Cell death (apoptosis) in the mouse small intestine after low doses: effects of dose-rate, 14.7 MeV neutrons, and 600 MeV (maximum energy) neutrons

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The production of dead (apoptotic) cells by low doses of γ -rays was independent of dose-rate between 0.27 and 450 cGy per min. The r.b.e. for doses of 14.7 MeV neutrons between 1 and 15 cGy was about 4, and for neutrons generated by bombarding a beryllium target with 600 MeV protons the r.b.e. was about 2.7. The dose-incidence curves for all three radiation types reached a plateau at about 3-4 dead cells per crypt section, and this occurred at about 20-40 cGy of γ -rays. These curves are compatible with exponential survival of the cell population at risk (D_0 of 24 cGy for γ -rays, 6 cGy for 14.7 MeV neutrons and 9 cGy for 600 MeV neutrons). Since the dose-response is exponential there is no indication of much higher r.b.e. values at very low doses, a point of concern in radiation protection. The spatial distribution of dead cells in the crypt was similar after doses of γ -rays or neutrons, indicating that the same population of target cells was affected in both cases.

1. Introduction

Cell death can be recognized either by the loss of the reproductive capacity of the cell or by the morphological appearance of a cell undergoing the classical sequence of events which is characterized by pycnosis, karyorrhexis, and phagocytosis and digestion of cellular debris. This whole sequence of events has been called apoptosis (Kerr *et al.* 1972), and apoptotic bodies can be recognized and scored at any of the above mentioned stages, including the terminal stage where groups of engulfed cellular fragments are visible in neighbouring cells. Recently, the dose-incidence relationship for apoptosis has been established for low LET radiation in the mouse intestinal crypt (Potten 1977) and for a similar process in the pericryptal fibroblast sheath (Neal and Potten 1981). Only a few cells in the tissue show this rapid death after radiation. The possible reasons for this and its significance have been discussed elsewhere (Potten and Hendry 1982, Hendry and Potten 1982) but they are not fully understood.

In view of the current use of fast neutrons in radiotherapy and in nuclear physics, and the lack of knowledge of the appropriate r.b.e. factors to use for radiation protection purposes, the dose-incidence curves for apoptosis have now been measured using 14.7 MeV (d + T) neutrons and neutrons generated by bombarding beryllium with 600 MeV protons. As the latter beam was of low intensity, the effects

of low dose-rates of γ -rays and 14.7 MeV neutrons were also investigated including a measurement of the time course of apoptosis induction after these protracted exposures.

2. Materials and methods

Male B6D2F₁ (Pat) mice were used at an age of 10–12 weeks. They were irradiated to the whole-body, with one of the following beams:

- (a) ^{60}Co γ -rays at 0.27, 0.53 or 82 cGy per min.
- (b) ^{137}Cs γ -rays at 450 cGy per min.
- (c) 300 kVp X-rays at 60 cGy per min (h.v.l. = 2.3 mm Cu).
- (d) A collimated beam of 14.7 MeV neutrons given at 0.25, 0.5 or 25 cGy per min, and generated using the (d + T) reaction in an experimental radiotherapy unit at Manchester (U.K.) as described previously (Greene and Thomas 1968).
- (e) Neutrons generated at 600 MeV (p(600) + Be) and given at a dose-rate between 0.1 and 0.3 cGy per min using the synchrocyclotron facility at CERN (Geneva, Switzerland).

The irradiations (a) to (d) (above) were performed at Manchester and irradiations (e) and in part (a) were performed at CERN. In both cases the absorbed dose was determined using tissue-equivalent ionization chambers (Greene 1971, Baarli *et al.* 1978). Neutron doses are quoted as total doses including γ contamination. The latter was estimated to be 10–20 per cent at Manchester and about 10 per cent at CERN. The data published in Potten (1977) are included as part of the data for irradiations (b) and (c).

