Acta Oncologica Vol. 30 No. 7 1991

FROM THE RADIOBIOLOGY RESEARCH GROUP OF THE NUCLEAR ENERGY BOARD, THE DEPARTMENT OF SURGERY, ST JAMES'S HOSPITAL, AND THE PHYSICS DEPARTMENT, DUBLIN INSTITUTE OF TECHNOLOGY, DUBLIN, IRELAND.

PROLIFERATION OF NORMAL AND MALIGNANT HUMAN EPITHELIAL CELLS POST IRRADIATION

C. MOTHERSILL, C. B. SEYMOUR, A. O'BRIEN and T. HENNESSY

Abstract

Fragments of human oesophageal mucosa, urothelium, squamous and adenocarcinoma of the oesophagus and carcinoma of the bladder have been plated in culture and irradiated. The cells growing from the explanted tissues have then been studied for four weeks post irradiation to assess the overall rate of growth from the irradiated explants and the fraction of proliferating cells. The results show that when using cell number as an endpoint it is possible to derive growth curves from this type of data which permit a doubling time to be obtained for the cell population surviving different doses. In an attempt to determine the proliferating fraction of the cell population, cultures were labelled at appropriate intervals with tritiated thymidine and were also stained with Ki-67 antiproliferating antigen. The results show an interesting relationship between the dose response obtained for cell labelling with tritiated thymidine and area of cellular outgrowth. Ki-67 staining when used carefully and analysed as described was a useful indicator of proliferating cells. The results provide a means of determining the post irradiation growth potential of fragments of tissue from human organs and may be important for determined overall response of the tumour bulk to proposed treatment.

Key words: Human cell culture, cell proliferation, repopulation post irradiation.

Radiation seldom kills 100% of cells in a tumour, partly because the dose needed would be too high for the rest of the body to bear but also because of the heterogeneity of tumour cell populations with respect to their radiation sensitivity (1, 2). Often therefore radiotherapy is aimed at delaying tumour growth or reducing tumour bulk, so-called palliative therapy, as opposed to the radical type of therapy which seeks to eradicate small tumours entirely.

Due to the difficulty of assessing tumour response to radiation in a scientific and controlled way, many workers have attempted to establish the response of different tumours in vitro (3, 4). Approaches have mainly concentrated on the use of established tumour cell lines (5, 6) or fresh tumour digests (7–9) and the endpoint used has usually been clonogenic survival—i.e. the number of treated cells which survive the dose and can subsequently divide at least 5–6 times (10). Occasionally other approaches have been used such as various biochemical assays which look at nucleotide precursor incorporation (11) which gives good correlation with clinical outcome. Incorporation of ^{3}H thymidine is measured following exposure of the cultures to various drugs which interfere with DNA synthesis. The advantage of this type of assay is that it allows the proliferation kinetics of the actual tumour cells to be included in the assay result. A fast version of this type of assay is the short-term predictive test developed by Volm et al., in 1979 (12), where a radioactive precursor of DNA is fed to tumour cell suspensions for 1 h and the binding of the radioactivity is then measured.

The problem with these tests is that they are based either on immediate cytocidal effects of treatments on cells and do not allow for long-term effects on reproductive survival to be measured or they use the much more rigourous endpoint of clonogenic survival in agar which attempts to measure only the stem cell response to the treatment. The main problem with the stem cell assays, apart from technical difficulty, is that they assume that all and only stem cells grow in agar. A partial solution to the problem was developed by Weisenthal et al. (13) who designed an assay which measured total cycling cell kill as opposed to stem cell kill in the tumour. The endpoint used was the ratio of dead to live cells detected from tumour cells four days

Submitted 21 August 1990.
Accepted for publication 16 March 1991.

after exposure to the drug compared to an internal Duck red blood cell standard. Live cells were detected using a modified dye exclusion assay and correlated with the labelling index, using autoradiography following ^3H thymidine uptake. Results give success rates for predictive testing in the region of 65–90%.

This assay again suffers from the problem that the long-term outcome from a drug cannot be predicted, only short-term response. The above techniques undoubtedly provide information on tumour cell dose—response rate in vitro but their relevance to the in vivo behaviour of a tumour is questionable. Tumour cell lines tend to be highly selected sub populations of the tumour which have the ability to grow indefinitely in culture. Cells which grow in soft agar from fresh tumour digests generally have an extremely low cloning efficiency and it is dubious to refer to them as stem cells. It is also very difficult to establish their origin, since the tumour digest contains several different types of cells, including fibroblasts, endothelial cells, normal epithelial cells and blood cells, any or all of which can theoretically grow in agar (14, 15).

A further problem with the clonogenic approach is the fact that information concerning the response of non-clonogenic cells is lost. Also such questions as the contribution of fibroblast or vascular radiosensitivity to the problem cannot be addressed. The relative effects on normal and tumour cells cannot be assessed and the question of the time taken to repopulate can only be approached if it is assumed that every stem cell and only stem cells grow in soft agar. Tumours which will not culture or clone cannot be evaluated at all. Clearly a different approach is needed.

