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The significance of spontaneous and induced apoptosis in the gastrointestinal tract of mice

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Abstract

The crypts of the gastrointestinal mucosa are highly structured and polarised organs with rapid cell proliferation and an hierarchical organisation with relatively few stem cells. These tend to be located at specific positions in the tissue — at the crypt base in the colon and about four cell positions from the base (above the Paneth cells) in the small intestine.

A small but constant level of spontaneous cell death occurs in the crypt. The levels of cell death are elevated by small exposures to radiation or cytotoxic drugs. The morphology of the cell death is typical of apoptosis. The maximum yield of cell death following cytotoxic exposure is observed at about 3–6 h after treatment and for many agents the death is characteristically located at the fourth (stem) cell position in the small intestine.

The significance and implications of these observations are discussed in relation to the internal screening and programming within damage cells and with respect to tissue homeostatic mechanisms.

Having been interested in cell proliferation control in the gastrointestinal tract for some time, we had more than passing interest in studies on cell death, for the following reasons.

1. We wished to develop a quantitative measure of the effectiveness of various cytotoxic treatments so that the efficacy of single dose and combination regimens of cytotoxic drugs and radiation could be assessed.
2. There is a small but significant level of spontaneous death in the crypts of the small intestine and we wished to investigate the possibility that this may be a part of the overall homeostatic processes that governs cell numbers and cell proliferation.
3. The levels of cell death clearly rise following exposure to cytotoxic insult and studies of this induced cell death may give some insight into the genetic basis of DNA injury. The deletion of a certain proportion of cells in a structure like the crypt results in a compensatory increase in cell proliferation and cell production in the neighbouring undamaged cells. The mechanisms involved in this triggering of regenerative changes represent another link in the growth regulatory networks within a tissue. These topics form the basis of this review.

Cells in a proliferative tissue may die in a variety of ways. Differentiation, maturation and function represent one form of death while the loss of reproductive capabilities represents another form of cell death. Differentiation is a normal process that oc-

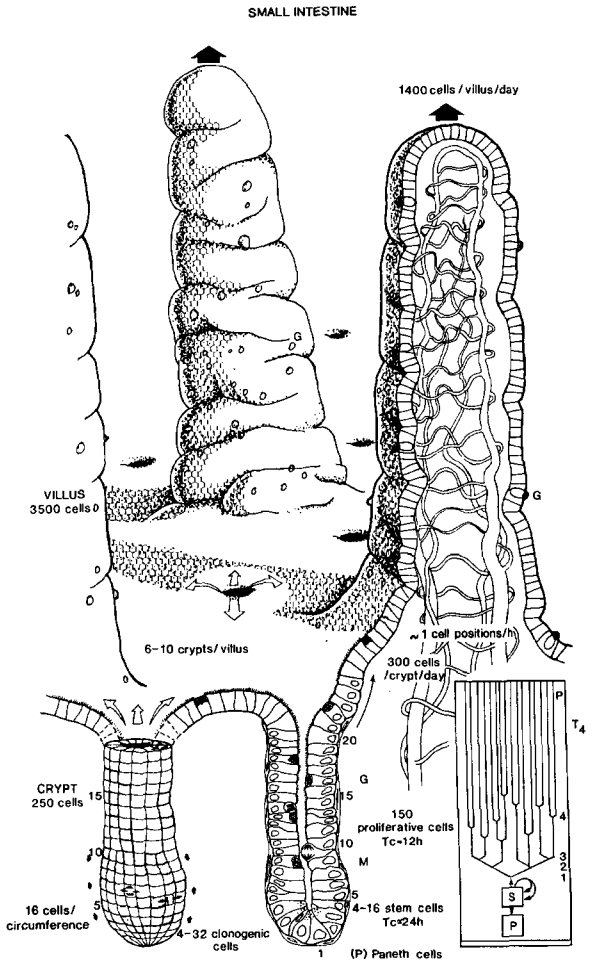


Fig. 1. Diagram illustrating the three-dimensional structural organisation of cells in the small intestine. 6–10 proliferative crypts feed cells on to each differentiated functional villus at a rate of about 300 cells emigrating from each crypt per day. The consequence of this is that cells are moving along the epithelium with a velocity of about 1 cell position per hour. A section cut through the middle of a crypt in the longitudinal plane shows the full cellular population of the crypt with differentiated functional Paneth cells at the crypt base, proliferation and hence mitotic (M) activity in the central region of the crypt where there is also differentiation into mature goblet (G) cells. The proliferative hierarchical cell lineage believed to most adequately explain the cell replacement process is illustrated in the lower right, and the position of cells in this lineage can be related to their topographical position within the crypt section such that stem cells occur near the fourth position from the base of the crypt and proliferative activity within the dividing transit population finishes between the 15th and 17th positions from the bottom of the crypt. There are likely to be between 4 and 16 stem cells per crypt, 16 being the number of cells in a full circumferential ring around the crypt. As a consequence of this, there will be between 6 and 4 dividing transit generations, respectively. This diagram has been modified and adapted from Potten and Loeffler [50] and Potten and Morris [6].

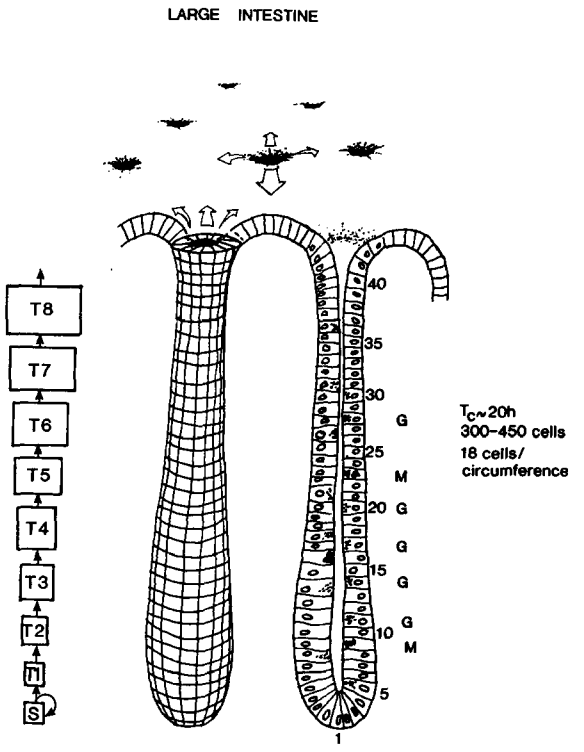
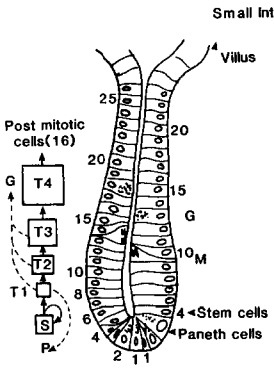


Fig. 2. Diagram illustrating the three-dimensional architecture in the colonic region of the bowel of the mouse. The crypts here are larger than in the small intestine and the cells are generally cycling more slowly. There is a greater proportion of goblet (G) cells per crypt which is probably associated with increased levels of differentiation within the dividing (or mitotic, M) transit population and hence, a lower growth fraction in the middle region of the crypt. A likely cell lineage is illustrated in the diagram on the left. This contains about eight transit generations since there are probably fewer stem cells here than in the small intestine (see also Fig. 1).

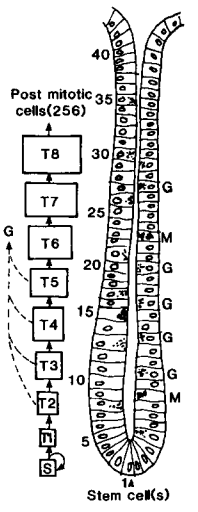
curs in all renewing tissues as part of the homeostatic mechanisms and the functional requirements of the tissue. However, cells may be additionally diverted down the differentiation pathway as a consequence of certain types of injury. This represents the cataclysmic destruction of cellular integrity, function and structure with characteristic changes in cell morphology. Cells may also undergo necrosis as a consequence of certain types of injury. However, with the agents and doses used in our studies, this mode of death which can be identified by its morphological appearance is rare. Finally, cells may undergo a more ordered form of death involving the production or release of a specific



	Mouse	Man
Cells/column	25	34
Cells/circumference	16	22
Cells/crypt	250+	450*[c]
Cell cycle	10-18h[b] 12h[d]	27-73h[b] 20-36h
Stem cell cycle	~24h	>36h[e]
Stem cells/crypt	4-16	?
Transit cell generations	4-6	>4-6
Crypts/villus	6-10	~6
Crypts/intestine	1-3x10[6]	?

