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INTESTINAL CELL KINETICS IN IRRADIATED MICE

A quantitative investigation of the acute reaction to whole body roentgen irradiation

by

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Surgery still appears to be the only rational means of completely removing malignant body cells. Ionizing radiation, chemotherapeutic agents and hormones are to a considerable extent of an empiric character, although certain specific effects on cell metabolism, cell growth and growth regulation have helped to support such forms of therapy with some degree of success (STEINFIELD 1967). A suitable selective agent has, however, yet to be found.

Radiobiologic investigations have provided data on radiation effects in cells, e.g. morphologic and functional changes, depression of mitosis, chromosome breakage, and cell death as an exponential function of dosage. The oxygen tension during radiation, effects on the nucleic acids, and cellular mechanism for repair of injury are of fundamental importance. Research in radiobiology has, however, in the main, not yet led to much change in established empiric radiotherapeutic practice. Cell population kinetics have assumed increasing importance in providing background material for a better understanding of homeostasis and growth as the balance between cell production and cell loss in the study of

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the biology of tumours, (TUBIANA et coll. 1968). LAMERTON & STEEL (1968) have recently reviewed the subject, and discussed the possibilities and limitations of the available methods.

The epithelial cells in a crypt of the small intestine may be regarded as forming a well defined and delimited cell population. Kinetic studies have provided much basic information, both under normal conditions and after injury, e.g. roentgen irradiation. The general features of radiation effects have been known for many years. Since REGAUD et coll. (1912) called attention to gastrointestinal changes in dogs following irradiation, clinical, morphologic, cytologic, biochemical and functional changes have been the subject of many publications. (References appear in reviews by PIERCE (1948) and RUBIN & CASARETT (1968).)

The principal events in the kinetics of the epithelial cells of the small intestine have for a long time been well understood from histopathologic and cytologic investigations. Recently introduced techniques with labelling of cells have provided more details and led to quantitative determinations that were not previously possible. In addition to cell counts, differential cell counts, mitotic index, abnormal mitoses, and number of dead cells, the duration of different phases of the cell cycle has been determined and the migration of cells from the crypts to the villi followed. Most information has been obtained from mice and rats, but it would appear to be applicable in general to other species, including man.

The cell population of an intestinal crypt is well suited for quantitative investigations. The size, cell production and cell loss of a cell population of an intestinal crypt were therefore chosen for the present investigation on changes in the cell population to total destruction or elimination following an increasing degree of injury by roentgen irradiation.

Material and Methods. The experiments were performed with male and female albino mice of the WLO strain, 2 to 3 months old, weighing about 25 to 30 g and fed a standard diet of pellets and water ad libitum.

Four mice at a time were given whole body roentgen irradiation in a plastic box, freely open to the surrounding air. The integral dose was measured during each irradiation with a Philips dosemeter with the ionization chamber placed between the mice. Two roentgen units had to be used; the physical conditions were as follows. (1) 250 kV, 12 mA, filter 1 mm Cu, FSD 55 cm, dose rate 38 R/min. The doses were 200 R (62 mice), 350 R (80 mice), and 700 R (120 mice). (2) 170 kV, 6 mA, filter HVL 0.85 mm Cu, FSD 28 cm, dose rate 140 R/min. The doses were 700 R (36 mice), 900 R (8 mice), 1 000 R (54 mice), 1 200 R (8 mice), 1 400 R (30 mice), 1 700 R (8 mice), 2 000 R

(30 mice) and 2 400 R (12 mice). A further 156 mice were used to determine the LD_{50} and study the migration of cells, and mitotic rates. There were 47 control mice of the total of 651 mice.

Of all the mice that received 700 R and more, 126 were anesthetized during irradiation with subcutaneous injection of Nembutal (Abbot), 100 mg/kg body weight, to permit comparison with localized irradiation in other experiments.

The mice were killed by cervical luxation, at time intervals as follows: 1/2, 1, 2, 3, 4, 6, 9, 12, 24, 36 and 48 hours, and 3, 4, 5, 6 and 7 days. For several dose levels, however, mice were killed only at two or a few more time intervals.

The material was fixed in Sanfelice's solution (16 parts CrO_3 1 %, 1 part acetic acid and 8 parts formalin 40 % mixed shortly before use), embedded in paraplast, sectioned serially at 5 μ, and stained with hematoxylineosin. Neutralized formalin 4 % was used for fixation for preparing autoradiographs of material labelled by intraperitoneal injection of tritiated thymidine (New England Nuclear Corporation thymidine-methyl-^{3}H, 10 Ci/mM, 25 μCi per mouse). The slides for autoradiography were coated with Kodak NTB 2 emulsion, and following exposure and development were lightly stained with hematoxylin and eosin. To determine mitotic rate, 1 mg/kg body weight vinblastine sulfate (Velbe, Lilly) was injected intraperitoneally (TANNOCK 1965) to arrest the mitoses; counts were performed at the time of injection and 3 2/3 to 4 hours after injection.

Counting. Counts were made in transverse sections of the small intestine mid-way between the doudenum and caecum, where crypts sectioned longitudinally through the middle, ten in each mouse, were selected for analysis. The cells counted were restricted to the cells cut through the middle part of the nucleus and lying in the same focal plane; sections were obtained in series to minimize the effect of variation in thickness. The results are given as number of cells, mitoses, dead cells and Paneth cells observed per section of one crypt.

The epithelial cells of the villi have not been counted, since the number of cells counted in a section of a villus and a crypt cannot be directly compared to each other. The crypts are more numerous than the villi, and it is estimated that there are three to four crypts per villus in the parts examined although this varies very much in the sections, as does the length of the villi.

Epithelial cells of the crypts are usually easily distinguished from those of the villi, the nucleus of the latter being more centrally located in the cell than in the former. The counts of the total number of cells comprised mitoses and Paneth cells, but did not include the dead cells.