Groups of three mice were irradiated at each dose, and one group of three was killed at each time thereafter. The small intestine was fixed, and segments of ileum were embedded in wax, sectioned at 5 μm , and stained with haematoxylin and eosin. Sections were cut longitudinally through the crypt so that a lumen, some Paneth cells and at least 17 nuclei along each side could be observed. Forty such crypts from each mouse were examined at $\times 500$ magnification for the presence of apoptotic bodies, each of a size representing the fragments from one cell. In some experiments the distribution of apoptotic bodies was assessed by mapping the cell position of the apoptotic bodies relative to the crypt base. The mean number of bodies observed per crypt section was plotted against dose. The average standard error on each value was about 8 per cent of the mean. The data have been also presented in terms of cell survival, by expressing the number of cells which did not die via apoptosis ($N_M - N_D$) as a fraction of those at risk ($N_M - N_C$) i.e. surviving fraction = $(N_M - N_D)/(N_M - N_C)$, where N_M is the maximum number of apoptotic bodies observed after high doses, N_D is the number observed after a dose D , and N_C represents the value determined in control animals.

3. Results

The time course of the appearance of apoptotic bodies is shown in figure 1 after various radiations, doses and dose-rates. After 36 cGy of ^{137}Cs γ -rays (450 cGy/min), the number of apoptoses per crypt section rose from the control value of about 0.25, to a value between 2.5 and 4 at 3 to 6 hours and thereafter declined (curve A). After a lower dose, 22 cGy (curve C), the number rose to a lower

Figure 1. Time course of the appearance and disappearance of apoptotic bodies in crypt sections. All irradiations in Manchester. Curve A and (○), after 36 cGy ^{137}Cs γ -rays at 450 cGy per min. Curve B and (□), after 36 cGy ^{60}Co γ -rays at 0.27 cGy per min. Representative standard errors are shown on curve B. Curve C and (●), after 22 cGy ^{137}Cs γ -rays at 450 cGy per min. Curve D and (△), after 5 cGy ^{60}Co γ -rays at 0.27 cGy per min. Note break in time scale between 12 and 24 hours. All times measured from the end of irradiation.

peak but the time course was about the same. Lower doses delivered at a lower dose-rate (curve D), where because of the small doses delivered the exposure times were quite short, showed a similar pattern. However, as expected, after 36 cGy γ -rays delivered at 0.27 cGy per min, where the exposure time was more than 2 hours, the subsequent time course was advanced (curve B). After 20 cGy neutrons (600 MeV) the number of apoptotic bodies per crypt section at 0, 1, 2, 3, 4 and 6 hours was respectively 1.9, 2.1, 3.3, 1.8, 2.2 and 1.6. It is unlikely that the higher value at 2 hours represents a true peak value because of the broad time course demonstrated by the more comprehensive curves shown in figure 1, especially curve B using the same low dose-rate. It is possible that scatter in the data accounts partially for this higher value. As it is not clear whether the start, middle or end of the irradiation period at low dose-rates should be taken as time zero for the time course, it was decided to use 3 hours after the end of the irradiation as the sampling time for all exposures. This could underestimate the damage if the incidence at 2 hours was truly higher than at 3 hours, and if a single sampling time gives a lower mean value for the sum of separate peak values accumulated over a 2 hour exposure time for the higher doses. However, both of these effects would produce a lower plateau value after high neutron doses compared to after γ -rays at high dose-rate and this was not observed.