+ absolute count; * assuming a 0.6 geometric correction factor; b Wright and Alison [5]; c Potten [13]; d Potten and Hendry [3], Potten [4].

Fig. 3. Diagram of a section through the small intestinal crypt illustrating the cell lineage together with various numerical parameters associated with cell proliferation. A comparison is made between these parameters for mouse and for man. Table taken with modification from Potten [8].



	Mouse	Man
Cells/column	42	82
Cells/circumference	18	46
Cells/crypt	300-450 #	2250*[c]
Cell cycle	15-23h[b] 24-36h[a]	72-96h[b] ~36h[c]
Stem cell cycle	>36h	>36h
Stem cells/crypt	1-4	?
Transit generations	5-9	>5-9

+ absolute count; * assuming a 0.6 geometric correction factor; # depends on position, 300 for caecum, 450 for rectum; a Kellett [45]; b Wright and Alison [5]; c Potten [13].

Fig. 4. Diagram illustrating a section cut longitudinally through a large intestinal crypt showing the cell lineage and parameters associated with cell proliferation. On the whole, these tend to be less accurately determined for the colon particularly the values for man. Figure taken with modifications from Potten [8].

endonuclease enzyme and a very specific sequence of morphological changes which culminate with the organised fragmentation of the cell and its nucleus and the phagocytic engulfment of these fragments by neighbouring epithelial cells or migrating macrophages. This form of death is believed to involve elements of programming and has been called apoptosis. Morphologically, the changes that we see in the intestinal crypts following a variety of treatments are indistinguishable from those described for apoptosis and hence we have adopted that term to describe the cell death that we observe. In this review, I should like to describe briefly the system with which we are concerned, make a few comments on the process of apoptosis, review some of our experimental findings and summarise the conclusions that can be drawn.

The epithelium lining the small and large bowel consists of a single layered sheet of cells folded into complex three dimensional configurations. In both areas, the proliferative cells are arranged into flask-shaped structures called crypts between which there is a small table of cells in the large bowel and large finger-like projections of functional cells — the villi — in the small bowel. The architectural and structural organisation at the cellular level is illustrated in Figs 1 and 2 and the size of the proliferative compartments and the cell kinetics are summarised in Figs 3 and 4. The small intestine (SI) represents one of the most dynamic tissues of the body, with cell division occurring every 5 minutes in each crypt in the mouse, the consequence of which is about one gram of tissue (10^9 cells) produced every 5 days in the mouse and perhaps every 20 minutes or so in the human. The cells in the crypts of the large bowel are generally proliferating more slowly. The system has been extensively reviewed over the last decade [1–8]. It has now become fairly widely accepted that the population of 150 proliferative cells in the SI crypt constitute a series of cell hierarchies or lineages of the sort illustrated in the lower right of Fig. 1 [2, 9]. The precise number of lineage ancestor cells (stem cells) remains unclear but is probably within the range of 4–16 for the small intestine and in the range 1–4 for the large bowel [8–11]. One of the unique features of this tissue is that the position of a

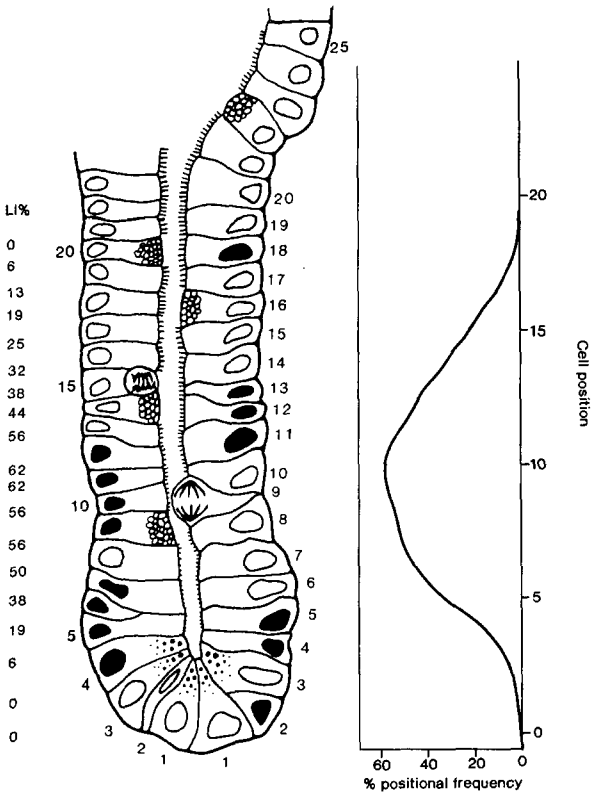


Fig. 5. Diagram showing a longitudinal section through a small intestinal crypt illustrating how the cell positions are numbered from the base. The diagram shows a typical distribution for the DNA synthesising (S-phase) cells in the crypt (shaded nuclei). An actual set of experimental data are illustrated (left) together with a labelling index frequency plot where labelling index as a percentage is plotted against cell position. This approach can be used to measure any parameter associated with crypt cells including the distribution of dead or dying apoptotic cells. These cell positional distributions are commonly presented with the frequency plotted on the vertical scale and cell position plotted on the horizontal scale with the base of the crypt on the left.

cell in the hierarchy can be related to its topographical position within the crypt in the manner that is illustrated in Fig. 1. Thus, in the small bowel, if stem cells are to be studied, they are most likely to be observed at around the fourth position from the base of the crypt, a position associated with the first columnar epithelial cells above the functional mature non-dividing Paneth cells. In the large bowel, the stem cells are most likely to be observed at the base of the crypt in cell positions 1 and 2. As a consequence of this, we have developed a scoring procedure for the cells at each individual cell position along the sides of longitudinal sections through the middle of the crypts. This is illustrated in Fig. 5 for a labelling index distribution (the spatial distribution of cells replicating their DNA or in S-phase). The scoring is initiated at the base of the crypt where the lowermost cell is designated 1 and information is recorded sequentially into an appropriately programmed BBC microcomputer [12, 13] along each side (along each crypt column). Generally, 25 such longitudinal crypt sections, or 50 half-crypt sections, are analysed for each mouse in an experiment and each experimental group usually involves at least four mice. This approach has been used extensively to study the distribution of proliferation associated markers, differentiation associated markers and the distribution of dead cells within the crypt following various treatments. The data on the spatial distribution of dead cells at each cell position can be then presented as a bar diagram, a frequency polygon or a smoothed distribution of the sort illustrated in Fig. 5.