Variation in counts occurred both along the small intestine, and between

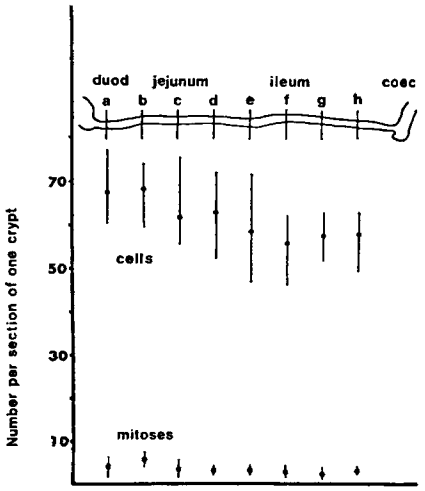

Fig. 1. Number of cells per crypt section along the small intestine at 8 places spaced at equal intervals between the stomach and the caecum. The vertical bars indicate the range of the mean values obtained from 6 mice.

individual mice. Fig. 1 gives the results of counts of the number of epithelial cells per section of a crypt from eight different parts along the small intestine in 6 control mice. The decrease in number of cells per section towards the aboral part is gradual and in no place abrupt. The exact location from where the specimen is taken is therefore not critical. The mean value ± 1 SD of cell counts from mid-way between the duodenum and caecum in 42 control mice was 57.6 ± 7.5.

Record was made of those crypts in a transverse section of the intestine that demonstrated the basal part of the lumen to obtain data on the relative changes in the cell population of the whole intestine. Since this number would depend on the diameter of the lumen, considerable variations were expected. In the preparations with the most marked changes this estimate was considered to be better than counting all the crypts.

The mitotic counts included the stages from prophase to early telophase, when the nuclear membrane was absent. Mitoses normally exhibited considerable diurnal variations with peak values in the afternoon and early morning in these mice. The mice were irradiated and sacrificed at different times of the day, but it was considered that corrections for diurnal variations in the irradiated mice were not necessary and would introduce an unnecessary complicating factor. The mean number ± 1 SD was 3.7 ± 1.9 mitoses per section of one crypt in 47 control mice.

The more central location towards the axis of the crypt of mitoses of many of the dead cells as well as of the Paneth cells, implies that counts performed

in sections represent and overestimate of their frequency relative to the other cells. TANNOCK (1965) estimated the true value for mitoses to be about 57 % of the counts observed in normal mice. Such corrections have not been made, however, because the factor obviously varies with the radiation reaction in the crypts, and in the case of Velbe administration numerous mitoses also occurred towards the periphery of the crypts.

Dead cells were easily identified by their eosinophilic or lysed cytoplasm, and pyknotic or fragmented nuclei with dense staining and loss of granulated structure; some of them were encountered in the lumen of the crypt. When dead cells were numerous, the exact number per sectioned crypt was difficult to assess because of the fragmentation of the nuclei. In sections from normal mice the number of dead cells varied considerably, the mean value ± 1 SD from 47 mice being 0.83 ± 0.85; the median value was 0.5, and two thirds of the values were between 0.3 and 1.2 per section of one crypt.

The Paneth cells with their granulated cytoplasm in the bottom of the crypt were in most sections easily distinguished from the other cells. The mean number per section ± 1 SD was 6.1 ± 1.5.

Goblet cells and argentaffine cells have not been recorded separately in the present work.

The counts were made by a trained assistant without knowledge of radiation dose and time intervals of the specimens. To test the reliability of the counting procedure the assistant was asked to count again 11 slides she had counted seven months previously, without knowing the identity of the slides. The average of the first counts was 47.3 cells per crypt section and on the second 48.4, and in no single slide was the deviation between the two values more than 7.3 % per crypt section.

Effect of narcosis. Since some of the mice were anesthetized during irradiation, it must be pointed out that anesthetics and narcotics may afford a slight protection to whole body irradiated animals (COTTIER 1966) probably due to induced hypoxia although increase in mortality has also been reported (ZAUDER 1959). The Nembutal narcosis was not deep enough to cause cyanosis, which is easily observed in experiments with hypoxia in albino mice. Pilot experiments with 45 mice revealed no clear difference in radiation lethality between anesthetized and non-anesthetized mice (the quotients indicate the mice surviving 30 days/mice irradiated):

Doses:	600 R	700 R	800 R
Anesthetized mice	4/7	1/8	0/7
Non-anesthetized mice	3/8	1/8	1/7

