj, and Miss A. Nielsen is gratefully appreciated.

REFERENCES

1. Nowell PC. The clonal revolution of tumor cell populations. *Science* 1976, **194**, 23–28.
2. Barranco SC, Ho DHW, Drewinko B, Romsdahl MM, Humphrey RM. Differential sensitivities of human melanoma cells grown *in vitro* to arabinosylcytosine. *Cancer Res* 1972, **32**, 2733–2736.

3. Heppner GH, Dexter DL, DeNucci T, Miller FR, Calabresi P. Heterogeneity in drug sensitivity among tumor cell populations of a single mammary tumor. *Cancer Res* 1978, **38**, 3758–3763.
4. Håkansson L, Tropé C. On the presence within tumors of clones that differ in sensitivity to cytostatic drugs. *Acta pathol microbiol scand. Sect A* 1974, **82**, 35–40.
5. Leith JT, Dexter DL, DeWyngart JK, Zeman EM, Chu MY, Calabresi P, Glicksman AS. Differential response to x-irradiation of subpopulations of two heterogeneous human carcinomas *in vitro*. *Cancer Res* 1982, **42**. 2556–2561.
6. Sinclair WK. Cyclic x-ray responses in mammalian cells *in vitro*. *Radiat Res* 1968, **33**, 620–643.
7. Skipper HE. The cell cycle and chemotherapy. In: Baserga R, ed. *The Cell Cycle and Cancer*. New York, Dekker, 1971, 358–387.
8. Steel GG. *Growth Kinetics of Tumours*. Oxford, Oxford University Press, 1977.
9. Spang-Thomsen M, Visfeldt J. Animal model of human disease: malignant tumors. *Am J Pathol* 1976, **84**, 193–196.
10. Steel GG, Courtenay VD, Peckham MJ. The immune-suppressed mouse as an alternative host for heterotransplantation. In: Fogh J. Giovanelle BC, eds. *The Nude Mouse in Experimental and Clinical Research*. New York, Academic Press, 1982, 207–227.
11. Povlsen CO, Visfeldt J, Rygaard J, Jensen G. Growth patterns and chromosome constitution of human malignant tumours after long-term serial transplantation in nude mice. *Acta pathol microbiol scand. Sect A* 1975, **83**, 709–716.
12. Selby PJ, Thomas JM, Monaghan P, Sloane J, Peckham MJ. Human tumour xenografts established and serially transplanted in mice immunologically deprived by thymectomi, cytosine arabinoside and whole-body irradiation. *Br J Cancer* 1980, **41**, 52–61.
13. Spang-Thomsen M, Nielsen A, Visfeldt J. Growth curves of three human malignant tumors transplanted to nude mice. *Exp Cell Biol* 1980, **48**, 138–154.
14. Spang-Thomsen M, Vindeløv LL. Proliferation kinetics of a human malignant melanoma serially grown in nude mice. *Cell Tissue Kinet* 1984, **17**, 401–410.
15. Vindeløv LL, Christensen IJ, Jensen G, Nissen NI. Limits of detection of nuclear DNA abnormalities by flow cytometric DNA analysis. Results obtained by a set of methods for sample-storage, staining and internal standardization. *Cytometry* 1983, **3**, 332–339.
16. Steel GG. Therapeutic response of human tumour xenografts in immune-suppressed mice. In: Sordat B, ed. *Immune-Deficient Animals*. Basel, S. Karger, 1984, 395–404.
17. Spang-Thomsen M, Brünner N, Engelholm SA, Vindeløv L. Estimation by flow cytometric DNA analysis of the effect of radiotherapy, hormone therapy and chemotherapy on human tumors grown in nude mice. In: Sordat B, ed. *Immune-Deficient Animals*. Basel, S. Karger, 1984, 409–415.
18. Engelholm SA, Vindeløv LL, Spang-Thomsen M, Brünner N, Tommerup N, Nielsen MH, Hansen HH. Genetic instability of cell lines obtained from a single small cell carcinoma of the lung. *Eur J Cancer Clin Oncol* 1985, **21**, 815–824.