Apoptosis [14] has been described in considerable detail in a variety of review articles [15–24] and

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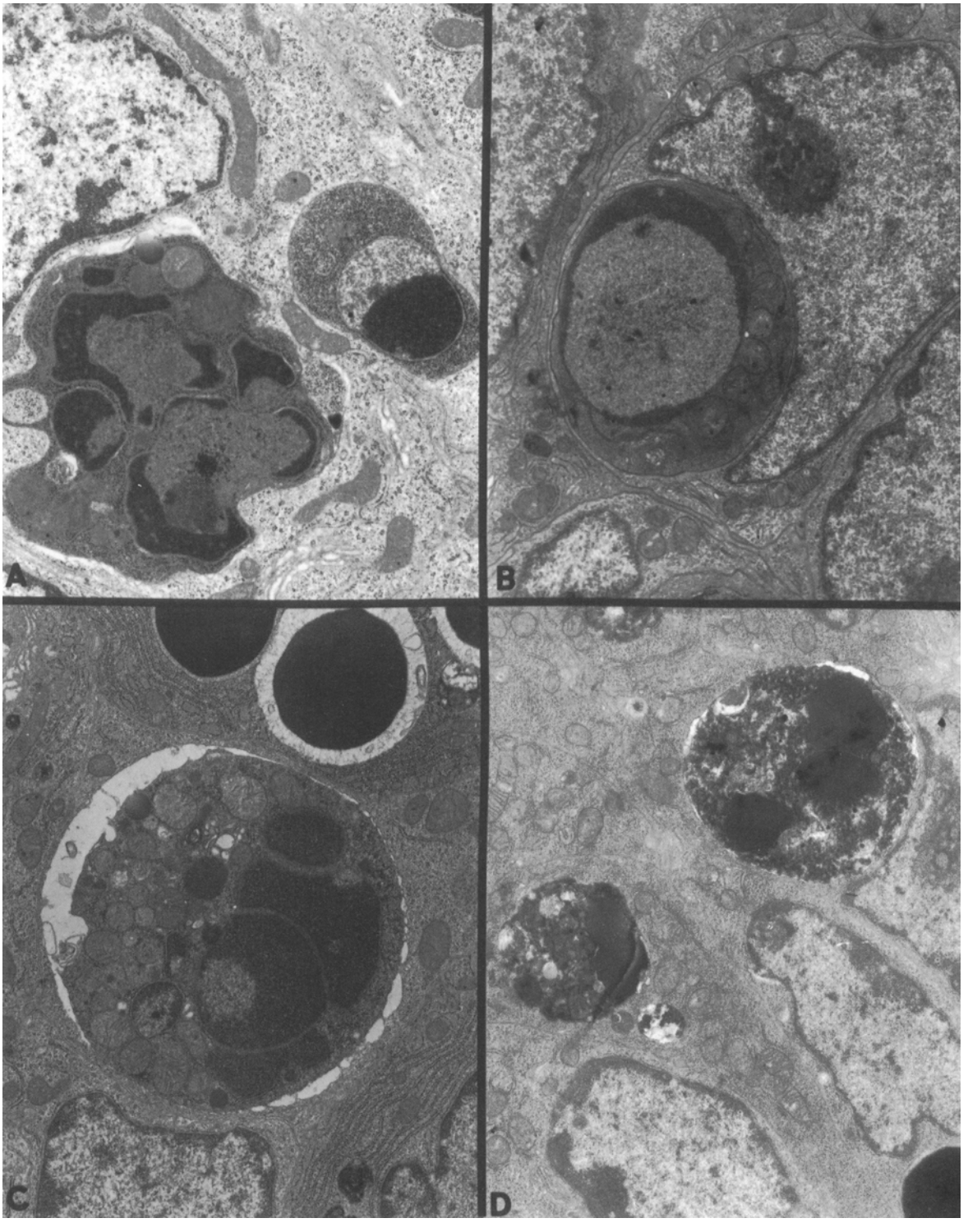
Fig. 6. Electron micrographs showing apoptosis in the crypt following treatment with cytotoxic agents. (A) an early stage in apoptosis (6 h following treatment with adriamycin 20 mg/kg); marginal chromatin condensation in the nucleus, nuclear fragmentation, cytoplasmic condensation are evident. An apoptotic fragment probably from another cell can be seen on the right. × 15 000. (B) an early stage in apoptosis with typical crescent shaped marginal chromatin condensation (2.5 h following 9 Gy irradiation). × 10 000. (C) intermediate stage of apoptosis showing fragmentation of nucleus and condensed cytoplasm with retention of organelle integrity (see mitochondria). Paneth cell granules are at the top of the picture (6 h following 20 mg/kg adriamycin). × 13 000. (D) late stage of apoptosis showing fragments engulfed by neighbouring cells. Remnants of nuclear chromatin are clearly visible (Paneth cell granule bottom right) (1 h after 12 Gy irradiation). × 10 000.

I am grateful to Drs N. Thakkar and T.D. Allen for help with these pictures.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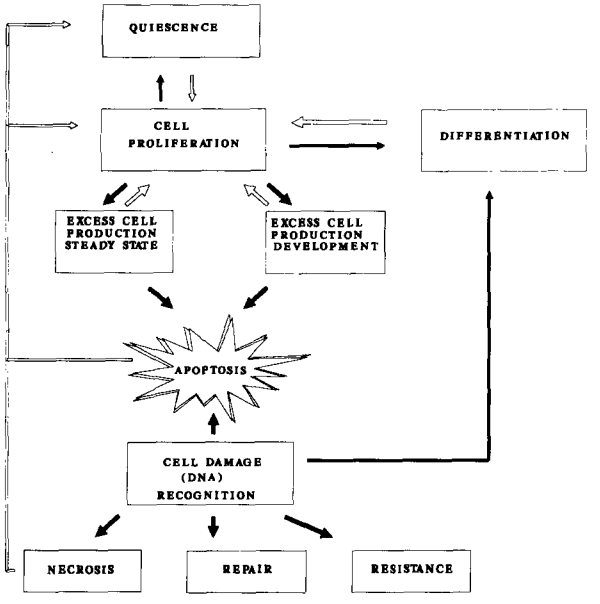

Fig. 7. Diagram illustrating the possible interrelationships between cell proliferation, differentiation, the removal of cells excess to requirement both in development and in steady state through apoptosis and the relationship between cell damage and the response of the cell through the induction of resistance, repair enzymes, necrosis, differentiation or apoptosis. Aspects associated with this diagram will be elaborated further in this presentation. This diagram is based on ideas outlined in a brief report by Allan [52].

a detailed description will not be given here. It is a mode of cell death that has been described as being programmed or controlled in an orderly sequence. It invariably involves single, isolated cells and does not elicit an inflammatory response. There are early changes in protein and RNA synthesis and an activation of a calcium- and magnesium-dependent endonuclease. This endonuclease cleaves the chromatin material in the internucleosomal region of the DNA, generating fragments of DNA that contain multiples of about 200 base pairs. As a consequence of this, when the DNA is extracted from cells that have been fairly synchronously induced into apoptosis and separated on a gel, a characteristic banding pattern (a ladder) of DNA fragments can be detected. While these biochemical changes are proceeding, the dying cell shrinks away from its neighbours and exhibits a condensation of chromatin material particularly at the margins of the nucleus and often into characteristic crescent-shaped patterns. There is also a condensation of the cytoplasmic material. The shrinkage process continues and develops into a fragmentation or budding of the cytoplasm and a disintegration of the nucleus into a number of fragments (karyorrhexis). The end result is a series of apoptotic fragments each of which may, or may not, contain remnants of the chromatin material and these are generally engulfed by healthy neighbouring epithelial cells, or macrophages, and are digested fairly rapidly. Most of the cell death that we observe in the crypt has the morphological features that have just been described (see Fig. 6).

The term programming is commonly associated with apoptosis, but its precise meaning and its implications have not really been defined. Apoptosis was commonly described in those situations during embryological development when tissue restructuring was required. This generally necessitates the removal of cells at particular locations that are no

Table 1. Comparison of the position of maximum mutagen cytotoxicity (apoptotic index) and the position of stem cells in small intestine and colon

Agent	Dose (mg/kg)	Time of peak AI (h)		Peak AI (%)		Cell position target cells at $t = 0$		Presumed cell position of stem cells	
		SI	LI	SI	LI	SI	LI	SI	LI
NEU	50	4.5	4	23.7	13.7	4.2	8.0	4	1–2
NMU	200	4	5	9.8	17.3	4.2	4.9	4	1–2
DMH	80	5	8	6.8	19.5	3.7	7.8	4	1–2
MDMA	50	6	6	15.0	9.0	5.1	10.5	4	1–2

AI, apoptotic index; SI, small intestine; LI, large intestine; NEU, *N*-nitroso-*N*-ethylurea; NMU, *N*-nitroso-*N*-methylurea; DMH, 1-2-dimethylhydrazine; NDMA, *N*-nitrosodimethylamine. Data from [39, 48].