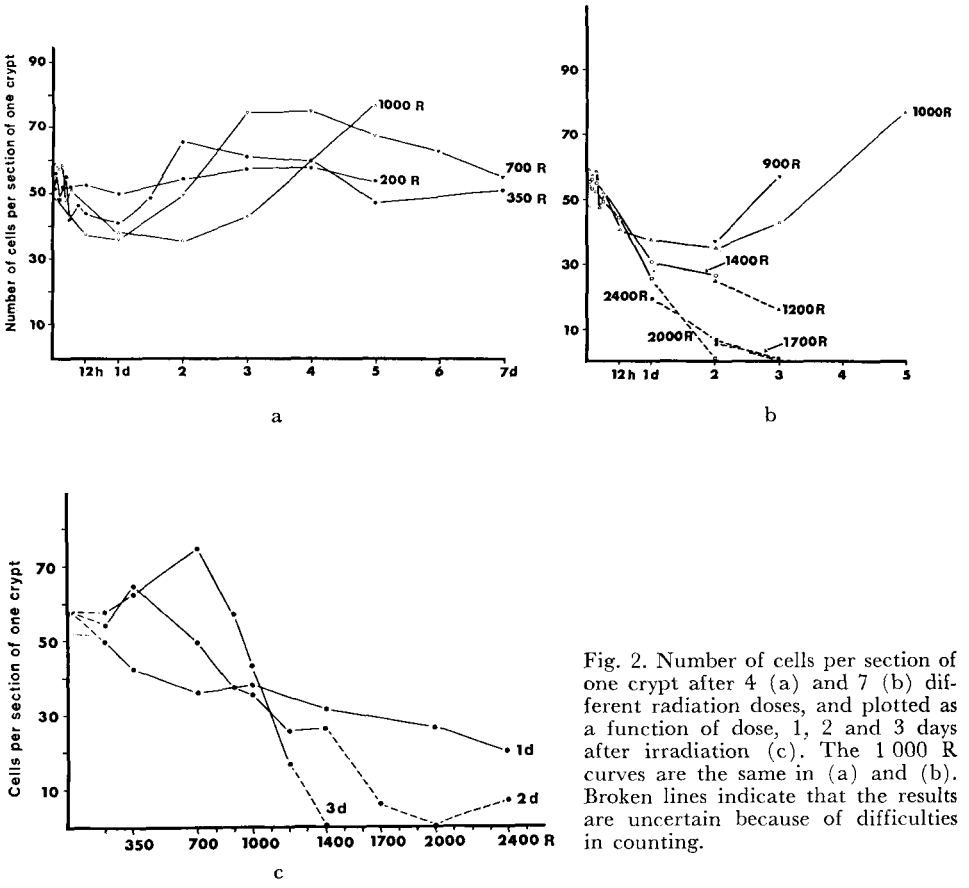

Fig. 2. Number of cells per section of one crypt after 4 (a) and 7 (b) different radiation doses, and plotted as a function of dose, 1, 2 and 3 days after irradiation (c). The 1 000 R curves are the same in (a) and (b). Broken lines indicate that the results are uncertain because of difficulties in counting.

Furthermore, differences in cell counts between such groups have not been significant. A possible effect of narcosis is therefore considered to be slight enough to be disregarded in these experiments, and the results from anesthetized and non-anesthetized mice have been pooled.

Results

Most points on the curves represent the arithemtic mean values from 4 mice, in some cases 6 or more, and in a few instances 2 mice. Comparison between results obtained at different doses and time intervals should ideally be made in mice from the same batches in experiments done at the same time, but for practical reasons it has been necessary to perform a number of smaller series

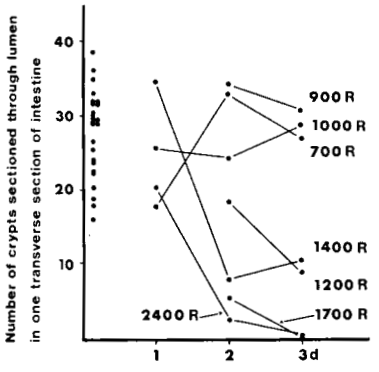

Fig. 3. Relative number of crypts in a transverse section of the intestine. The points close to the ordinate illustrate the distribution in normal mice.

of separate experiments. It has been found justifiable to pool the results since they are coherent and well reproducible.

The number of crypt cells is given in Fig. 2, a and b. With doses above 200 R there is a reduction in cell number during the first day followed by an increase after two days in the 350 and 700 R series and after three days in the 900 and 1 000 R series. The increase continues to values higher than normal after 350, 700 and especially after 1 000 R. After 1 200 and higher doses the initial decrease continues during the three days of observation, approaching zero values for doses of 1 400 R and above.

The data may be plotted in a different way, as in Fig. 2c with dose instead of time along the abscissa, and each curve referring to a given time interval instead of a dose. It is seen how the decreased number of cells the first day does not change much with increasing dose. A compensatory increase is indicated on the second day following low doses, the increase becoming marked on the third day and after higher doses, when the values on this day decrease steeply towards zero around 1 400 R. It should be noted that counting of cells was difficult in sections with marked radiation changes and that the very low counts are indications rather than accurate figures.

The relative number of crypts did not decrease below normal after doses of 700, 900 and 1 000 R (Fig. 3). A decrease was observed after 1 200 R and higher doses. This did not become apparent until the second day, and was considerable on the third day when, following 1 700 and 2 400 R, crypts were almost absent.

The results of mitotic counts per crypt after seven dose levels are given in Fig. 4. The well known initial depression has not been detailed. Except for the highest dose levels, an increase may be noted after two days, and maximum counts are recorded on the third day; thereafter the number decreases.

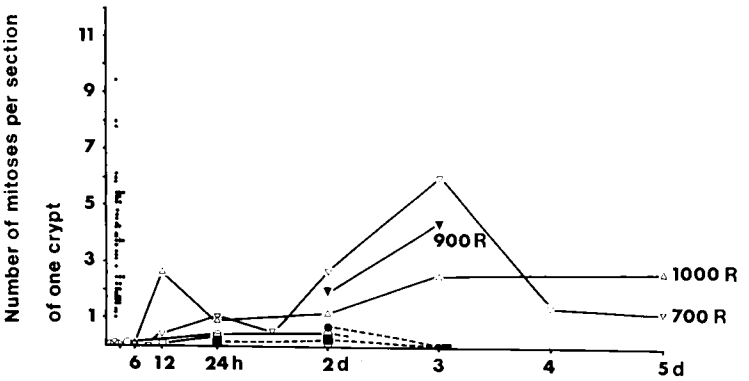

Fig. 4. Number of mitoses per section of one crypt. The points close to the ordinate illustrate the distribution in normal mice. Broken lines indicate that the results are uncertain because of difficulties in counting.

