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Letter to the Editor

Stromal Damage as a Complication in the Interpretation of Tumour Growth Delay*

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THE RESPONSE of tumours to radiation can be assessed by local control or regrowth *in situ* or by excision assays in which the fraction of clonogenic cells can be determined, either *in vitro* or by reinjection into other recipient mice (for reviews see [1, 2]). It is tempting to suppose that the response of a tumour left *in situ* is determined mainly by the surviving fraction of clonogenic malignant cells. However, repair of potentially lethal damage, changes in the physiological milieu, host immune reactions and radiation damage to the stroma may all influence the clonogenic probability for cells which survive the initial insult [3, 4]. In this communication we are concerned with the effect that stromal damage may have on the shapes of dose–response curves obtained by measuring regrowth delay *in situ*.

Figure 1 shows typical dose–response curves for tumours irradiated in air breathing mice. The top two panels show the biphasic response that is usually attributed to the existence of a hypoxic subpopulation in the tumour [5–7] which dominates the response above the break point. The bottom two panels show two examples where there is no break into two components. This could indicate that no hypoxic cells were present, or that they represented such a large proportion of the population that they dominated the response at all dose levels. Of 43 sets of dose–response curves that we have analysed for 19 different tumour types, 17 showed a definite break; 12 showed a probable break; 6 showed only a hint of a break; and 8 showed absolutely no evidence of a break.

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All these dose–response curves result from irradiated tumour cells regrowing in an irradiated stroma. It has long been recognised that stromal damage can itself lead to a slowing of tumour growth. This 'tumour bed effect' (TBE) has been investigated separately from tumour cell damage by implanting untreated tumours into normal or previously irradiated sites (e.g. [8–13]). These studies have generally shown that stromal damage is dose-dependent, with a plateau being reached at doses between 10 and 30 Gy (Fig. 2, Table 1). Growth rate reductions for tumours regrowing after irradiation *in situ* show a similar dose-dependency (Fig. 2, Table 1). This 'break point' for stromal injury usually lies within the same dose range as the break towards a more resistant response that is seen in regrowth delay experiments (e.g. in Fig. 1). We have therefore considered here the possible influence of stromal damage on the shapes of regrowth delay dose–response curves to see whether this factor rather than hypoxia could be the cause of biphasic curves.

Figure 3 shows schematically the influence of a TBE on the growth delay curves. The top panels illustrate TBE curves showing (a) a linear dependence on dose; (b) an initial linear dependence followed by a plateau; and (c) a threshold below which no TBE is demonstrable and above which the response is linear.

The middle panels illustrate the effect this TBE dose-dependency would have on a linear dose–response curve for regrowth delay, e.g. one resulting from the killing of a homogeneously oxygenated population of tumour cells (dashed line). In panel (d) the TBE serves to increase the steepness of the line and makes it curve upwards;