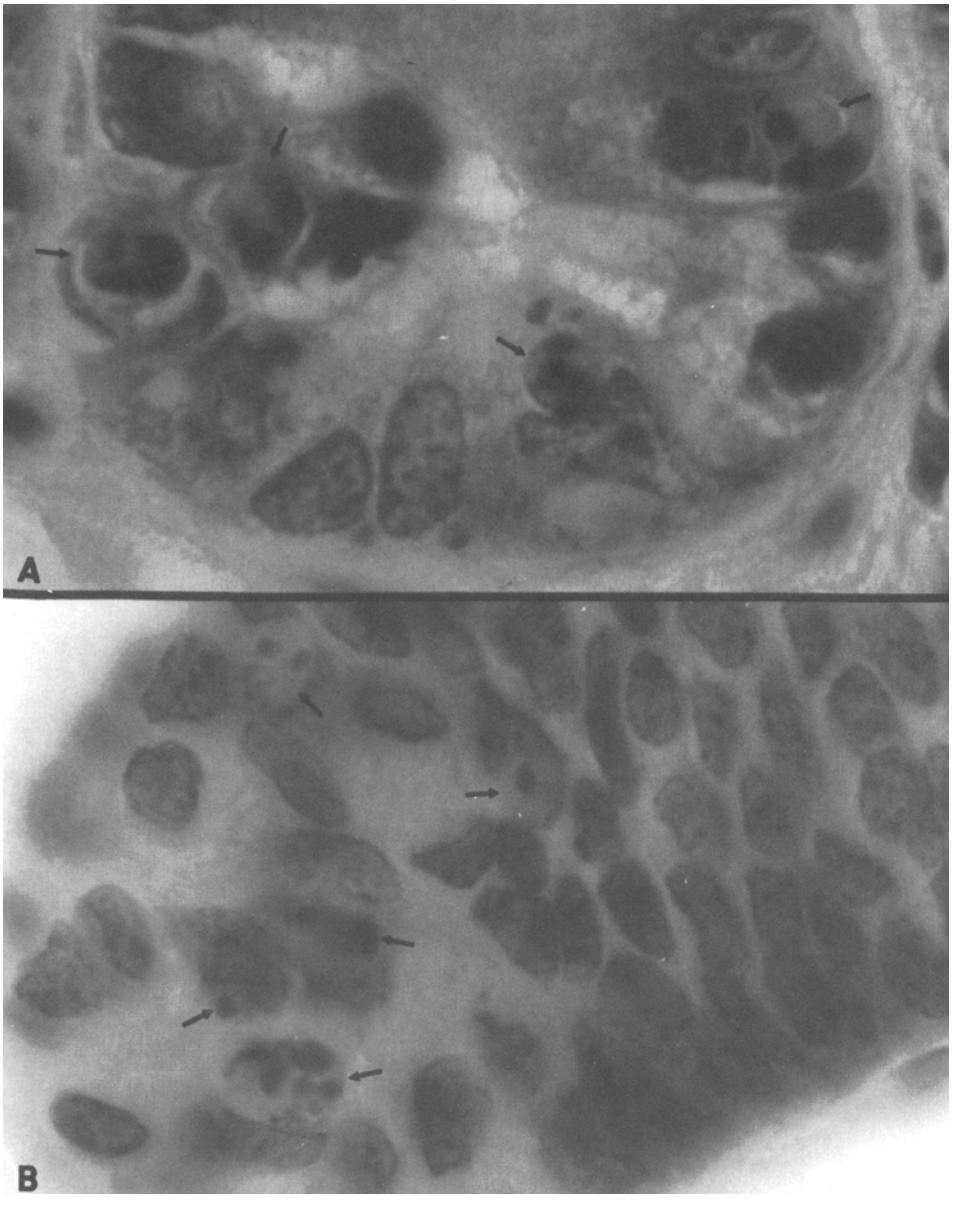

Fig. 8. Haematoxylin and eosin stained paraffin section (A) through the middle of a small intestinal crypt from a mouse 4 h after treatment with ethylnitrosourea illustrating apoptotic cells in the lower regions of the crypt (arrows) particularly around the fourth position. (B) Optical section through a whole crypt showing several apoptotic cells (arrows). × 1000.

I am grateful to Y.Q. Li for help with these pictures.

longer required (ie, are excess to requirements). The cells themselves that are about to be deleted are perfectly healthy cells and may be present in large numbers (ie, not isolated cells). They respond to some external (hormonal) stimulus which triggers an altruistic cell suicide, ie, the removal of cells for the good of the tissue and the organism in general. The term 'programming' here clearly has an element associated with the external triggering of cells into apoptosis. But, the triggered cell responds to the activation of its surface receptors, or the receipt of a signal, by internal changes that have

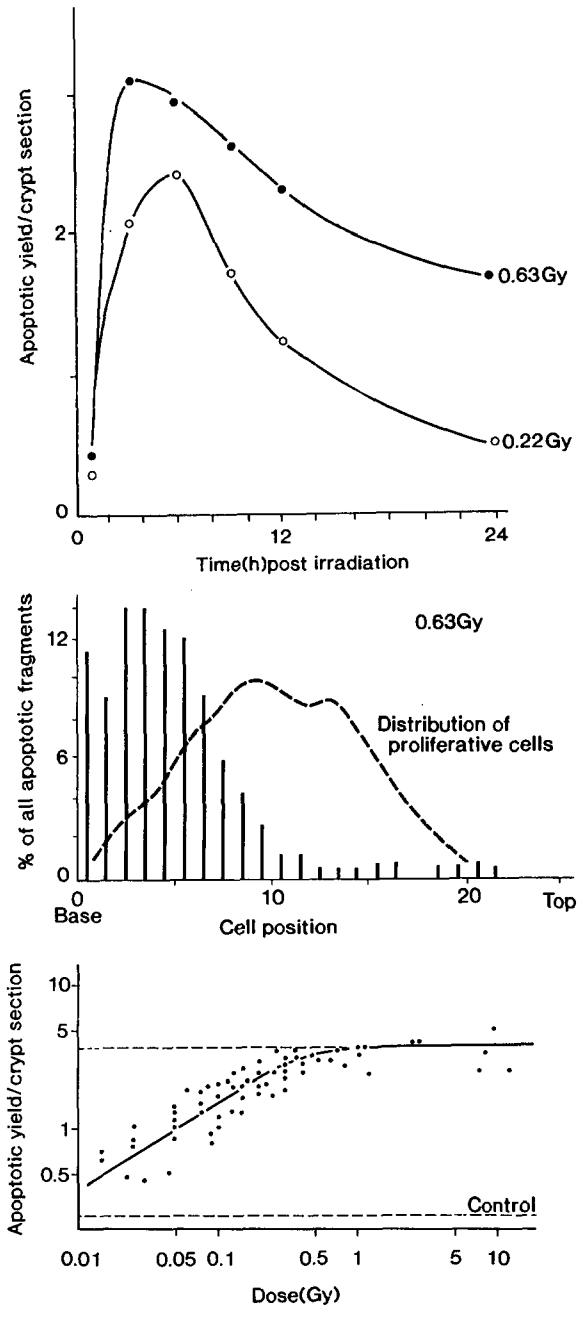

Fig. 9. Graphs illustrating: (Top) The relationship between the yield of apoptotic figures per crypt section at various times following small doses of radiation. Peak yields are obtained within 3–6 h of exposure. (Centre) Diagram illustrating the differential yield of apoptotic cells at each cell position in the crypt 3 h after a dose of 0.63 Gy. The maximum yields can be observed at around the fourth position. This frequency plot is compared with that for labelling index which identifies the proliferative compartment-line.