19. Spang-Thomsen M, Visfeldt J, Nielsen A. Effect of single-dose x irradiation on the growth curves of a human malignant melanoma transplanted into nude mice. *Radiat Res* 1981, **85**, 184–195.
20. Vindeløv LL, Christensen IJ, Keiding N, Spang-Thomsen M, Nissen NI. Long-term storage of samples for flow cytometric DNA analysis. *Cytometry* 1983, **3**, 317–322.
21. Vindeløv LL, Christensen IJ, Nissen NI. A detergent-trypsin method for the preparation of nuclei for flow cytometric DNA analysis. *Cytometry* 1983, **3**, 323–327.
22. Vindeløv LL, Christensen IJ, Nissen NI. Standardization of high-resolution flow cytometric DNA analysis by the simultaneous use of chicken and trout red blood cells as internal reference standards. *Cytometry* 1983, **3**, 328–331.
23. Barlogie B, Göhde W, Johnston DA, Smallwood L, Schumann J, Drewinko B, Freireich EJ. Determination of ploidy and proliferation characteristics of human solid tumors by pulse cytophotometry. *Cancer Res* 1978, **38**, 3333–3339.
24. Christensen IJ, Hartmann NR, Keiding N, Larsen JK, Noer H, Vindeløv L. Statistical analysis of DNA distributions from cell populations with partial synchrony. In: Lutz D, ed. *Pulse-Cytophotometry*. Ghent, European Press Medicon, 1978, Vol. 3, 71–78.
25. Steel GG, Hanes S. The technique of labelled mitoses: analysis by automated curve-fitting. *Cell Tissue Kinet* 1971, **4**, 93–105.
26. Spang-Thomsen M, Vindeløv LL. Changes in proliferation kinetics after x-irradiation of a human malignant melanoma grown in nude mice. *Eur J Cancer Clin Oncol* 1984, **20**, 849–855.
27. Steel GG. Cell loss from experimental tumours. *Cell Tissue Kinet* 1968, **1**, 193–207.
28. Steel GG. Cell loss as a factor in the growth of human tumours. *Eur J Cancer* 1967, **3**, 381–387.
29. Bunn PA, Jr, Carney DN, Gazdar AF, Whang-Peng J, Matthews MJ. Diagnostic and biological implications of flow cytometric DNA content analysis in lung cancer. *Cancer Res* 1983, **43**, 5026–5032.
30. Vindeløv L, Hansen HH, Spang-Thomsen M. Growth characteristics and heterogeneity of small cell carcinoma of the lung. In: *Recent Results in Cancer Research*. Berlin, Heidelberg, Springer, 1985, Vol. 97, 47–54.

31. Barendsen GW. Effectiveness of combined radio and chemotherapy in the treatment of experimental tumours and an evaluation of some factors influencing response. In: *Radiobiological Research and Radiotherapy*. Vienna, International Atomic Energy Agency (STI/PUB/441), 1977, Vol. I, 351–368.
32. McNally NJ, Sheldon PW. The effect of radiation on tumour growth delay, cell survival and cure of the animal using a single tumour system. *Br J Cancer* 1977, **50**, 321–328.
33. Spang-Thomsen M, Vindeløv LL, Nielsen A. Effect of x irradiation on a heterotransplanted human colonic carcinoma before and after a change in the cellular DNA content. *Radiat Res* 1983, **93**, 299–311.
34. Denekamp J. The relationship between the 'cell loss factor' and the immediate response to radiation in animal tumours. *Eur J Cancer* 1972, **8**, 335–340.
35. Jung H. Radiation effects on tumours. In: Broerse JJ, Barendsen GW, Kal HB, van der Kogel AJ, eds. *Radiation Research*. Amsterdam, M. Nijhof, 1983, 427–433.