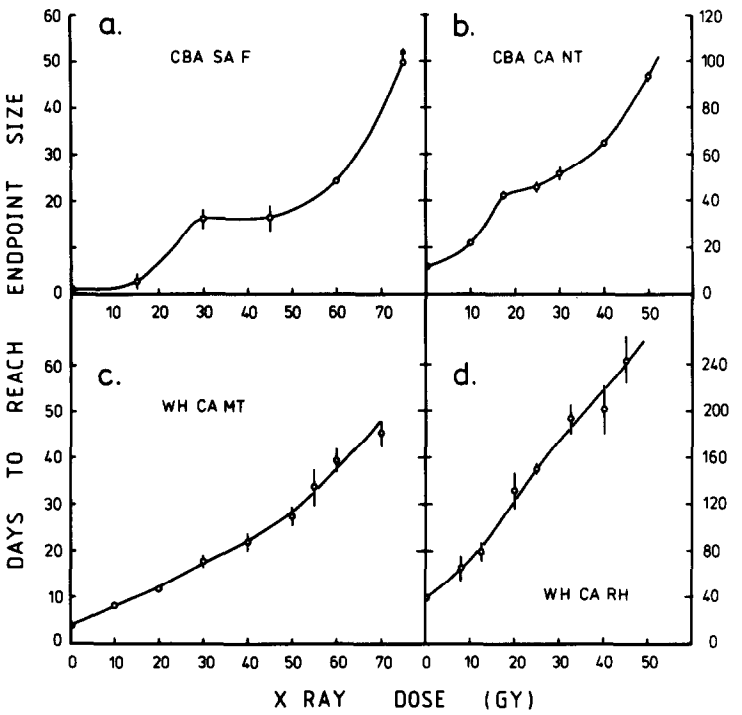

Fig. 1. Time to regrow to a fixed size after irradiation for four different types of murine tumour. (a) Regrowth to R + 2 mm *for an anaplastic sarcoma. A distinct break is seen in the curve at* 30 Gy *(data from* [33]*); (b) regrowth to* R + 4.5 mm *for a mammary carcinoma. A break point is observed at* 16 Gy *(data from* [34]*); (c) regrowth to* R + 4.5 mm *for an anaplastic tumour of mammary origin. No break point exists (data from* [6]*); (d) regrowth to* R + 4.5 mm *for slow-growing mammary carcinoma. No definite break point is indicated (data from* [34]*).* R *is the tumour size at irradiation.*

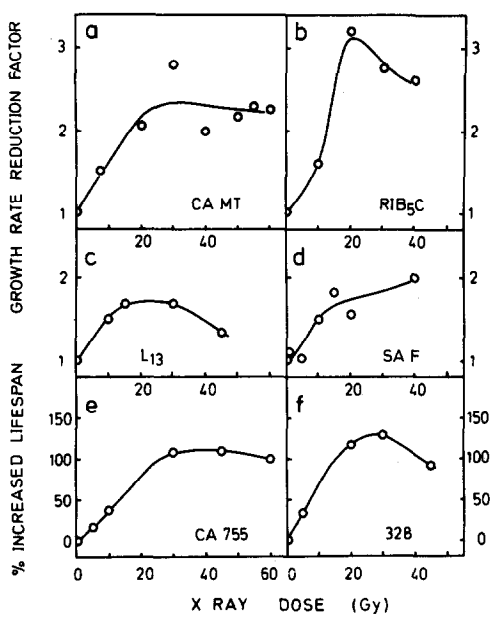

Fig. 2. Examples of stromal damage for 6 different experimental tumours. Panels (a and b) represent growth rate alterations for tumours recurring after irradiation in situ. *Panels (c–e) show TBE data (untreated tumours implanted into pre-irradiated sites), assessed either by tumour size measurements (c and d) or from the time taken to reach a lethal size (e and f). The magnitude of TBE increases with increasing dose, reaching a peak or plateau value at* 15–30 Gy. *Data from (a)* [26]; *(b)* [32]; *(c)* [8]; *(d)* [11]; *(e)* [10]; *and (f)* [10].

the overall response in this case results from 2 dose-dependent processes and hence is proportional to dose squared. Panels (e) and (f) show the influence of the biphasic dose–responses for TBE shown in (b) and (c). The straight line response for tumour cell kill becomes transformed into a biphasic curve, which is curvilinear in the dose region where the TBE increases with X-ray dose. This may give rise to an initial steep portion followed by a more resistant tail (panel e), or an initial straight portion which becomes concave upwards at higher doses (panel f).

The lower panels show the influence of the same three modes of TBE dose-dependence on a biphasic curve resulting from the killing of a mixed population of sensitive and resistant tumour cells. In panel (g) both portions of the dashed curve are steepened and made curvilinear, but the break-point dose is not altered. In panel (h) the biphasic nature of the tumour cell kill curve has been exaggerated by the superposition of a TBE break occurring at the same dose. If the break point for TBE occurred at a higher or lower dose it would introduce a second kink into the growth delay curve, making it triphasic. However, experimental scatter might make it difficult to detect the kinks so that a gradual curve could

Table 1. Studies on the dose-dependence of stromal damage in tumours

Tumour	Reference	Assay type*	Tumour bed effect: Threshold dose (Gy)	Tumour bed effect: Plateau dose (Gy)	Max. dose tested (Gy)
SA FA	[23]	A	5	10	25
SA F	[11]	A	—	15	40
CA NT	[13, 23]	A	—	20	40
L 13	[8]	B	—	15	45
CA 755	[10]	B	—	30	60
Tum. 328	[10]	B	—	20	45
TAK. SA	[24]	C	—	30	45
H.P. MEL	[25]	C	—	—	30
AT 7	[25]	C	—	—	35
ADENO CA. 284	[25]	C	—	—	40
AT 17	[25]	C	(No TBE)	—	50
SSK 2	[25]	C	—	—	40
TVC	[25]	C	—	12	50
CA MT	[26]	C	—	30	60
CA NT	Hill (unpublished data)	C	—	20	40
CA SQD	Hill (unpublished data)	C	—	15	35
C3H BA MAM.CA	[27]	C	—	30	45
C3H(Z) MAM.CA	[9]	C	—	—	60
H-4-II-E	[28]	C	—	—	30
OSTEO SA XENO.	[29]	C	—	—	20
R-1	[30]	C	—	—	40
R-1/LBL	[31]	C	—	25	60
RIB5C	[32]	C	—	20	40
SA S	Terry (unpublished data)	C	10	—	30
RIB_5	[5]	C	—	20	45