The dose-incidence curve for apoptoses using various dose-rates of low LET radiation is shown in figure 2. A logarithmic ordinate is used so that standard errors,

Figure 2. Incidence of apoptotic bodies in crypt sections at 3 hours after various doses of low LET radiation. (○), ^{137}Cs γ -rays at 450 cGy per min (Manchester). (◇), ^{60}Co γ -rays at 82 cGy per min (Manchester). (△), ^{60}Co γ -rays at 0.53 cGy per min (Manchester). (▽), ^{60}Co γ -rays at 0.53 cGy per min (CERN). (□), ^{60}Co γ -rays at 0.27 cGy per min (Manchester). (■), 300 kVp X-rays at 60 cGy per min (Manchester). The curve is based on the exponential line in figure 3.

when expressed as a fraction of the mean, stay approximately constant. A logarithmic abscissa is used to display adequately the whole range of doses. The incidence of apoptoses was above control values after doses of 1.5 cGy or greater, whereas between 30 and 1200 cGy the incidence tended to reach a plateau at a value between two and five apoptoses per crypt section, with a mean value between three and four. There was no significant effect of dose-rate between 0.27 and 450 cGy per min, and the data from the two γ -ray experiments carried out at Manchester and at CERN, using the same dose-rate, were very similar. The deduced survival curve is shown in figure 3. This is expressed in terms of S or $(N_M - N_D)/(N_M - N_C)$, because the value of D_0 is critically dependent on the number of cells at risk (N_M). For example, the D_0 is about 12 cGy if N_M is equal to 3, whereas it is about 24 cGy if N_M is equal to 4, and about 33 cGy if N_M is equal to 5. Using four cells at risk per crypt section, the line was drawn by eye (and checked using a computer program (Gilbert 1969)). This line was transformed using the expression for S reported above to get the interpolated values of N_D which were then drawn on figure 2. The observations in figure 3 correspond to those in about the first decade of survival when N_M is equal to 4, as in the second decade the errors on the values, which are associated with the difference between two numbers differing by less than 10 per cent, become very large.

The exponential line would tend to have a small shoulder if three cells were chosen to be at risk per crypt section. This is because changes in N_M do not reflect precise dose-modification. When N_M is changed from N_1 to N_2 , and D_0 is changed correspondingly from D_1 to D_2 , then for an equal number of dead cells, $N_1\{1 - \exp(-D/D_1)\}$ must equal $N_2\{1 - \exp(-D/D_2)\}$. Thus the relationship between N_M and the deduced D_0 is partially dependent on the dose. This is discussed in detail elsewhere (Hendry and Potten 1982).

Figure 3. Deduced survival curve for low LET radiation, calculated as described in the text. Symbols as in figure 2. The ordinate is a logarithmic scale where $S = N_M - N_D/N_D - N_C$. The curve was calculated for $N_M = 4$, where $D_0 = 24$ cGy and $a = 1$. When N_M is different from 4, the ordinate can be scaled accordingly by changing the value of a . When $N_M = 3$, $D_0 = 12$ cGy (see Results section), and $a = 24/12 = 2$. When $N_M = 5$, $D_0 = 33$ cGy, and $a = 24/33 = 0.73$. However, these scaling factors are not exactly dose-modifying (Hendry and Potten 1982).

The incidence of apoptoses observed after 14.7 MeV neutron irradiation is shown in figure 4. The incidence was above control values after a dose of 0.25 cGy. At doses between 10 and 70 cGy the incidence tended to reach a plateau at between 3 and 4 apoptoses. There was no significant effect of dose-rate between 0.25 and 25 cGy per min. A similar procedure was carried out with these data to express them as cell survival values, and the curve drawn in figure 4 represents exponential inactivation of four cells at risk per crypt section, giving a D_0 value of 6 cGy. However, the incidence of apoptoses for doses between 0.25 and 1 cGy tended to be preferentially above the fitted line.

The dose-incidence curve for irradiations with the higher energy neutrons (600 MeV maximum energy), is shown in figure 5, and this comprised three experiments performed on separate occasions. The data from the first and third experiments were in agreement, whereas in the second experiment the cells appeared much less sensitive and unexpectedly their sensitivity was even lower than reported for γ -rays (figure 2). The reason for this discrepancy has not been found. An inspection of the data from all the experiments reported in this paper, together with other experiments reported elsewhere, does not reveal marked circadian or seasonal variations, nor marked inter-experimental variation, e.g. the combined data from several experiments shown in figure 2 are quite consistent. For the two experiments in agreement, survival values were calculated using four cells at risk per crypt section and a single exponential inactivation curve was drawn through the data. The D_0 was 9 cGy. This was redrawn through the data in figure 5. For the two experiments in

Figure 4. Incidence of apoptotic bodies in crypt sections at 3 hours after various doses of 14.7 MeV neutrons (Manchester). ($\blacktriangle$), 0.25 cGy per min. ($\blacktriangledown$), 0.5 cGy per min. ($\blacklozenge$), 25 cGy per min. The curve is based on exponential survival (see text).