This paper sets out to look at the problems outlined above using fresh explant cultures according to the methods previously outlined (16–18).

This technique allows pieces of normal and tumour tissues from various areas of the tumour to be plated and their radiation response evaluated. The technique has been shown to produce cultures of cells which grow from the explant (16) and this growth can be monitored at suitable time intervals using autoradiographic techniques.

Material and Methods

Tumours were obtained during routine surgical operations. Normal tissue was obtained from the parts of the resection specimen remote from the tumour and were confirmed as histologically normal by the hospital pathology department. Tissue samples were collected in complete growth medium (16) and rushed to the tissue culture laboratory. There they were chopped into small pieces 2–3 mm^2 and incubated in balanced salt solution containing 0.25% trypsin and 10 mg/ml collagenase for 30 min at 37°C. The pieces (one per flask) were then plated in gridded tissue culture flasks (Lux) with a 25 cm^2 growth area, in 2 ml growth medium. The cultures were incubated at 37°C in an atmosphere of humidified CO_2 in air for two days prior to treatment. This allowed explants to attach firmly to the flask before being disturbed and allowed elimination of any explants giving no growth or not attaching. Irradiation was performed 48 h after plating using a cobalt-60 teletherapy unit delivering 1 Gy/min at 60 cm flask-source distance.

Cultures were incubated without medium change because of the adverse effect of medium changes on growth of epithelial cells (16). The degree of growth inhibition was assessed at various times by measuring the total area of growth in each flask and expressing the area as a percentage of the untreated control growth ± standard error of the mean (SEM). At least six flasks were measured per experimental point and experiments were repeated three times using tissue from different patients. The figures represent the pooled data from these patients except where otherwise stated. The success rate for culture of explants was in the region of 90%. Within sample variation was reduced by screening samples on day 2 eliminating any which had not attached. The mean variation on control samples was in the region of ± 15% (range ± 6–20%). The average outgrowth area for controls after two weeks was 160 mm^2 ± 34 (SEM) for normal tissues and 138 mm^2 ± 32 for tumour tissues, based on the pooled results of three experiments, performed on three different patients. Population doubling times were calculated by drawing tangents to relevant parts of the growth curve.

Autoradiography. Cultures were labelled one week after explantation with ^3H thymidine (10 μCi/ml) for one hour, after which the cultures were carefully washed before being coated with liquid emulsion (Ilford) and stored for two weeks to allow development of grains.

Immunocytochemistry. To ensure that cultures were composed mainly of epithelial cells which where proliferating immunocytochemical analysis was routinely performed using cytokeratin and Ki-67 antibodies (Fig. 1) Cultures were fixed in situ on plastic flasks using a solution of acetic acid: methanol (1 : 3) and air dried. They were rehydrated before staining by three washings in phosphate-buffered saline (PBS). 20 μl of a general mouse antihuman-cytokeratin antibody (anti-type II from Amersham International) was added to the culture and incubated for 40–45 min at room temperature in a humidified chamber. After incubation the slides were washed well in PBS. The second antibody, also supplied for 40–45 min at room temperature, was an anti-mouse immunoglobulin monoclonal labelled with fluorescin. After further washings in PBS the cultures were rinsed briefly in 1 mol/l Tris HCl buffer (pH 9.0) and mounted in Aquamount before viewing with a fluorescent microscope. Positive and negative controls were carried through the whole procedure every time it was done. Proliferating cells were demonstrated as above using Ki-67 (DAKO) as the primary antibody (19).

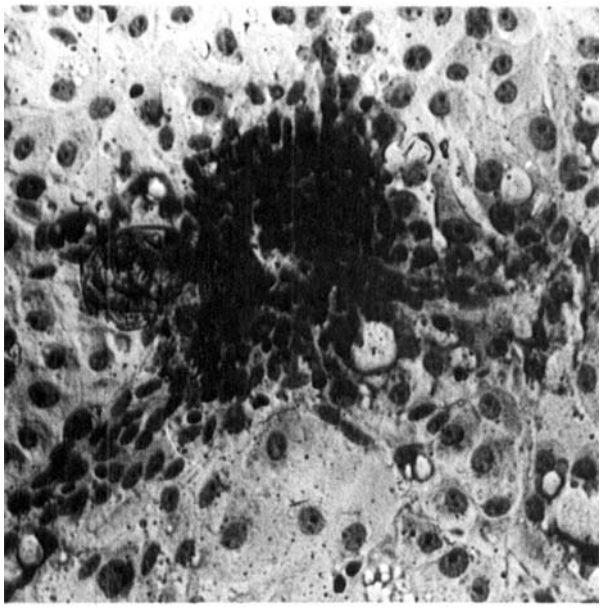

Fig. 1. Dark staining (Ki-67 positive) focus on a monolayer of urothelial cells × 100.