Since mitotic counts do not give information on the rate of cell production unless the mitotic duration is known, vinblastine was injected to stop mitoses, and arrested mitoses were counted at four hours, on the first, second and third days after 1 000 R. The results are given in Table 1. The mitotic rate has in this case been calculated by the formula:

$$\frac{M_T - M_0}{T} = \text{number of mitoses per hour,}$$

where M_0 and M_T are the mitotic index before injection of vinblastine and after T hours. To be valid the method, inter alia requires steady state conditions, and since in this instance the conditions at best may be considered only as an approximation, the figures in Table 2 must be evaluated with reserve.

The number of dead cells observed after six dose levels are given in Fig. 5. About three hours after irradiation, there is a marked peak increasing in size with dose; the numbers then gradually return to near normal values. The dead cells are most abundant in the lower part of the crypts, their distribution being very similar to the distribution of mitoses. The dead cells are also frequent in the Paneth cell region.

The number of Paneth cells (Fig. 6) seems to be influenced relatively little by the radiation as long as there is no marked decrease in cell number. If so, they also disappear; depopulated crypts with only Paneth cells left were not observed.

In the overall cell population kinetics of the crypts the drain of cells to the villi is important, and may be studied by following the migration of labelled

Table 1

Estimated mitotic rate, duration of mitosis and mean generation time 1, 2 and 3 days after 1 000 R — The figures represent average values from 4 mice

	Controls	1 day	2 days	3 days
Before Velbe injection				
Mitoses per crypt section	1.5	1.0	0.5	1.6
Cells per crypt section	58.6	33.5	29.8	38
Mitotic index M_0 $\frac{\text{mitoses}}{100 \text{ cells}}$	2.56	3.0	1.7	4.2
Interval T	3 2/3 h	4 h	4 h	4 h
After Velbe injection				
Mitoses per crypt section	6.1	1.7	3.8	14.2
Cells per crypt section	60.2	28.6	32.0	43.9
Mitotic index M_T $\frac{\text{mitoses}}{100 \text{ cells}}$	10.1	6.2	11.9	32.3
Mitotic rate: mitoses per hour per 100 cells	2.1	0.8	2.6	7.0
Duration of mitosis $= \frac{\text{mitotic index}}{\text{mitotic rate}}$	1.25 h	3.75 h	0.67 h	0.6 h
Mean generation time $= \frac{1}{\text{mitotic rate}}$	48 h	125 h	39 h	14.2 h

cells. Fig. 7 indicates that for two days there was little difference between the migration in the normal intestine and after radiation with doses of 700 and 2 000 R. The distance of migration recorded was from the base of the villus to the most advanced of the labelled cells on the villus. The changes seen in sections of the small intestine up to five days after 1 000 R are illustrated in Fig. 8.

Discussion

A full account of the population kinetics of the irradiated epithelial cells in the small intestine is a complex task because of the great variability in size, shape and number of villi. Since it is usually easy to distinguish between cells in crypts and cells on villi, the present study has limited the quantitative investigations to the cells in the crypts; these have tentatively been regarded as a closed population in which it is possible to characterize quantitatively several

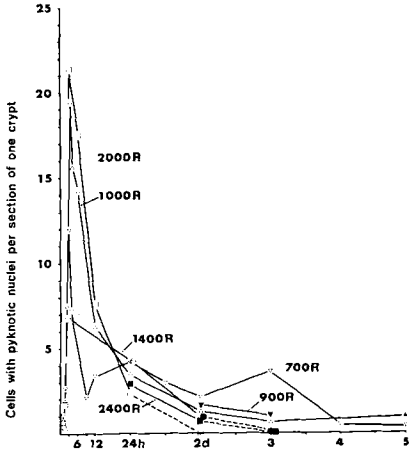

Fig. 5. Number of dead cells recorded per section of one crypt. Broken lines indicate that the results are uncertain because of difficulties in counting.

main parameters of population kinetics. The number of cells on the villi and the total life span of the epithelial cells have been determined. It should also be mentioned that characteristics of crypt cells under abnormal conditions may be found on villi, e.g. cell divisions, but this has only been observed occasionally in the present experiments when extreme changes existed. The present results are in accordance with previously published information on radiation effects in the intestines, where such information is available.

The immediate and strong influence on the number of dividing cells corresponds closely to the detailed data from the rat published by WILLIAMS et coll. (1958). A temporary and compensatory 'overshoot' of mitoses is observed after doses that are not too high. For cells in vitro (ELKIND et coll. 1963) mitoses disappear for a time where the number of hours is about the same as the dose expressed in hundreds of rad (e.g. four hours following 400 rad), and a similar relationship seems to apply to the mitoses in the crypts of the mouse.

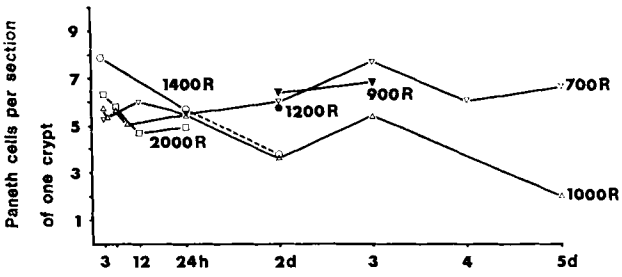

Fig 6. Number of Paneth cells per section of one crypt. Broken line indicates that the result is uncertain because of difficulties in counting.