Diagrams taken from Potten [3] and Hendry [37]. (Bottom) The relationship between the yield of apoptotic figures at 3–6 h following a range of radiation doses. The spontaneous level of apoptosis is around 0.2 apoptotic figures per crypt section. The yield increases progressively with increasing dose up to about 1 Gy at which point about four cells are killed through apoptosis per section and a plateau is apparently reached. Doses of a few cGy can be easily detected.

been assumed to have a genetic basis ie, involve derepression, transcription, translation and protein synthesis of material such as the specific endonuclease. It may also involve the release of preformed endonuclease or the activation of partially formed enzyme but both these processes may require the synthesis of the releasing or activating protein. Thus, there is an implicit internal programming as well as the external triggering. A further element of this programming process is the question of how the developing organism discriminates between those cells that need to be deleted and those to be retained, which cells are to have apoptosis initiated and how does the tissue or organism discriminate between cells to delete and cells to keep and furthermore how is cell deletion terminated and restricted to only specific cells. How does the tadpole know that the deletion of its tail must stop at the base of its tail? One possibility here is that all cells are programmed to die but specific mechanisms exist to prevent expression of this death [25–27]. For example, the product of bel-2 in mammals [26, 27], and ced-9 in *Caenarhabditis elegous* [28 and review 25]. It is also possible that the products of some tumour suppressor genes such as p53 could be involved in releasing cells for apoptosis or initiating cell death [29].

One further possibility that we have considered is that this mechanism which was evolved for the removal of excess cells during tissue restructuring in embryological development is retained as part of the overall tissue homeostatic process in adult replacing tissues. The small intestinal crypt has a spontaneous level of apoptosis equivalent to seeing one dying cell in every fifth crypt section [30] and this level of cell death shows circadian rhythms [31]. One possibility is that this is a reflection of an excess in cell production particularly in the stem cell population since this is the position in the tissue where the spontaneous cell death is observed. Apoptosis is also a process that occurs at very significant spontaneous levels during spermatogenesis.

An alternative explanation for the spontaneous level of death observed in crypts and possibly also in testis is that this represents the removal of cells that contain random genetic defects either as a

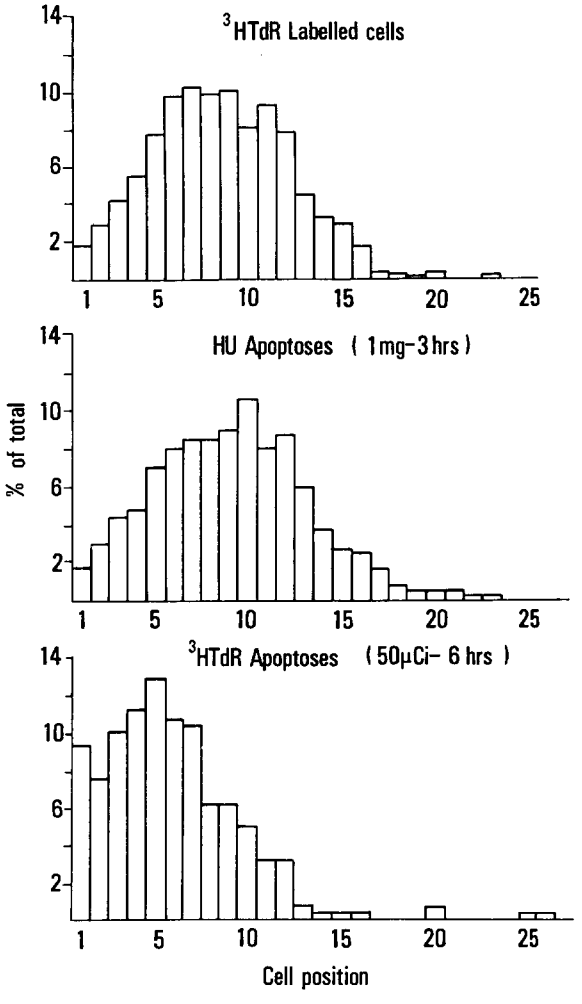

Fig. 10. Bar diagrams illustrating the frequency of apoptosis at each cell position following treatment with radioactively labelled (tritium) thymidine which causes internal irradiation of the cells in comparison with the yield of apoptosis following treatment with hydroxyurea. The distribution of S-phase cells as labelled with tritiated thymidine in an autoradiograph is also illustrated which demonstrates the pattern of proliferative (S-phase) cells in the crypt. The distribution of cells killed by hydroxyurea reflects very closely the distribution of S-phase cells. However, the distribution of cells killed by tritiated thymidine is very similar to the distribution observed for external irradiation (see Fig. 9). Even though tritiated thymidine is incorporated into the S-phase cells.

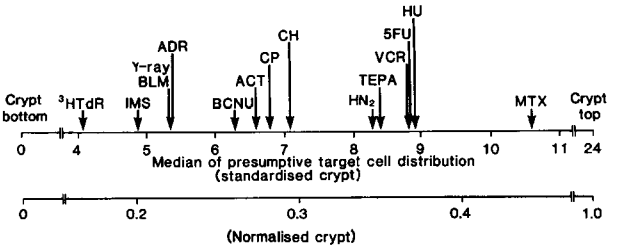

Fig. 11. Diagram illustrating the estimated position of the target cells for a range of different cytotoxic agents. The position of the median of a presumed target cell distribution at the time of administration is presented (see Fig. 10). This value is obtained by a back-extrapolate where a range of median values is plotted at different times following each cytotoxic treatment. For CP and TEPA the median values were obtained by back-extrapolation to time = 3 h. Diagram taken from Ijiri and Potten [33] where the technique is described in full and the data are presented in full.

Abbreviations: 3HTdR, tritiated thymidine; IMS, isopropylmethane sulphonate; BLM, bleomycin hydrochloride; ADR, adriamycin; BCNU, bis-chlorethylnitrosourea; ACT, actinomycin-D; CP, cyclophosphamide; CH, cycloheximide; HN2, michlorethamine, nitrogen mustard; TEPA, triethylene thiophosphoramide, thiotepa; VCR, vincristine; 5FU, 5-fluoruracyl; HU, hydroxyurea; MTX, methotrexate.

consequence of errors in processes such as DNA replication or as a consequence of random interaction with cytotoxic materials, chemicals, temperature for the testis, or background radiation. Certainly, the levels of apoptosis can be elevated very significantly by small changes in the levels of these cytotoxic agents (see reviews in Potten, 1987 [22]). This deletion of damaged cells implies other elements of programming. It implies that the cells possess self-screening mechanisms in order to detect minor or major genetic errors or damage to the DNA. Having detected these errors, a range of options are open for the cell. It may ignore these errors and proceed to function relatively normally provided the defects do not involve the functional or housekeeping genes. It may activate those regions of the genome involved in repair processes (repair enzymes) or, it may activate that part of the genome required for the altruistic removal of this defective cell from the tissue. Such considerations are summarised in Fig. 7 and will be discussed further later in this paper.

Apoptosis can be easily detected in paraffin embedded 3–4 μm haematoxylin and eosin stained sections (see Fig. 8) [8, 30, 32–34]. As part of the initial phase of a programme of work directed towards the development of a quantitative index of cytotoxic efficacy, a series of experiments was performed with low LET irradiation. The results are