Results

In Fig. 2 data from several experiments involving normal and tumour bladder mucosa or oesophageal mucosa have been pooled to produce average dose response curves for cultures fixed two weeks after irradiation. These can be seen to resemble conventional clonogenic survival curves for mammalian cells and show as previously found by this group (16) that explants from the tumour are more resistant to radiation than explants from normal surrounding tissue. When measurements are only made at a single time point post radiation treatment there is always the possibility that differences in postirradiation growth rates could obscure the true radiation response of the cells. That this possibility is real is shown in Fig. 3, where outgrowth areas obtained from normal oesophageal explants at 1, 2, 3 and 4 weeks post irradiation (5 Gy) are compared with unirradiated controls. It is apparent that the control areas increase rapidly during the first week while the irradiated areas increase more slowly initially but then reach the control size by four weeks after irradiation. Since it is highly likely that tumour and normal tissues will grow and respond differently following irradiation, it was decided to plot growth curves for normal and tumour explants over a four-week period for cultures irradiated to a range of doses. The results (Fig. 4a and b) show apparently very little difference in the growth rates although the effect of radiation doses relative to the control may be different. Replotting of the data to try to show this involved normalising each irradiation point to the control for that time. This reveals that growth rates can be derived from these data by analysing the slopes of the growth curves for irradiated cells as they approach the normalised control

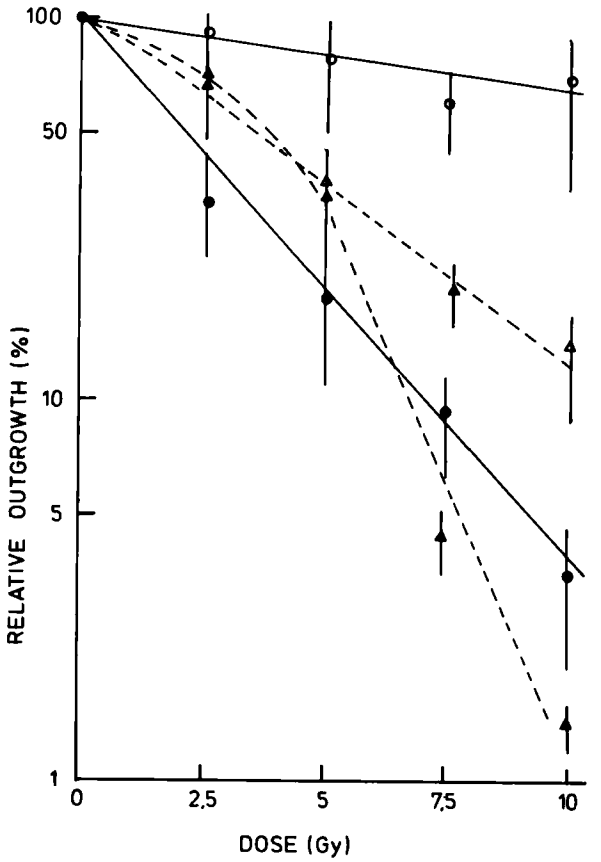

Fig. 2. Dose—response curves obtained for tissue cultures derived for normal (▲, ●,) and tumour (△, ○) tissues from the oesophagus (○—○) and bladder (△---△).

(Fig. 5). The data show that tumour and normal tissues do respond quite differently. In the tumour the radiation-induced reduction in growth area is not apparent for nearly 12 days and reaches a maximum at about 20 days after irradiation. The rate of growth then increases sharply and the rate of increase is greatest in the cultures receiving the highest radiation doses. In contrast, the normal tissue surrounding the tumour shows a much earlier acute response and recovers much earlier as well.

The data for repopulation rates derived from Figs 4 and 5 are summarised in Table 1, which shows the maximum growth rate detected for each tissue.

Due to the difficulty of obtaining sufficient bladder tumour tissues, results using this system could only be obtained for normal urothelium. The data (Fig. 6) show that there is an acute response to radiation which is maximal at about five days after exposure. After this time there is a rapid increase in the proliferation rate which is just as fast in cultures which received 7.5 Gy as in those which received 5 or 2.5 Gy. In cultures receiving 2.5 Gy or 5 Gy the total cell numbers observed eventually exceed those detected in control cultures. This data suggests that the technique works well for this system and that it is also possible to generate repopulation data using bladder explants.