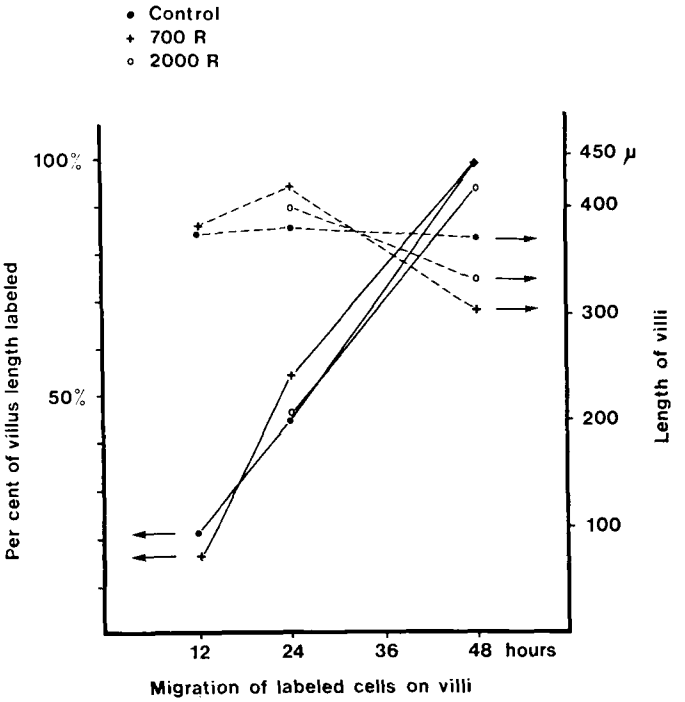

Fig. 7. Migration of labelled cells along villi. Fully drawn lines refer to the ordinate to the left, which indicates the percentage of length of villi, and broken lines refer to the ordinate to the right, which gives the length of the villi observed.

Abnormal mitoses with chromosome aberrations are conspicuous when cell divisions begin again after the mitotic depression, but their relative number decreases rapidly, and two days after 700 R most of the mitoses in the crypts have normal appearances (DEVIK 1968).

Regarding the dead cells with pyknotic nuclei, it is interesting to see the abrupt increase in their number about two hours after irradiation at all dose levels, with a maximum at about three hours. The maximum apparently correlates with dose in spite of the counting difficulties when many dead cells are present. The value of the maximum as a possible indicator of radiation dose is limited, however, by the narrowness of the peak on the time scale. It is not known how long the dead cells remain in the crypts after they can be recognized as dead. The steep falling part of the curves indicate that this 'clearance' time is of the order of a few hours or even less for many of them; others may remain for several hours.

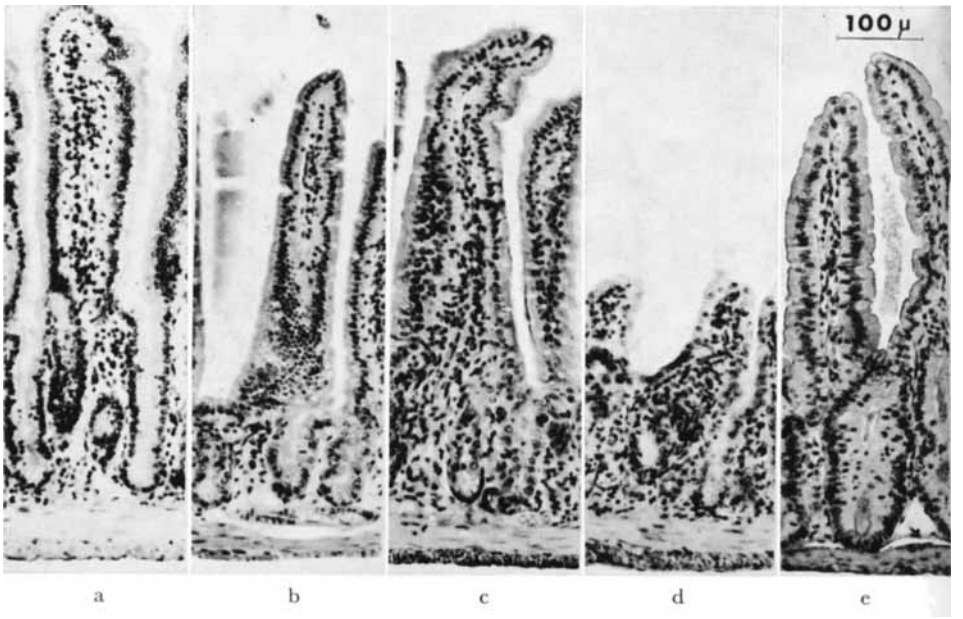

Fig. 8. Changes in the small intestine after 1 000 R whole body irradiation. a) Control. b), c) and d) The crypts present marked changes 1, 2 and 3 days, respectively, after irradiation. The villi are little different from normal after 1 and 2 days, but on the 3rd day their size is greatly reduced. e) Regeneration of villi and crypts is almost complete by the 5th day.

The cells that become pyknotic after irradiation seem to have been in a state closely or directly related to the processes preparatory to mitosis. A great part of the pyknotic nuclei are labelled when tritiated thymidine is injected before irradiation (SHERMAN & QUASTLER 1960, DEVIK 1968). When the label was injected five hours before a dose of 350 R, 68 % of the pyknotic nuclei were found to be labelled two hours later. These cells had been in the S phase at the time of labelling, but died before entering mitosis. The curves in Fig. 5 clearly demonstrate that cell death may be frequent before the mitotic activity is resumed.

A connection between early pyknosis and mitosis is also indicated by the inverse relationship of the number of cells acutely destroyed and the number of mitoses (WILLIAMS et coll.), particularly after relatively low doses (DEVIK). It is illustrated in Fig. 9 with curves from a set of experiments with split doses; 350 R was given twice with an interval of twelve hours.