Figure 5. Incidence of apoptotic bodies in crypt sections at 3 hours after various doses of 600 MeV neutrons given at 0.27 cGy per min (CERN). (+), experiment 1. (x), experiment 2. (*), experiment 3. The curve is based on exponential survival, using the data from experiments 1 and 3 (see text).

agreement, the data below 1 cGy tended to fall above the fitted line, as with 14.7 MeV neutrons.

Values of r.b.e. can be deduced as ratios of the above D_0 values, or directly from figures 2, 4 and 5. The values are independent of dose-rate down to 0.25 cGy per min.

The r.b.e. value is about 4 for 14.7 MeV neutrons compared to ^{60}Co or ^{137}Cs γ -rays, and about 2.7 for 600 MeV neutrons. These comparisons of effectiveness of course assume that target cells with similar characteristics are at risk. The spatial distribution of apoptotic bodies in the crypt, after doses of different radiations which produced the same apoptotic incidence, was very similar and was not affected by the LET of the radiation (figure 6). Furthermore, the distribution of damage is independent of dose up to at least 1400 cGy (Potten unpublished), which implies no spatial heterogeneity in sensitivity. In view of the high proportion of dose produced by nuclear fragmentation 'stars' (Alsmiller *et al.* 1970) in the 600 MeV beam, an analysis of the clustering of apoptotic bodies was undertaken using statistical techniques discussed elsewhere (Potten *et al.* 1982). The analysis demonstrated that after 15 cGy X-rays, the degree of clustering was not significantly different from that expected in a random distribution. After 15 cGy of 600 MeV neutrons, the degree of clustering tended to be higher, but the increase was only bordering on significance at the 5 per cent level.

Figure 6. Distribution of apoptotic bodies according to cell position from the base of the crypt (position 1). (O), 22 cGy ^{137}Cs γ -rays at 450 cGy per min (Manchester). (■), 15 cGy 300 kVp X-rays at 60 cGy per min (Manchester). (▼), 7.5 cGy 14.7 MeV neutrons at 0.5 cGy per min (Manchester). (x), 15 cGy 600 MeV neutrons at 0.27 cGy per min (CERN). The lines envelop all the observations.

4. Discussion

The incidence of apoptoses in crypts in the small intestine following low LET radiation is very similar to that measured previously (Potten 1977). The deduced value of D_0 depends critically on the number of cells which are assumed to be at risk. The lowest value of D_0 which can be calculated is about 12 cGy considering three cells at risk per section, and this is very similar to the value of 10 cGy quoted previously (Potten 1977). The present data indicate that there is no effect of dose-rate

down to 0.25 cGy per min, and this is expected as the survival curve is virtually exponential.

The high sensitivity of some cells in the intestinal crypt has been reported and discussed previously (Potten 1977). We now believe (Hendry and Potten 1982) that either, as suggested by Potten (1977), they represent a subpopulation of clonogenic or non-clonogenic cells which have a sensitivity vastly different from the resistant clonogenic cells which are assayed using the microcolony technique (Withers and Elkind 1970), or, they represent the *early* death of a discrete subpopulation (about 8 per cent) of the clonogenic cells all of which have the same sensitivity regarding clonogenic capacity.