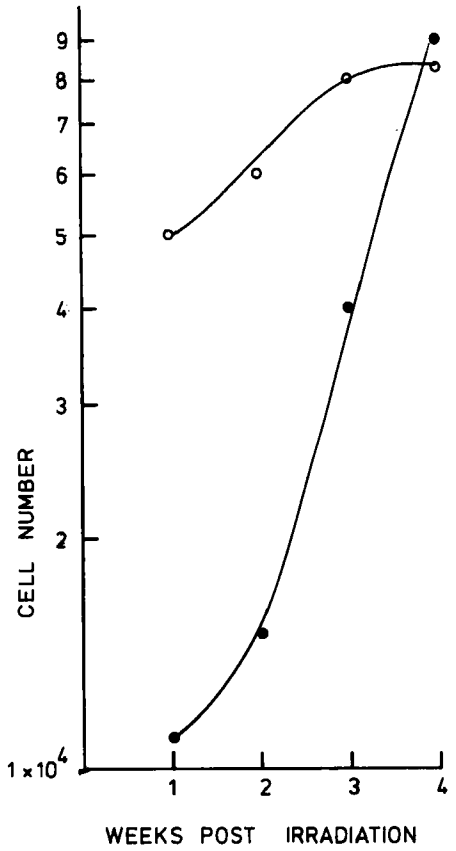

Fig. 3. Increase in cell number with time in control normal oesophageal cultures (○) and after irradiation to 5 Gy (●).

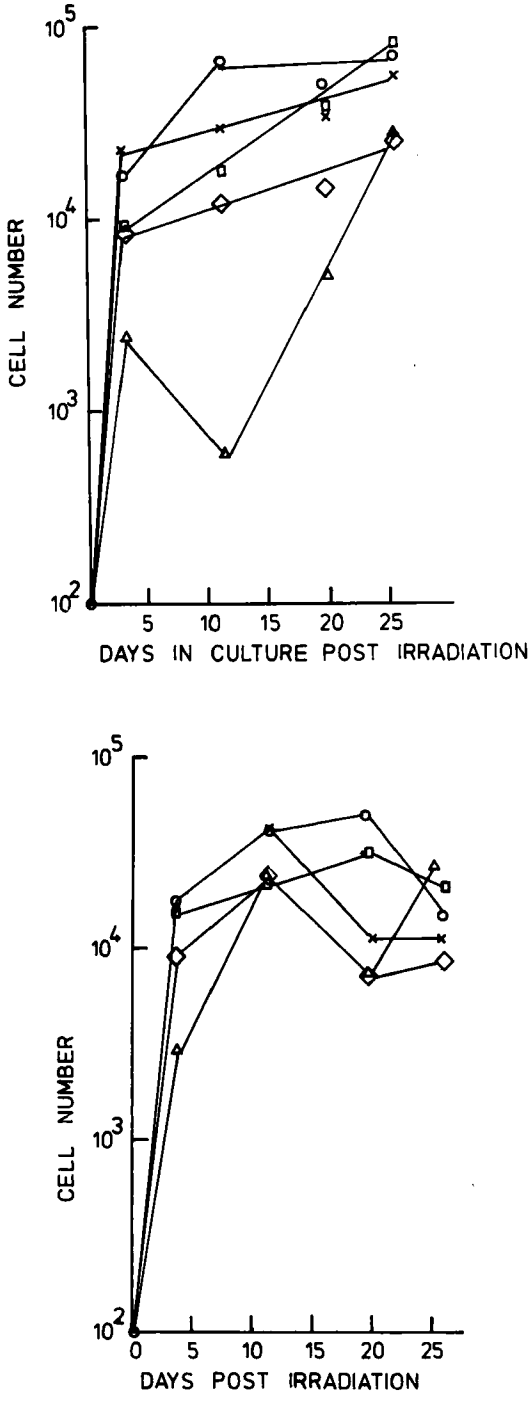

Fig. 4. a) Increase in cell number with time after irradiation in normal oesophageal cultures; ○ = control, x = 2.5 Gy, □ = 5 Gy, ◇ = 7.5 Gy, △ = 10 Gy. b) Ditto for tumour oesophageal cultures.

The technique as described uses a rather crude estimation of cellular outgrowth to determine radiation response. In an attempt to refine this and to reduce variability between duplicate samples cultures in some experiments were exposed to tritiated thymidine followed by autoradiography or to Ki-67 antigen to detect proliferating or DNA synthesising cells in the outgrowths. Results for percentages of ^3H labelled cells per culture and per unit area are shown in Table 2 for normal and tumour oesophageal cultures. These indicate that both the actual size of the outgrowth and the percentage of cells labelled with tritiated thymidine per unit area show a similar relationship to dose although after four weeks in culture, labelling in normal cells has fallen considerably while in the tumour cultures the labelling is maintained. If the data in Table 2 is plotted to show the relationship between total cell number and dose of radiation each week, the results (Fig. 7) show that the tumour cell survival is severely affected initially by the dose of radiation but then recovers rapidly relative to the normal tissue. By week 4 there is a huge diffference between the response of normal and tumour cultures and the number of cells labelled in the tumour cultures remains high irrespective of dose. Table 3 shows similar data where Ki-67 immunocytochemical staining was used to determine the number of proliferating cells in the cultures. The results are not so clear using this method and high numbers of weakly but positively staining cells which appear grossly abnormal are detectable in the cultures.