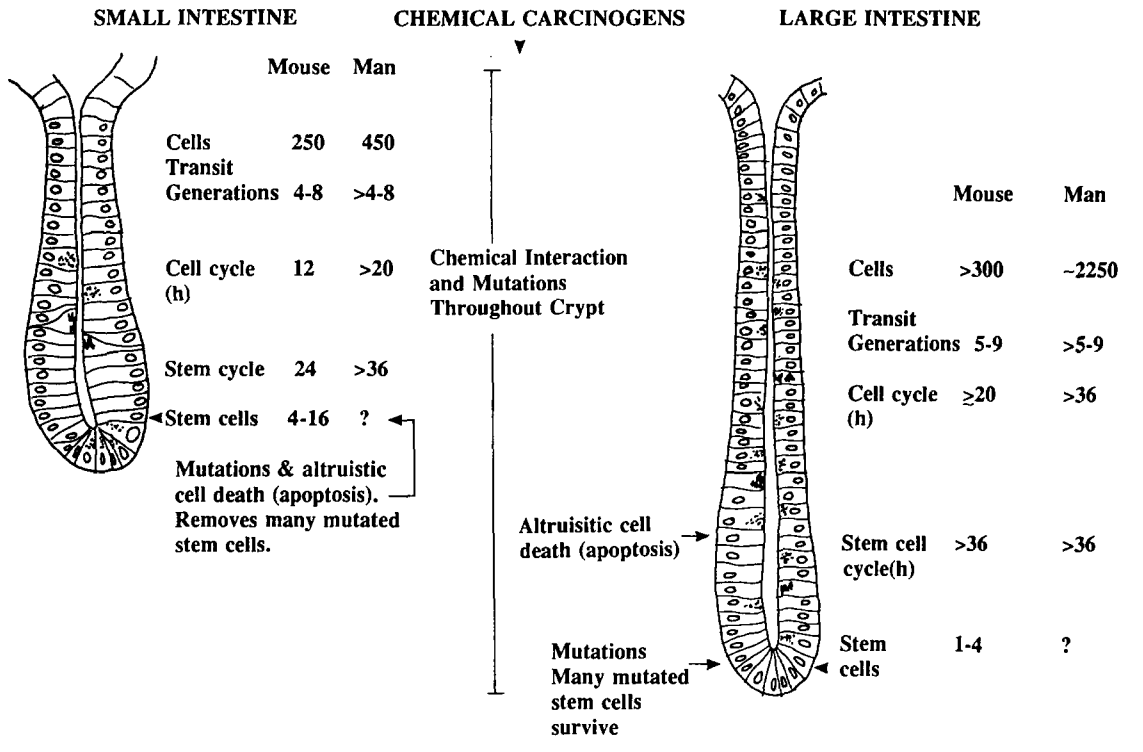

Fig. 12. Diagram illustrating the small intestinal crypt and the large intestinal crypt and the differences in the position of the target cells at times 0 based on a back-extrapolate as described in the text and for Fig. 11 for apoptosis induced by a range of chemical carcinogens (see Table 1). In the small intestine, the mean position of the target cells is 4.3 which is almost coincident with the presumed position of the stem cells. However, in the large intestine, the mean position of the target cells is at cell position 7.8, while the presumed location of the stem cells is cell position 1 or 2.

summarised in Fig. 9. Initially a temporal study was performed using small doses of radiation, the total yield of dead cells was measured in sections at various times after radiation. The results show clearly that the levels rise from the spontaneous incidence of apoptosis to reach peak values within 3–6 h of irradiation exposure. Thereafter, there is a decline which after the lowest dose approaches the spontaneous level within 24 h [30, 32, 35, 37]. The decline in the number of apoptotic bodies is attributable to the fact that the fragments are phagocytosed and digested by healthy cells and the healthy cells themselves are moving up and out of the crypt. Analysis of the decay portion of the incidence versus time plot following small doses of radiation and certain cytotoxic drugs suggested that the half-life for apoptotic bodies in this system

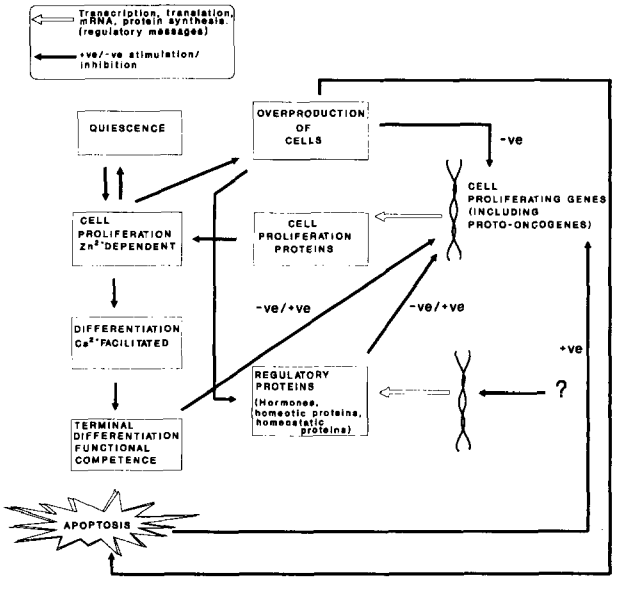

Fig. 13. Diagram illustrating a possible range of interrelationships and regulatory feedbacks determining cell proliferation.

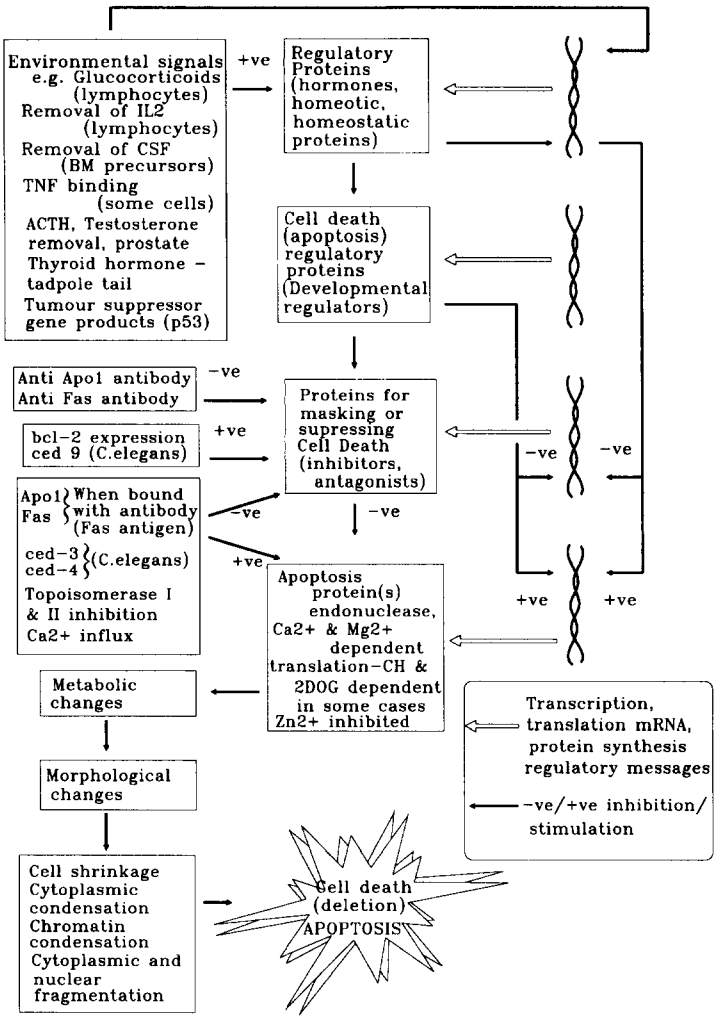

Fig. 14. Diagram illustrating a speculative range of pathways and regulators that might be involved in apoptosis associated with developmental restructuring of tissues. This diagram is not intended to be comprehensive in the sense of dealing with all published observations, but is intended to give an idea of a possible sequence of events and the complexity of this system. The basis of this diagram developed from a similar scheme illustrated in Bowen and Bowen [24].

is about 5 h. Studies performed in another somewhat analogous proliferating system, the growing hair follicle where the cell cycle time is the same as in the small intestinal crypt, the time of appearance of apoptotic bodies is slower with the peak levels being reached at about 12 h following irradiation [38]. Thus, the time of appearances of apoptosis is unrelated to the cell cycle but is more an intrinsic property of the cells of a particular tissue.