In connection with the post-irradiation pyknosis in the crypts, a similar, though less marked appearance of degenerate cells was noted in bone marrow squash from mice, between two and six hours after irradiation with 200 R (DEVIK

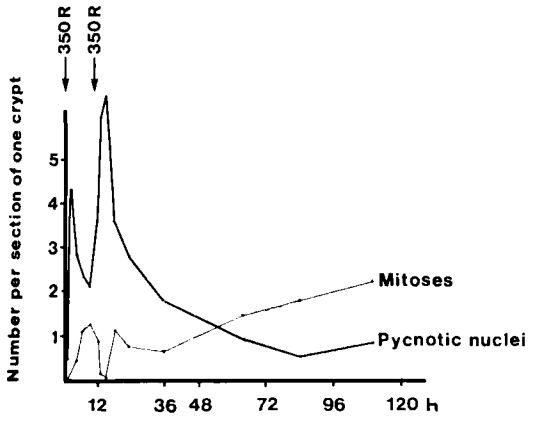

Fig. 9. Inverse relationship of mitoses and dead cells after two doses of 350 R, spaced 12 hours apart.

& LOTHE 1955). This mode of cell death thus occurs in other tissues as well, although it is usually less conspicuous.

Lymphocytes are known to be very sensitive to irradiation, as well as to many other types of injury or influences, and extensive degeneration of these cells may be demonstrated after a short time. Since lymphocytes are highly differentiated cells, they are generally regarded as an exception to BERGONIÉ-TRIBONDEAU's 'law' (1906). However, it is known that lymphocytes may be induced to divide at short notice, and with the relationship between pyknosis and mitosis in the crypts in mind, it is possible that radiation-induced degeneration of lymphocytes may be connected with a preparedness for mitosis. This might be a confirmation of rather than a contradiction to the law.

As a result of cell death, depression of mitoses, and migration of cells to the villi, the number of cells in the crypts is rapidly reduced until cell production is again restored.

A repopulation of the crypts to cell numbers above the normal after the temporary depression occurs after 1 000 R, 700 R, 350 R, and may be indicated after 200 R. LESHER (1968) has pointed out that there may be a marked 'overshoot' after 1 000 R. The results indicate that in these mice the epithelial cells in the crypts can survive and regenerate after lethal doses of whole body irradiation, at least up to 1 000 R. When the dose is increased to 1 200 R, 1 400 R and more, depopulation of the crypts becomes marked in the course of two to three days, and regeneration is not apparent in histologic sections before the mice die. A dose of about 1 200 R seems to be a critical level with respect to intestinal death of the mice. After a single local irradiation of the intestine, regeneration of epithelial cells in the crypts may take place after doses approaching 2 000 R (WITHERS & ELKIND 1968).

Cells may die in ways other than passing through a stage recognized as pyknosis, e.g. by lysis, which is not so easily followed in the microscope. Abnormal cells produced by abnormal mitoses may suffer a delayed death. However, it appears that the early reduction in the number of cells in the irradiated crypts may mainly be accounted for by the pyknotic cells, apart from migration.

The number of cells per section of one crypt may be taken as a good relative indicator of the total number of cells in the crypt as long as the conditions are not much different from normal. When the number per longitudinal section is reduced, however, the number per transverse section will also be reduced, most likely by a similar factor. The total number of cells in a crypt will therefore represent a greater reduction than indicated by the cell counts in the curves in Fig. 2, a and b. A reduction in the number of crypts makes the difference still more obvious (Fig. 3). Cell counts in the transverse sections were made after 1 000 R to calculate approximately the total number of cells in a crypt. The results are given in Table 2. The present results are in good agreement with results obtained by KONONENKO (1968) in mice irradiated with 700, 900 and 1 200 R.

Two to three days after high doses, morphologic changes of the cells left in the crypts may be marked with increase in size, bizarre forms with nuclear irregularities, and changes in stainability. A reduction in size of the villi after a few days takes place when the cell production is severely restricted and there are not enough cells to meet the demand for migration. In view of the profound radiation effects in the crypts, it is somewhat surprising to find that migration of cells from the crypts to the villi is at first little affected even by considerable doses of radiation, i.e. up to 3 000 rad (HUGHES et coll. 1958, SHERMAN et coll. 1958) (cf. also Fig. 7).

Investigations of functional changes in the intestines after irradiation are less numerous than morphologic studies. Reviews have been published by BOND (1963) and BOUCKAERT (1968). Differentiation between effects on epithelial cells of villi or crypts cannot usually be made in investigations of functional changes.

The radiation response of the small intestine in terms of cell survival data may be considered with the present results.

An exponential dose survival curve in cell cultures may be demonstrated for many cell types, and the D_0 or D_{37} (the dose that reduces the number of cells capable of proliferation to 37 %) has usually been stated to be around 100 R. WITHERS & ELKIND (1968) reported an exponential function with a D_0 of 100 R for epithelial cells in the intestinal crypts of mice in experiments performed with local irradiation in vivo. Their data will be used in the following discussion.

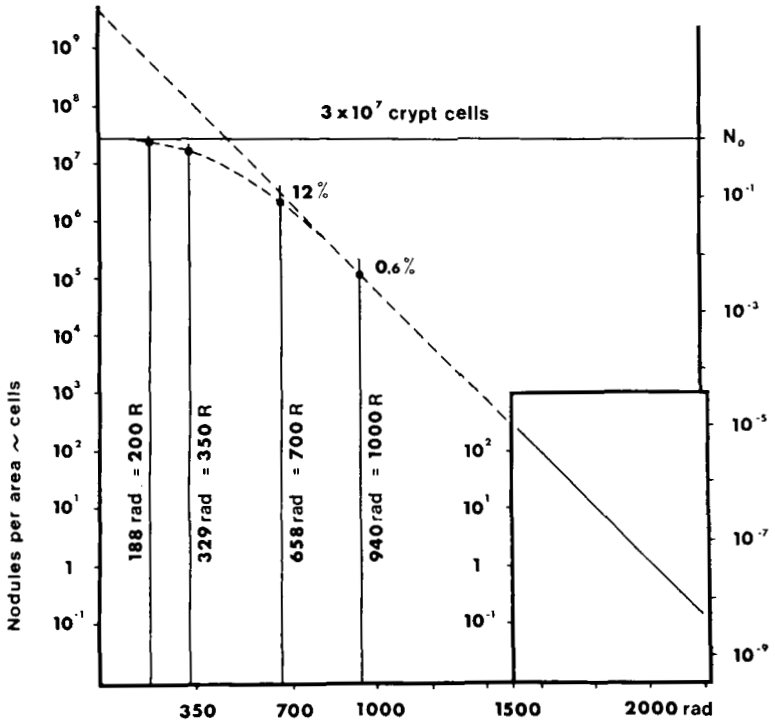

Fig. 10. Extrapolation of the cell survival curve of WITHERS & ELKIND (rectangle to the right).