Bettter results are obtained using Ki-67 if counts are made of the number of dark staining foci of cells

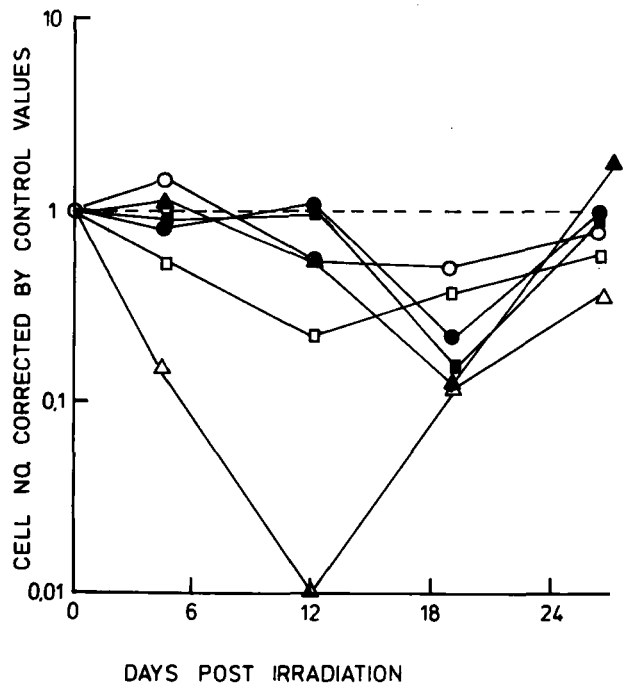

Fig. 5. Cell numbers (from Fig. 4a and b) plotted relative to the control at each time point post irradiation. Normal (open symbols) Tumour (closed symbols) ○, ● = 2.5 Gy; □, ■ = 7.5 Gy; △, ▲ = 10 Gy Dashed line represents unirradiated control.

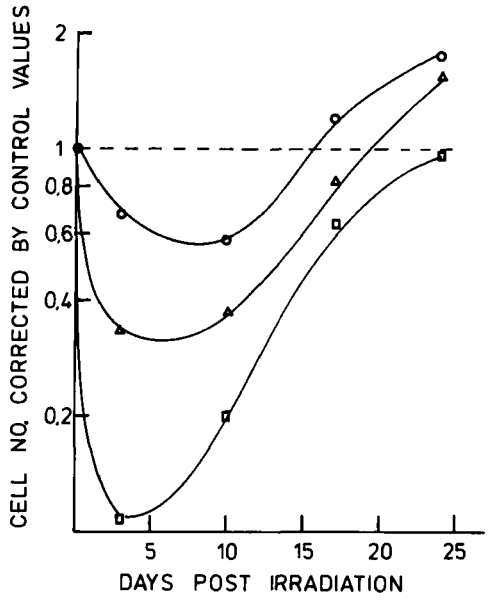

Fig. 6. Cell numbers relative to the control at each time point post irradiation for normal urothelium; ○ = 2.5 Gy, △ = 5 Gy, □ = 7.5 Gy Dashed line represents unirradiated control.

(see 1) per unit area. These can be regarded as proliferating units of cells. Fig. 8 shows results for oesophageal cells stained two weeks after irradiation where the number of proliferating foci per unit area is plotted as a function of dose. Results indicate that in the normal cultures the number of foci per unit area increases with increasing dose. This confirms the data showing increased proliferation after irradiation. In the tumour cultures a similar increase with dose was seen. This measurement should relate well to a measurement of the size of the clonogenic population.

Discussion

The results presented in this paper represent an extension of a technique developed by our group to try to overcome problems associated with the measurement of radiation response in human tissues which are non clonogenic. In earlier papers the cells were proved to be epithelial and to be growing not migrating. Data was obtained for several tissues but survival was usually only measured at a single time. Quite dramatic variations in radiation response were obtained both between tissues and between normal and tumour samples (18, 20). This was thought to be due to difference in intrinsic radiosensitivity but the results presented in this paper strongly suggest that they reflect rather differences in expression time of the damage. Differences in the rate of 'repopulation' or growth of survivor cells after exposure to radiation show that it is possible to obtain not only acute dose—response data but also growth rate information from the primary explant culture model. The acute dose response varies depending on the time after initial exposure when measurements are taken. Careful analysis of the dose response at intervals over 4 weeks shows clearly that growth of the surviving

Table 1

Average population doubling times detected in the period immediately following the maximum response to the radiation dose and the time taken to show the maximum response

Dose (Gy)	Normal		Tumour	
	Doubling time days	Maximum response after (days)	Doubling time days	Maximum response after (days)
2.5	9	15	4	19
5.0	6	12	7	16
7.5	6	12	3.5	19
10.0	1.5	12	1.8	19