There are few comparisons with other systems which can be made concerning the r.b.e. values determined at the low doses used here. The present data for intestinal cell killing do not suggest vastly higher r.b.e. values at doses of neutrons lower than 10 cGy, as found for example in the induction of lens opacities (Bateman *et al.* 1972, Di Paola *et al.* 1980), mammary tumours (Shellabarger 1974), and micronuclei in bean roots (Diehl-Marshall and Bianchi 1981). Using survival of type B spermatogonia in mice, r.b.e. values of 2.6–3, 3.8, and 3.2 respectively for 14, 400 and 600 MeV neutrons were measured at 50 per cent survival which corresponds to a dose of 20 cGy of γ -rays (Bianchi *et al.* 1974, Baarli *et al.* 1976). The values *decreased* at lower doses of 14 MeV neutrons, but *increased* at lower doses of 400 or 600 MeV neutrons. The latter increase was due to a biphasic survival curve reported for neutrons produced by 400 and 600 MeV protons (Bianchi *et al.* 1974), but this effect was not observed with bone marrow stem cells (Hendry *et al.* 1979). There is no strong evidence for a marked biphasic survival curve in the present work (figures 4 and 5), although for doses less than 1 cGy there is a tendency for the data points to be preferentially above the fitted line. This could be an artefact, for example influenced by sampling time, or it could represent heterogeneity in target cross-sections between cells (Goodhead 1980). The analysis of clustering of apoptotic bodies indicates that the influence of a 'star' in killing more than one cell is very small, with the reservation that in the present work the clustering is measured in only one dimension (up and down a crypt column) rather than in three dimensions.

It is interesting that in the control mouse the level of apoptoses per crypt section was about 0.25, i.e. about 0.42 per crypt (since 60 per cent of all crypt apoptoses are seen in a true longitudinal section). If the observable life of a spontaneous apoptotic body is about 5 hours (i.e. the same as a drug-induced body (Potten *et al.* 1978)), about two cells are aborted each day per crypt. In human terms this represents about 1×10^9 cells aborted per day, based on the values of about 375 crypts per mg in man (Potten 1982), and an intestinal weight of about 1.5 kg (and assuming larger crypts and the same apoptotic incidence and lifetime: the life of radiation-induced apoptoses might be longer e.g. 10 hours (Potten 1977)). From this we can estimate the numbers of aborted cells induced by whole-body occupational exposure. The maximum permissible dose is 50 mSv per year of low LET radiation, i.e. on average a maximum of 0.2 mSv per working day over 250 days. Assuming that the exponential dose-response relationship for γ -rays (figure 3) applies also at extrapolated lower doses, a dose of 0.02 cGy (20 mrem) would produce at most an additional 0.005 cells aborted per crypt per day i.e. an increase by about 0.25 per cent. In absolute terms this would represent about 2.5×10^6 additional cells aborted per man per 0.2 mSv (2 mrem). (Clearly, background radiation of about 0.1 cGy per year (0.00027 cGy per day) would make a negligible contribution to the spontaneous abortion rate in

controls.) However, in view of the extrapolation of the dose-effect curve, these values should be regarded only as very rough estimates. With neutrons, the applied quality factor of 10 for radiation protection is higher than the r.b.e. values of 3–4 observed here in mice, and the quality factor probably encompasses the tendency towards a greater incidence than expected at very low doses (figures 4 and 5). Hence the yield at the dose limit is not expected to be higher than that for γ -rays.

If the mean lethal dose (D_0) is as low as 10 cGy, it is *possible*, but in no way established, that the killing of this particular subpopulation of cells is of no consequence to the animal. This is because it has been calculated that a D_0 value of 10 cGy for low LET radiation could correspond to only one track per nucleus being required to kill one of these cells (Potten 1977). The data with neutrons are also compatible with this effect (J. Booz, personal communication). If it is assumed that a single fast-neutron recoil particle passing through the cell will kill it, then $D_0 = Z_F = 20.4 Y_F/d^2$, where Y_F is the mean lineal energy transfer and d is the diameter of the critical target. Using 10 keV per micron for Y_F , with 14.7 MeV neutrons, and mean target diameters less than about 10 μm , then a measured D_0 of 6 cGy with four cells per crypt section at risk corresponds to a target diameter of 6 μm . This is similar to the size of cells in the crypt, where for example, in other experiments (unpublished) the maximum and minimum dimensions of the oval nuclei in the region of the crypt where the apoptotic incidence is highest, were respectively 7.7 and 3.4 μm .