Discussion

The technique used by WITHERS & ELKIND is restricted to relatively high doses and to that part of the exponential curve where relatively few nodules representing single surviving cells can be counted; it would hardly be possible to make counts after doses that left survivors in every crypt. With this reservation, an extension of their dose survival curve has been plotted in Fig. 10. The lower end seems well established; the upper part must terminate at the number of cells exposed to irradiation, and at dose 0. The total irradiated population of crypt cells in their experiments can be estimated to have been about 3×10^7, a figure that the present author considers unlikely to be wrong by more than a factor of 2 either way. (About 20 mm intestine was irradiated. It is assumed that with an average diameter of 50 μ there are 400 crypts along 20 mm, that there are 150 crypts per transverse section of the intestine, and that each crypt contains 500 cells.)

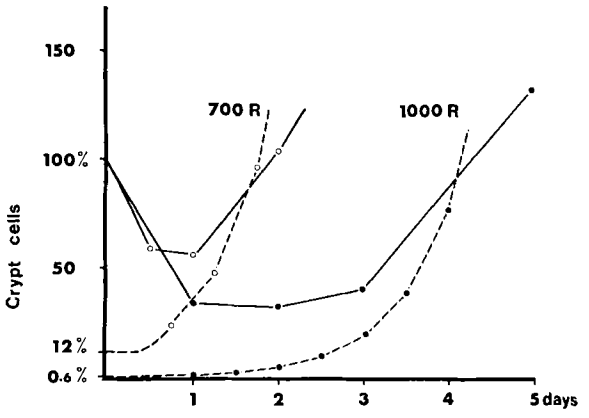

Fig. 11. Cell numbers in crypts after 700 and 1 000 R as percentages of normal values (full lines) and hypothetic repopulation in crypts (broken lines).

The experimental results after 700 R and 1 000 R are compared in Fig. 11 to hypothetic curves derived from the cell survival curve of WITHERS & ELKIND. The numbers of cells following 1 000 R are as in Table 2. The figures for 700 R have been calculated by assuming that transverse sections of the crypts had the same relative change in number of cells as the longitudinal sections. The hypothetic curves have been constructed as follows. The theoretic number of surviving cells following 1 000 and 700 R is estimated to be 0.6 and 12 % respectively (Fig. 10). It is assumed that following 1 000 R the surviving cells divide once in the first 24 hours, and thereafter every 12th hour. Seven cell divisions in the course of 4 days would bring the number of cells to the level observed. The fact that the cell cycle may be even shorter than 12 hours, when the repopulation begins, has been ignored. LESHER & BAUMAN (1969) reported a cell cycle time of less than 7 hours at three days after 1 000 R determined by counting the labelled mitoses. After 700 R the first division is assumed to take place within 18 hours and thereafter every 12th hour. Three cell divisions in the course of 2 days would bring the number of cells to the level observed.

There is an apparent discrepancy between hypothetic and experimental curves in their earlier parts. The degree of depopulation of cells in the crypts after irradiation is much less than expected, even after 1 000 R, if it is assumed that the lethally injured cells disappear and only the proliferating ones remain. The initial degree of cell depletion does not present great variation within the dose range 350 to 1 000 R (Fig. 2a); the cell number alone would not be a good indicator of dose.

The migration of labelled cells along the villi seems to indicate that the migration rate is not much different from the normal for two days both after

Table 2

Estimated total number of cells per crypt following 1 000 R — Each figure represents four mice

	Controls	1 day	2 days	3 days	5 days
Cells per longitudinal section of one crypt (a)	58.6	38.0	35.3	40.7	77.7
Cells per transverse section of one crypt (b)	19.5	10.2	10.4	11.3	19.5
Total number of cells per crypt $\frac{(a)}{2} \times (b)$	571	194	185	230	757

700 and 2 000 R, and therefore presumably also following 1 000 R. However, since a normal rate of migration means a daily cell production of the same order as the normal crypt population, and since the cell production is much decreased during the first two days, the true rate must be lower. A moderate re-arrangement of the anatomic form of the villi may greatly affect the surface of the villi, as pointed out by CREAMER (1964), and it is likely that a reduction takes place. One day after 1 000 R the cell number observed is still about a third of the normal, whereas the surviving cells are expected to constitute only 1.2 % of the normal or 3 % of the actual population at this time. The rest of the cells are supposed to be non-viable, and a further rapid decrease towards a still slowly rising hypothetic curve would be expected. A decrease would also be likely because migration still goes on along the villi (cf. Fig. 7) and presumably also out of the crypts.

This initial discrepancy between the observed and the hypothetic number of cells becomes less marked when relevant modifying factors are taken into account:

1. If lethally injured cells were able to divide one or several times before they succumb, a fall in the number of cells would be counteracted. Abnormal cell divisions in the crypts produce abnormal cells, many of which may be recognized by nuclear abnormalities, fragmented nuclei and micronuclei. Such cells are found in the crypts one, two and three days after 1 000 R. Some of them may lie at the base of the villi after one day, and a little higher after two days, when most of the cell on the villi appear normal. After three days, when the villi are shrunken, their epithelium is abnormal, and many cells have fragmented nuclei and micronuclei, indicating that by now the normal-appearing cells have been shed and replaced with what is available of abnormal cell offspring. The presence of such cells indicates that they are at least a contributing factor to explain the discrepancy.