Table 2

Total cells (× 10^4) and percentage of ^3H labelled one to four weeks following increasing doses of radiation. N-normal oesophageal mucosa, T = tumour from the same patient

Dose (Gy)	Week 1				Week 2				Week 3				Week 4			
	Total cells		% labelled		Total cells		% labelled		Total cells		% labelled		Total cells		% labelled	
	N	T	N	T	N	T	N	T	N	T	N	T	N	T	N	T
0	7.5	1.0	67	55	10.3	4.5	87	80	12.5	25	85	85	17.0	28	2.5	90
2.5	1.0	0.8	22	40	2.0	4.0	50	75	20.0	18	33	75	10.5	10.5	10	85
5.0	1.5	0.5	7.5	32	1.0	3.5	30	60	9.0	16	3.0	65	8.5	20	19	85
7.5	0.3	0.1	7.0	10	7.2	2.2	7.5	50	0.5	15	2.0	60	0.7	22	5	83
10.0	0.5	0.03	5.0	5	5.0	2.0	6	35	1.5	10	6.0	50	0.5	17	5	75
12.5	0.25	0.01	7.0	0.5	0.3	1.0	1	20	1.5	5	5.0	50	0.01	15	7.5	75
15.0	0.3	0.01	4.5	0.1	1.5	0.5	18	10	1.0	1	10.0	45	0.01	11	15.0	65

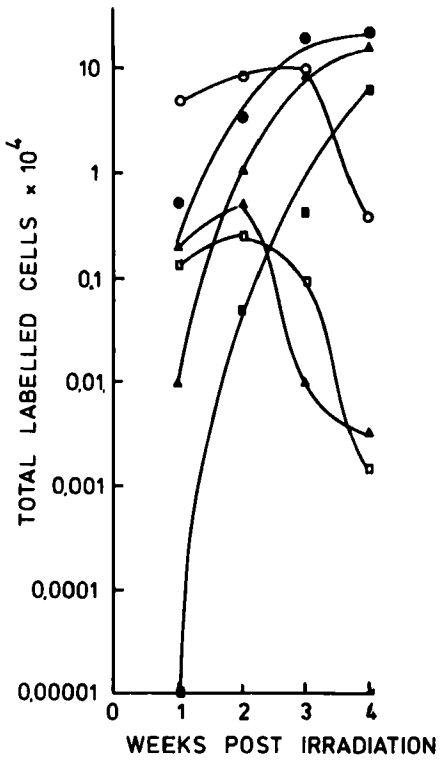

Fig. 7. Total labelled cells as a function of weeks post exposure K 0 Gy (○,●), 7.5 Gy (△,▲) or 15 Gy (□,■). Open symbols = normal oesophageal cultures closed symbols = tumour oesophageal cultures.

cell population or repopulation is taking place with time after irradiation. This is a very interesting feature of the system since it allows the time for the surviving cells to regain the pretreatment cell number and to regain the pretreatment cell growth rate to be determined. In addition, accelerated growth rate can be detected and in certain cases results can be compared to similar data for normal cells. There features give the method several clear advantages over existing in vitro methods of predicting or studying tumour response to therapy.

Looking at the data in Fig. 5 it is apparent that once the cells do start to grow exponentially following the dose, they tend to show a faster growth rate when the radiation dose is high. This can be seen by measuring growth areas and also by calculating labelling indices using autoradiography of ^3H thymidine-labelled cultures. The results are also in agreement with data published by Duchesne & Peacock (21) using clonogenic assay techniques. Cultures tend to reach a saturation cell density at about the same time and this saturation density is dose-dependent and lowest when the dose is highest. These data therefore suggest that a number of different effects are being observed in the cultures. The enhanced growth rate is probably real and could relate to an amplification or activation of processes involved in proliferation in radiation-damaged tissues. This process is well-known to occur in vivo in events such as wound healing or liver regeneration. The occurrence of saturation densities which are dose-dependent may be artifactual since the effect could be due to exhaustion, deficiency or decay of nutrients in the medium. It is also possible that the effect is due to the late expression of lethal damage (reduced probability of division success, which has been observed to occur in a number of cell lines (22, 23). If the latter suggestion is true, the effect could be relevant in vivo.