A dose of 1 cGy of low LET radiation is believed to generate about seven single strand breaks per mammalian cell genome (e.g. Sawada and Okada 1972). For most cells these must be very rapidly and efficiently repaired. For the sensitive cells in the crypt this repair must be absent, deficient or slow, the consequence being that a few single strand breaks in these cells result in the activation of the cell deletion (apoptotic) sequence. The sensitivity of the cells and the speed of removal suggest that little risk in terms of accumulated genetic damage can be expected. In fact the extreme sensitivity could in a sense almost be regarded as a protective mechanism against genetic damage (Potten 1977). The loss of these cells has no, as yet, detectable effect on the crypt. However, some compensation for their loss must presumably occur, and if they are part of the clonogenic population their loss is likely to be important in chronic exposures.

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Aux faibles doses de radiations γ , la production de cellules qui meurent par apoptose reste la même pour des débits de dose compris entre 0,27 et 450 cGy/min. Une valeur de l'e.b.r. d'environ 4 a été trouvée pour les neutrons de 14,7 MeV, à des doses entre 1 et 15 cGy, tandis que pour les neutrons produits par interaction de protons de 600 MeV avec une cible de Be, la valeur de l'e.b.r. était de 2,7. Les courbes dose-effet atteignent un plateau d'une valeur de 3–4 cellules mortes par crypte intestinale; ce plateau est obtenu avec une dose d'environ 20–40 cGy de rayons γ . A partir de ces courbes d'incidence on peut déterminer que la courbe de survie de la population cellulaire à risque est du type exponentiel (les valeurs de D_0 obtenues étant respectivement 24,6 et 9 cGy pour les rayons γ , les neutrons de 14,7 MeV et les neutrons de

600 MeV). Comme, pour la radiation de référence, la courbe de relation dose-effet est de type exponentiel, les valeurs de l'e.b.r. n'augmentent pas au niveau des petites doses qui intéressent la radioprotection. La localisation des cellules mortes dans les cryptes intestinales est la même après irradiation aux γ ou aux neutrons; c'est donc la même population de cellules qui sont endommagées dans les deux cas.

Bei kleinen Dosen von Gammastrahlen war die Produktion toter (apoptotischer) Zellen unabhängig von der Dosisleistung, die von 0.27 bis 450 cGy/min betrug. Für 14.7 MeV Neutronenstrahlen bei Dosen zwischen 1 und 15 cGy ergab sich ein R.B.W. Wert von ungefähr 4, wogegen er bei Neutronen, die durch 600 MeV Protonen auf ein Be-Target produziert wurden, ca. 2.7 betrug. Bei allen drei Bestrahlungsarten erreicht die jeweilige Dosis-Effekt-Kurve ein Plateau bei ca. 3-4 toten Zellen pro Darmkrypta; bei Gammastrahlen wird das Plateau mit einer Dosis von ca. 20-40 cGy erreicht. Aus diesen Kurven lässt sich ableiten, dass die Überlebenskurve der gefährdeten Zellpopulation exponentiell ist (mit D_0 Werten von 24 cGy für Gammastrahlen, 6 cGy für 14.7 MeV Neutronenstrahlen und 9 cGy für 600 MeV Neutronenstrahlen). Da die Dosis-Effekt Beziehung ebenfalls exponentiell ist, besteht kein Anzeichen dafür, dass bei sehr kleinen Dosen, die für den Strahlenschutz von Bedeutung sind, die R.B.W. Werte sehr viel höher sind. Sowohl Gamma als auch Neutronen Bestrahlung verursachen eine ähnliche räumliche Verteilung der toten Zellen in den Darmkrypten. Daraus ist zu schliessen, dass in beiden Fällen die gleichen Zellarten betroffen wurden.