2. Lethally injured cells may stay alive for some time and prevent a further depletion in the number of cells until regeneration is well established. It seems likely that this factor is of significance.

3. Repair of lethally injured cells in addition to repair expressed by the 'shoulder' of the exponential curve could take place at these dose levels. It may be that this factor is significant. It is not possible at present to decide which of these three factors is more important, but it is considered likely that together they may account for the values observed.

4. Substitution of cells by migration from outside could also be mentioned but this possibility is considered so unlikely as to be disregarded.

When regeneration starts and the number of cells again increases, the time for the increase observed seems to coincide closely with the hypothetic increase, which therefore could account for this part of the curves. It may be concluded that the experimental results are compatible with an exponential cell survival curve, as illustrated in Fig. 10. It would however be difficult to postulate compatibility if the curve had a course that gave markedly fewer survivors than the 0.6 % and 12 % as indicated for the two higher dose levels.

The suggested extrapolation of the curve is also in agreement with the observations on the numbers of crypts. Allowing for considerable variability of this parameter, it is evident from Fig. 3 that it is hardly affected by doses up to and including 1 000 R. A decrease is observed with doses of 1 200 R and more. Assuming that one intact cell is enough to repopulate a crypt (which has a population of about 500 cells), a reduction in the number of crypts would be expected when the probability for survival is less than 1 : 500. This value on the exponential curve corresponds to 1 000 rad or 1 060 R, which is rather close to the dose level at which the number of crypts becomes reduced.

The extrapolated curve in Fig. 10 indicates an extrapolation number of the order of 100, which is a high value for such a figure. A lower extrapolation number would be obtained if the number of cells had been much underestimated, or if the slope of the curve were less steep. Since all the cells in the crypts are regarded here as potentially dividing stem cells, the number of cells seems to be overestimated rather than underestimated. Any significant change in the slope would mean a considerable departure from the data obtained by WITHERS & ELKIND.

A high extrapolation number suggests a high degree of ability of the cells to repair injury. Based on data from irradiation with split doses, WITHERS & ELKIND described a recovery factor of about 60 at 24 hours after 700 R. On the basis of split dose experiments, HORNSEY & VATISTAS (1963) also suggested a high degree of recovery, with an extrapolation number of more than 28, assuming a D_0 of 120 R. From the hypothetic curve in Fig. 10 it may be

estimated that two thirds of the cells would survive a dose of 350 R, and practically all cells a dose of 200 R. The experimental data (Fig. 2a) seem to agree fairly well with this. Available experimental evidence therefore appears to agree that an exponential cell survival curve of the crypts has a high extrapolation number, perhaps of the order of 100.

The ability of the intestinal epithelium for repair and regeneration is also well illustrated by LAMERTON's report on experiments with continuous irradiation of rats (1966), in which he called attention to the 'remarkable capacity for recovery of normal crypt population even after a total accumulated dose of about 2 000 rad in 5 days'.

The quantitative determination of cellular changes in a closed cell population after irradiation provides information of general interest, both with respect to time and mode of death of cells, cell production, cell migration, size of cell population, and regarding the extrapolation number N and the D_0. If radiobiology is going to provide a better theoretic background for radiotherapy, it is especially important to know the latter parameters for as many cell types as possible. It seems likely that an analysis of the crypt cell population may also provide information of value with respect to the mode of action of drugs and cytotoxic agents.

The quantitative data mainly provide an illustration of injury suffered by actively dividing cells, which constitute the majority of the cells of the crypts. The Paneth cells are differentiated cells, which in part consist of cells that either have a long generation cycle, or that no longer divide (DEVIK & IVERSEN 1970). Their number is found to be little affected during the first days after irradiation, but they disappear when the number of cells in the crypts is much decreased. The results indicate that the Paneth cells either are destroyed by irradiation in the course of a few days when the dose is high enough, around 1 200 R, or that their existence depends upon the presence of the other epithelial crypt cells.

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SUMMARY

Quantitative estimates were made of the number of epithelial cells, mitoses, dead cells and Paneth cells in the intestinal crypts of mice following increasing doses of whole body

roentgen irradiation at intervals of up to seven days. The results are compatible with an exponential cell survival curve with a high extrapolation number. The crypt cell population may prove useful in investigating reactions of rapidly proliferating cell populations to various external influences.

ZUSAMMENFASSUNG

Es wurde die Zahl der epithelialen Zellen, der Mitosen, der toten Zellen und der Paneth Zellen in den Darmkrypten von Mäusen nach steigenden Dosen von Ganzkörper-Röntgenbestrahlung in Intervallen bis zu sieben Tagen bestimmt. Die Resultate lassen sich mit einer exponentiellen Zell-Überlebenskurve mit einer hohen Extrapolationszahl vereinen. Die Population der Krypten mag bei Untersuchungen der Reaktionen einer rasch proliferierenden Zell-population unter verschiedenen äusseren Einflüssen anwendbar sein.

RÉSUMÉ

L'auteur a fait des estimations quantitatives du nombre de cellules épithéliales, de mitoses, de cellules mortes et de cellules de Paneth dans les cryptes intestinales de souris après des doses croissantes d'irradiation totale du corps par les rayons de roentgen à des intervalles allant jusqu'à sept jours. Les résultats sont compatibles avec une courbe exponentielle de survie des cellules avec un nombre d'extrapolation élevé. La population de cellules des cryptes peut se montrer utile dans l'étude des réactions de population cellulaire à prolifération rapide soumise à différentes influences externes.

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