The data in Fig. 5, showing different responses in cultures from normal and tumour tissues, also indicate the importance of time of measurement when using the explant technique. The data suggest that for this patient the normal cells responded earlier to radiation than tumour cells and that they have begun to repopulate before the damage is expressed by the tumour cells. The patient used for the data shown in Tables 2 and 3 and Figs 7 and 8 has a different pattern of response. The tumour response is maximally expressed soon after irradiation and regrowth is well established by two weeks. If it is possible to validate this assay clinically this type of data could be extremely relevant to treatment planning since it could indicate the optimum time to administer second doses of radiation or

Table 3

Total cells (× 10^4) and percentage of Ki-67 positive cells one to four weeks following increasing doses of radiation. N = Normal oesophageal mucosa T = Tumour from same patient

Dose (Gy)	Week 1				Week 2				Week 3				Week 4			
	Total cells		% positive		Total cells		% positive		Total cells		% positive		Total cells		% positive	
	N	T	N	T	N	T	N	T	N	T	N	T	N	T	N	T
0	1.5	1.5	100	84	4.75	3.5	97.5	85	8.5	8.0	26.00	80	10.0	25	0	100
2.5	1.0	1.3	100	20	2.4	1.0	85.0	80	4.1	0.05	100.0	90	7.0	16.0	10	100
5.0	0.1	0.5	100	100	0.25	0.3	47.5	55	0.5	0.2	55.0	65	2.7	0.3	100	100
10.0	0.01	0.75	100	100	0.15	0.05	90.0	100	0.6	0.01	65.0	50	0.6	0.01	6	100

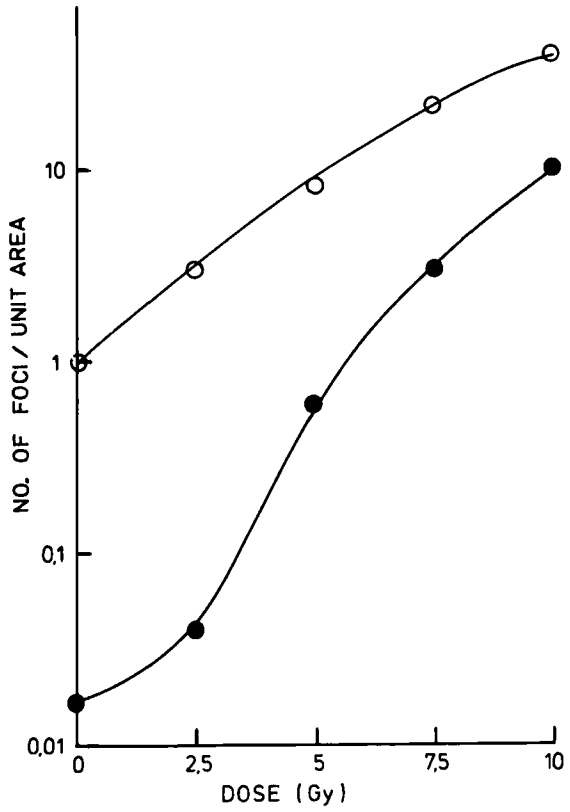

Fig. 8. Number of Ki-67 positive foci per unit area of the culture as a function of radiation dose. ○ = normal cultures, ● = tumour cultures. Measurement taken 3 weeks after exposure to radiation.

other cytotoxic treatment (e.g. give the second treatment when the tumour cells are maximally affected by the first treatment or when proliferation of normal cells is low relative to tumour cells).

A major objection to tests of the type developed by this group and others which look at the response of all the cells in a culture from a tumour is that the important cells are the clonogenic or stem cells and that only information which exclusively concerns stem cell response to a proposed treatment regimen is useful. This idea has lead to the development of tests such as the tumour stem cell assay developed by Hamburger & Salmon (8) or Courtney (9). However, as discussed in the introduction such assays are difficult to perform and rely on the ability of the tumour stem cells to grow in agar, and the technique has a limited success rate for many tumour types (24). For this reason the attempts to detect proliferating units in the cultures were undertaken. Quantification of this endpoint may allow an estimate to be made of the numbers of cells which are capable of repopulating a tumour. The test has the advantage over soft agar assays that a single cell suspension is not required. Therefore the therapeutic agent can be administered while the tumour cells are organised in a solid piece of tissue as occurs in vivo. The proliferating unit endpoint can be measured using ^{3}H thymidine labelling followed by autoradiography or immunocytochemistry, although in a clinical situation the latter would be more practical.

The assay may also allow the problem of tumour heterogenity to be addressed. This is an acknowledged problem in any type of predictive assay and particularly where growth of cells in vitro occurs since selection for aggressive or resistant cell types may take place, as may clonal dominance (1, 2, 25, 26). Using the assay described here a good indication of variation may be obtained from the range of response between individual replicate samples since growth and response in several pieces of the tumour are measured and each piece is relatively large (containing over 8 × 10^6 cells).

In conclusion, the assay described can detect growth of normal and tumour cell response to radiation in terms of growth rate absolute or relative growth of number of surviving proliferating units. The test, if validated, may eventually be useful in planning radiotherapy treatment for patients who have had surgery.

Corresponding author: Dr Carmel Mothersill, Physics Department, Dublin Institute of Technology, Dublin 8, Ireland.