References

- ALSMILLER, R. G., ARMSTRONG, T. W., and COLEMAN, W. A., 1970, *Nucl. Sci. Engng.*, **42**, 367.
- BAARLI, J., BAKKE, O., SULLIVAN, A. H., and TUYN, J. W. N., 1978, *Proceedings of the Third Symposium on Neutron Dosimetry in Biology and Medicine*, Euratom Eur. 5848 DE/EN/FR edited by G. Burger and H. Ebert, p. 243.
- BAARLI, J., BIANCHI, M., SULLIVAN, A. H., and DI PAOLA, M., 1976, *Biological and Environmental Effects of Low-Level Radiation*, Volume 1 (Vienna: I.A.E.A.), p. 195.
- BATEMAN, J. L., ROSSI, H. H., KELLERER, A. M., ROBINSON, C. V., and BOND, V. P., 1972, *Radiat. Res.*, **51**, 381.
- BIANCHI, M., BAARLI, J., SULLIVAN, A. H., DI PAOLA, M., and QUINTILIANI, M., 1974, *Biological Effects of Neutron Irradiation* (Vienna: I.A.E.A.), p. 349.
- DIEHL-MARSHALL, I., and BIANCHI, M., 1981, *Br. J. Radiol.*, **54**, 530.
- DI PAOLA, M., COPPOLA, M., BAARLI, J., BIANCHI, M., and SULLIVAN, A. H., 1980, *Radiat. Res.*, **84**, 453.
- GILBERT, C. W., 1969, *Int. J. Radiat. Biol.*, **16**, 323.
- GOODHEAD, D. T., 1980, *Br. J. Radiol.*, **53**, 1104.
- GREENE, D., 1971, *Phys. Med. Biol.*, **16**, 489.
- GREENE, D., and THOMAS, R. L., 1968, *Br. J. Radiol.*, **41**, 455.
- HENDRY, J. H., BAARLI, J., BIANCHI, M., and SULLIVAN, A. H., 1979, *Int. J. Radiat. Biol.*, **35**, 393.
- HENDRY, J. H., and POTTEN, C. S., 1982, *Int. J. Radiat. Biol.*, **42**, 621.
- KERR, J. F. R., WYLLIE, A. H., and CURRIE, A. R., 1972, *Br. J. Cancer*, **26**, 239.
- NEAL, J. V., and POTTEN, C. S., 1981, *Int. J. Radiat. Biol.*, **39**, 175.
- POTTEN, C. S., 1977, *Nature, Lond.*, **269**, 518; 1978, *Stem Cells and Tissue Homeostasis*, edited by B. I. Lord, C. S. Potten and R. J. Cole (Cambridge University Press), p. 317; 1982, U.C.L.A. Symposia (in the press).
- POTTEN, C. S., AL-BARWARI, S. E., and SEARLE, J., 1978, *Cell Tissue Kinet.*, **11**, 149.
- POTTEN, C. S., CHWALINSKI, S., SWINDELL, R., and PALMER, M., 1982, *Cell Tissue Kinet.*, **15**, 35.
- POTTEN, C. S., and HENDRY, J. H., 1982, In: *Stem Cells: Their Identification and Characterisation*, edited by C. S. Potten, Churchill-Livingstone, Edinburgh (in the press).
- SAWADA, S., and OKADA, S., 1972, *Int. J. Radiat. Biol.*, **21**, 599.
- SHELLABARGER, C. J., 1974, *Biology of Radiation Carcinogenesis*, edited by J. M. Yuhas, R. W. Tennant and J. D. Regan (Raven Press), p. 31.
- WITHERS, H. R., and ELKIND, M. M., 1970, *Int. J. Radiat. Biol.*, **17**, 261.