*A, untreated tumours implanted into irradiated sites; assessment by growth rate reduction; B, untreated tumours implanted into irradiated sites; assessment by increased lifespan; C, tumours irradiated *in situ*; assessment by growth rate reduction of recurrent tumours.

result. In panel (i) the biphasic dose-response curve has been straightened by the opposing effects of the two non-linear dose-dependencies.

Thus it can be seen from Fig. 3 that a dose-dependent stromal effect could lead to a steepening of dose-response curves, or it could either introduce a biphasic appearance into a uniform response or mask the existence of a mixed population. These examples serve to illustrate that tumour regrowth curves can be complicated by stromal injury, making it difficult to derive conclusions about the radiosensitivity of the constituent tumour cells from the shapes of these curves. Nevertheless, regrowth delay is a useful assay for comparing radiomodifiers, or different fractionation schedules, in a single tumour system. It has generally been found to correlate well with local tumour control, as would be expected at high doses since local control represents an infinite growth delay [14]. The growth delay assay will most closely correlate with a cell survival assay when (a) there is little or no TBE, or (b) a low end-point size is chosen to minimize the effects of a significant TBE. The latter condition is most easily satisfied with rapidly shrinking tumours in which any 'slow shrinkage artefact' [4] is minimal.

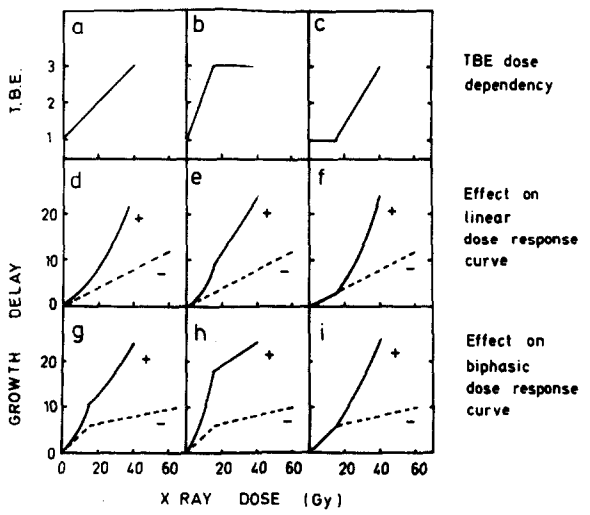

Fig. 3. Schematic representation of the influence of different TBE dose-response relationships (top row) on the shape of growth delay dose-response curves for uniform cell sensitivity (middle row) or for mixed cell sensitivity (lower row). The TBE can steepen curves, introduce apparent breaks or obscure existing breaks. − = dose response without influence of TBE; + = dose response with influence of TBE.

Slower regrowth rates after treatment in comparison with untreated tumours can have a major influence on the response of tumours after X-irradiation, in contrast to hyperthermia, where no such growth rate reductions are seen [15, 16]. Slower regrowth rates of tumours have been

observed after treatment with certain chemotherapeutic agents, e.g. bleomycin, acinomycin D, cyclophosphamide [17]. The correlation between growth delay and clonogenic cell survival after chemotherapy is different for different types of drug. Because chemotherapy is a systemic treatment it is not possible to attribute these effects solely to local stromal injury. Changes in the cellular proliferation rates, cytostatic effects, potentially lethal damage repair, the host immune response and disaggregation artefacts may all contribute to these differences [18, 19].

Providing the experimenter is aware of the limitations of the assays, misinterpretation can be avoided. Additional analyses of regrowth data, using growth rate as well as growth delay, can even provide extra information about the tumour damage relative to the damage in the supporting stromal tissue [20, 21]. As far as deductions about subpopulations are concerned, Fig. 3 shows that it would be dangerous to assume that a resistant subpopulation existed simply on the basis of a biphasic curve. Equally, a monophasic curve need not imply a uniform sensitivity of all tumour cells. For radiobiological experiments the deduction and quantification of hypoxic fractions must be based on a comparison of dose–response curves from unclamped tumours in air-breathing mice with those made uniformly resistant by occluding the blood supply, or with those after treatment with hyperbaric oxygen or radiosensitizers [5, 7, 22].

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