REFERENCES

1. Michelson S, Leith JT, Glicksman AS. A technique to analyse the emerging zonality of heterogenous solid tumors. Cell Tissue Kinet 1987; 20:449–506.
2. Michelson S, Leith JT. Unexpected equilibria resulting from differing growth rates of sub populations within heterogeneous tumours. Mathemat Biosc 1988; 91:119–29.
3. Hofmann V, Berens ME, Martz G, eds. Predictive drug testing on human tumour cells. Berlin, Heidelberg, New York, Tokyo: Springer-Verlag 1984.
4. Peters LJ, Inherent radiosensitivity of tumour and normal tissue cells as a predictor of human tumour response. Radiother Oncol 1990; 17:177–90.
5. Steel GG, Peacock JH. Why are some human tumours more radiosensitive than others? Radiother Oncol 1989; 15:63–72.
6. Fertil B, Malaise EP. Inherent cellular radiosensitivity as a basic concept for human tumour radiotherapy. Int J Radiat Oncol Biol Phys 1981; 7:621–9.
7. Steel GG, Courtenay VD, Peckham MJ. The response to chemotherapy of a variety of human tumour xenografts. Br J Cancer 1983; 47:1–13.
8. Hamburger AW, Salmon SE. Primary bioassay of human tumour stem cells. Science 1977; 197:461–3.
9. Courtney VD. A replenishable soft agar colony assay for human tumour sensitiveity testing. In: Hofmann, Berens, Martz, eds. Predictive drug testing on human tumour cells. Berlin, Heidelberg, New York, Tokyo: Springer-Verlag, 1984:17–34.
10. Puck TT, Marcus PI. Action of x-rays on mammalian cells. J Exp Med 1956; 103:653–66.
11. Sanfilippo O, Daidone MG, Costa A, Canetta R, Silvestrini R. Estimation of differential in vitro sensitivity of non-Hodgkin's lymphomas to anticancer drugs. Eur J Cancer 1981; 17:217–26.
12. Volm M, Waiss K, Kaufmann M, Mattern J. Pretherapeutic detection of tumour resistance and the results of tumour chemotherapy. Eur J Cancer 1979; 15:983–93
13. Weisenthal LM, Marsden JA, Dill PL, Macaluso CK. A novel dye exclusion method for tesing in vitro chemosensitivity of human tumours. Cancer Res 1983; 43:749–54.
14. Freshney RI, Hart E. Clonogenicity of human glia in suspension. Br J Cancer 1982; 46:463–70.
15. Laug WE Tokes ZA, Benedict WF, Sorgente N. Anchorage independent growth and plasimogen activator production by bovine endothelial cells. J Cell Biol 1980; 84:281–93.
16. Mothersill C, Cusack A, Seymour CB. Radiation induced outgrowth inhibition in explant cultures from surgical specimens of five human organs. Br J Radiol 1988; 61:226–30.
17. Mothersill C, Seymour CB, Bonnar J. Effect of radiation and other cytotoxic agents on the growth of cells cultured from normal and tumour tissues from the female genital tract. Gynaecol Oncol 1990; 37:210–8.
18. Seymour CB, Mothersill C, Cusack A, Hennessy TP. (1988). The effect of radiation on the growth of normal and malignant human oesophageal explant cultures pretreated with bleomycin. Br J Radiol 1988; 61:383–7.
19. Gerdes J, Schwab U, Lembe H, Stein BH. (1983). Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. Int J Cancer 1983; 31:13–20.
20. Mothersill C, Cusack A MacDonnell M, Hennessy TP Seymour CB. Differential response of normal and tumour oesophageal explant cultures to radiation. Acta Oncol 1988; 27:275–80.
21. Duchesne GM, Peacock JH. Radiation cell survival and growth delay studies in multicellular spheroids of small-cell ling carcinoma. Int J Radiat Biol 1987; 51:365–75.
22. Seymour CB, Mothersill C, Alper T. High yields of lethal mutations in somatic mammalian cells that survive ionising radiation. Int J Radiat Biol 1986; 53:319–30.
23. Mendonca MS, Kurohara W, Antoniono R, Redpath JL. Plating efficiency as a function of time post-irradiation. Evidence for the delayed expression of lethal mutations. Radiat Res 1989; 119:387–93.
24. Rozencweig M, Hofmann V, Sanders C, Rombaut W, Fruch U, Martz G, In vitro growth of human malignancies in a cloning assay in predictive drug testing on human tumour cells Hofmann, Berens, Martz, eds. Berlin, Heidelberg, New York, Tokyo Springer-Verlag, 1984:1–7.
25. Goldie JH, Coldman AJ. (1979). A mathematic model for relating the drug sensitivity of tumours to their spontaneous mutation rate. Cancer Treat Rep 1979; 63:1727–31.
26. Dexter DL, Leith JT. Tumour heterogeneity and drug resistance. J Clin Oncol 1986; 4:244–57.