The dose–response relationship in the small intestine at 3–6 h following irradiation, shows a strong dose-dependence over the range 0 to about 1 Gy, and this dose–response relationship shows that this system is among the most sensitive of any mammalian *in vivo* system [30, 37]. Doses of the order of 5 rads (0.05 Gy) can be easily detected. The dose–response curve reaches a plateau at about 1 Gy and at a level of about four apoptotic cells per crypt section at the fourth cell position (ie, in the stem cell zone). For geometric reasons this

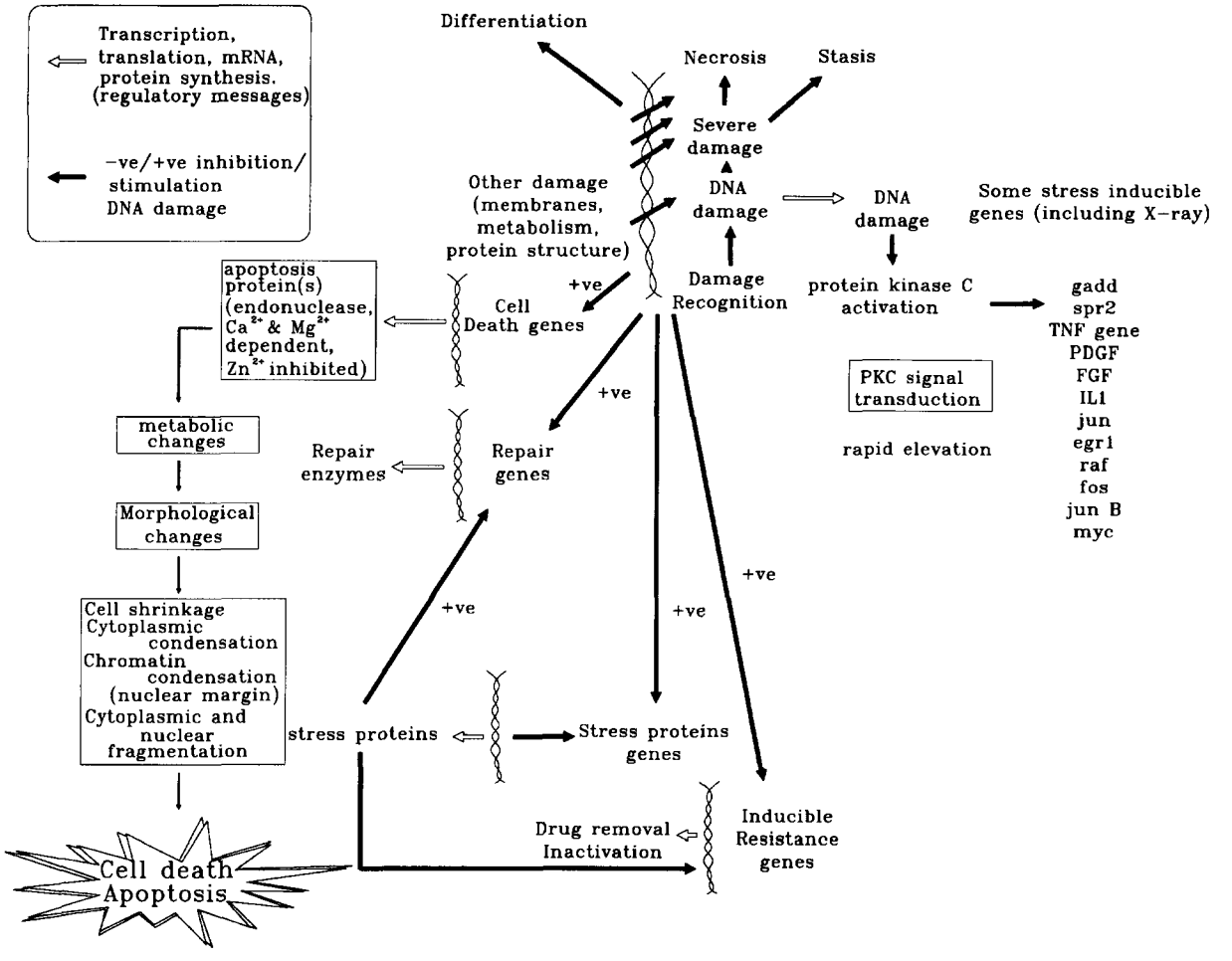

Fig. 15. Diagram illustrating the range of options open to cells that are subjected to DNA damage and the possible pathways and regulatory networks that might be involved. The diagram is not intended to be comprehensive but merely to give an indication of the range of options that might be considered and the complexity of the system. The options may well depend on the severity of the damage that is induced and almost certainly depends on the type of cell that is being considered. Following stress imposed upon the cell, a wide range of genes may be activated. The diagram shows 12 such genes that were noted in a recent symposium on stress-inducible genes. The list is unlikely to be comprehensive, but is intended to illustrate the fact that many genes may be potentially activated.

can be equated to a total of about six cells in the entire crypt [30, 39]. There is thus a small subpopulation of the crypt that is susceptible to small doses of radiation and which activates the apoptotic sequence after incurring damage. The sensitivity exhibited by these cells is equivalent, at the lower doses, to a situation where a single radiation interaction with a target the size of the entire DNA triggers the cell deletion or apoptotic process [30]. The sensitivity of these cells may be related to the possibility that they lack repair enzymes which might also be involved in sister chromatid exchange, a process which is at odds with the concept of selective DNA strand segregation [30, 40]. This was an hypothesis proposed as a mechanism to conserve the genetic integrity of stem cells. There is some limited evidence in support of the concept for a few cells at the crypt base [36].

The final point to note from these experiments which is also illustrated by the picture in Fig. 8 is that the death induced by radiation and also that induced by certain chemicals is not randomly dis-

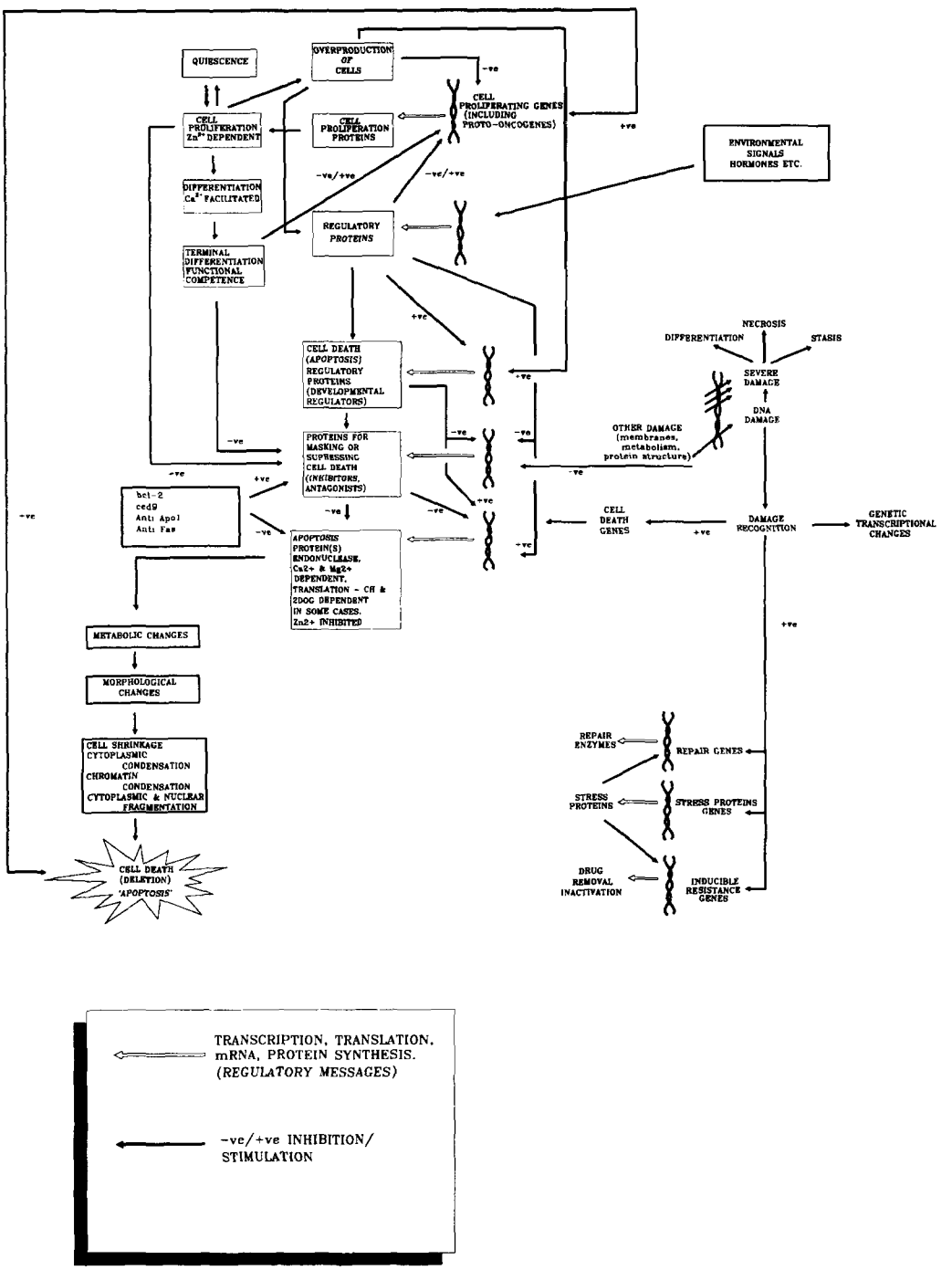

Fig. 16. As suggested in Fig. 7, cell proliferation, cell deletion via apoptosis whether during development or in steady state and the altruistic deletion of cells that have sustained damage to their DNA may be linked within a common homeostatic network. This figure brings together the elements illustrated in Figs 13, 14 and 15 in a speculative manner to illustrate how these processes might interact and interregulate. The diagram is intended more to illustrate the complexity of the interactions than as a comprehensive definitive outline of all the interrelationships.

tributed throughout the proliferative compartment of the crypt. The greatest yield of cell death is observed at around the fourth or fifth position from the base of the crypt which is close to the area that is expected to contain the stem cells [32, 33]. In contrast, the maximum yield of proliferating cells occurs over cell positions 5 to 15. This is also illustrated in Fig. 10, where the distribution of cells killed by hydroxyurea is compared with the distribution of cells labelled with tritiated thymidine. They are coincident. However, the distribution of cells killed by the dose of tritiated thymidine is typical of the radiation distribution shown in Fig. 9. This figure illustrated the fact that although cells in the middle region of the crypt are damaged by radiation, they do not activate the apoptosis sequence, however, they possess the ability to activate the sequence since they initiate apoptosis following hydroxyurea exposure. This point was elaborated in considerable detail by Ijiri in a series of experiments where 18 different cytotoxic agents were analysed following different doses and at different times [32, 41]. Frequency distributions were obtained at each dose and time by Ijiri and a measure of the central tendency, the median, was determined. The median value was then plotted against time to determine the extent of the movement of apoptotic fragments and these plots then were used to determine the hypothetical median for the cells at time 0, ie, the position of the cells that are susceptible to damage. The results of these experiments are illustrated in Fig. 11, where it can be seen that each agent tends to target cells at a specific position within the crypt. Thus, most cells have the ability to activate the apoptosis sequence, but only do so if damaged by a particular agent. The observation that in the rabbit bacterial toxins may precipitate apoptosis even on the villus [42] indicates that any cell is capable of activating the apoptosis sequence. Under normal circumstances, in most cells of the tissue there must be some suppression of this ability and if we consider radiation, virtually all the cells in the epithelium barring a small proportion of the stem cell population are either incapable of activating apoptosis or have this ability heavily suppressed. These ideas are in accord with similar conclusions reached elsewhere based on other experimental observations (see above and 25–27).

Various observations have demonstrated that cells in the lower few positions of the crypt have a cell cycle time significantly longer than the cells in the middle of the crypt (24-hour cycle time compared with 12 hours) [43, 44]. Using small doses of radiation to kill just a few cells in the lower few positions of the crypt (ie, the stem cell region), we have studied the changes that this elicits in cell proliferation. Three independent and totally different types of experiment have all illustrated that the killing of a very small number of cells in the stem cell region elicits rapid (immediate) changes in the proliferation in the remaining cells [7, 8]. The cell cycle in this region of the crypt is shortened dramatically [43, 45, 46]. Using a functional assay for stem cells it can be shown that they are recruited into rapid proliferation. Analysing the changes in the total population at the top of the crypt (a cell migration type of experiment), we have concluded that the stem cells changed their self-renewal probabilities following very minor levels of cytotoxicity. Experiments with larger doses of radiation show that the entire population of apoptosis sensitive cells (ie, about six per crypt) are killed, and this triggers an early compensatory proliferation in the remaining cells in the stem cell region that is initiated at a time coincident with the peak yield of apoptosis [47]. This all suggests that apoptosis may be part of the overall homeostatic process, ie, the system detects cell death and responds accordingly by increases in cell proliferation and self-maintenance as is illustrated in Fig. 7.

We have recently undertaken a major programme of work looking at the induction of cell death following exposure to a variety of chemical mutagens [39, 48]. These agents have been assumed to be distributed evenly throughout the tissue and are likely to cause mutations at all levels within the tissue. Mutations in the rapidly dividing transit population however, are short lived since these cells only have a life span in the tissue of a few days. In contrast, mutations in the stem cell population are potentially carcinogenic. It is a common observation that cancer is surprisingly a rare event in the rapidly proliferating small bowel, however,

is relatively common in the more slowly proliferating large bowel. A variety of mechanisms have been proposed to explain this differential cancer incidence [49], but none is totally adequate to explain all the observations. Amongst the mutagens studied by us, most will cause cancers in the large bowel, but rarely cause cancers in the small intestine. We have used cell positional approaches to determine the levels of cell death at various times following various doses (as shown in Fig. 8). Cell death is easily recognised and is common with the maximum yield in the small bowel being commonly observed at around the fourth position, ie, in the stem cell zone. We have used the median extrapolation approach to determine the position of the target cells in both the small and large bowel. The results are summarised in Table 1 and Fig. 12, where it can be seen that these chemicals induce damage that triggers altruistic cell death in the stem cell region of the small intestine. Thus, stem cells that have changes in their DNA (mutations) are altruistically removed from the system which is thus protected. This is similar to the response to very small doses of radiation [30]. In contrast, in the large bowel the altruistic cell death is triggered in the mid-crypt region and thus many mutated stem cells may survive in the colon and ultimately go on to produce tumours. This hypothesis is implicit also in Fig. 7, where it is postulated that the presence of DNA damage is detected in cells and one of the options open for the cell containing damage is the activation of the apoptosis sequence.

Figure 7 suggests a link between proliferation, the production of cells excess to requirement either during development or under steady state conditions, the process of self-screening for genetic errors and the removal of cells bearing any genetic errors. Each of these processes, is itself likely to involve a complex sequence of events including regulation of gene expression and feedback signals. Each segment can be looked at in greater detail and Fig. 13 illustrates some of these interactions for the process of cell proliferation. Figure 14 attempts to display some of these genetic regulatory processes for the apoptosis involved in developmental tissue restructuring. Part of this sequence of events may be retained for controlling the cells excess to requirement under steady state conditions. Figure 15 illustrates the complexity of interactions and options open to cells that are exposed to cytotoxic insults, while Fig. 16 attempts to link these three processes together in a highly speculative fashion. The implications of this diagram are that apoptosis is a controlled 'natural' mechanism that is invoked when there is an overproduction of critical cells in the tissue or when critical cells sustain DNA damage, ie, it is part of the overall tissue homeostasis. If the controls on apoptosis are disturbed, too many, or too few, cells will be deleted. The former would result in a mild and local compensatory proliferative response while the latter would result in distortion of the tissue structure since extra cells and their progeny would have to be accommodated and this could generate a situation similar to hyperplasia (eg, expansion of crypt size). If apoptosis is part of an overall homeostatic process with its own regulatory mechanisms then there is the possibility for manipulation of these regulatory processes to facilitate tissue regeneration or tumour regression. The intention of this review and these diagrams is to indicate the complexity of the interactive networks and to stimulate future discussion and experimentation.

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