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CELL POPULATION STUDIES ON THE INTESTINE OF CONTINUOUSLY IRRADIATED RATS¹

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INTRODUCTION

Several studies have been made on changes that occur in the epithelium of the gut in rats exposed to continuous irradiation (1-3), the analysis of the intestinal cellular kinetics being based on data taken from tissue sections. However, cell counts from sections, even when very carefully aligned and cut, do not provide a measure of total cell population in crypt or villus. In the present study a new method is introduced in which whole mounts of crypts are made and data taken from the entire cell population, i.e., total number of cells, cells in division and cells in DNA synthesis. Some of the findings obtained with this method have previously been published (2): they include a time course study on rats exposed to 415 rads per day up to 5 days and up to 10 days recovery following irradiation. The present report gives data on animals exposed for 5 days to 84, 176, and 415 rads per day. Irradiation for 5 days was chosen because, at 415 rads, the population is in a steady state at this time and it was considered likely that was also the case with the lower doses. However, this point has not yet been tested.

Since this study is a continuation and expansion of earlier results, a brief enumeration of the previous findings is in order:

1. Cell debris was common in the crypt during the first and second day of irradiation but was infrequent thereafter.
2. The percentage of cells labeled by a single injection of H^3 -thymidine fell slightly on the first day and then returned to normal levels by the fifth day. In contrast, the number of mitoses per crypt

was greatly lowered on the first and second day and then rose slowly.

3. The cell generation time (using the labeled mitosis method (4)) appeared to be unchanged from normal in animals subjected to 5 days irradiation and in animals having 2 days recovery time after 5 days at 415 rads per day; however, the data do not exclude the possibility of a small to moderate change in G_1 (5).

4. The number of cell rows in tissue sections (see Fig. 2b) of crypts was not found reduced during exposure up to 415 rads per day. With the method of crypt squashes, it was found that at the same dose rate the total number of cells per crypt was reduced to one-half the control level by the second day of irradiation and was maintained at this number through the fifth day. Recovery of the crypt to normal size and cell numbers occurred several days after the removal of the animal from the radiation field.

5. The villus row count was found to decrease during the first two days of irradiation, and then appeared to reach a constant value which was smaller the higher the dose rate.

It was concluded that a steady state of cell renewal had been reached in the rat gut at about 2-3 days at 415 rads per day. The evidence suggested that in some way or other the cells became adapted to the continuous irradiation and that cell death became less frequent after an initial period of exposure.

This paper will discuss the cell population changes in the intestinal crypt of the rat after 5 days of irradiation at dose rates from 84 to 415 rads per day. An increase in the incorporation of H^3 -thymidine into

the nuclei of the intestinal epithelium at 415 rads per day will also be reported.

MATERIALS AND METHODS

Male rats of the "August" strain, weighing 100-120 grams and approximately 6 weeks old, were used in this work. Except where otherwise stated, all animals were exposed to continuous gamma irradiation for 5 days in the Cesium¹³⁷ unit at the Physics Department, Institute of Cancer Research, Royal Cancer Hospital (1); dose rates were 84, 176 and 415 rads per day. Animals used for autoradiographic studies of DNA metabolism were injected intraperitoneally with 25 μ c H³-thymidine (1.9 c/ μ l) in normal saline (100 μ c/ml) and sacrificed under ether anesthesia one hour later. A small piece of the lower ileum was removed and fixed in acetic-alcohol (1 part glacial acetic acid and 3 parts absolute ethyl alcohol) for a minimum time of 24 hours. Tissues were then stored in 70% ethanol a few days or if slides were immediately prepared, the tissue was treated for 10 minutes in each of 70%, 30% ethanol and distilled water. The Feulgen staining reaction was used; the tissue was hydrolyzed for 15 minutes in HCl at 60° C, followed immediately by an ice water rinse, and placed in Schiff's reagent for at least one hour. The stained tissue was placed on a slide in a drop of 45% acetic acid, slit open, laid back and positioned with the villi pointing up. The crypts were removed from the tissue under a dissecting microscope by gently probing the tissue with a fine wire. A syringe needle cleaning wire mounted in a holder is suitable for this purpose. The tissue was held in place by a spear-shaped dissecting tool

or the wire. After the crypts or villi were loosened from the surrounding tissue they were removed from the acid by working them above the surface of the drop onto the dissecting tool by gentle use of the small wire. The tissue on the needle was transferred to another drop of 45% acetic acid on a clean, subbed slide and floated off. A square cover slip was placed on top of the drop after several crypts were transferred. The crypts were squashed by firm pressure from the fingertips on the cover slip; a folded piece of filter paper absorbed the excess moisture. The slide was then carefully warmed over an alcohol lamp, the cover slip removed by the dry ice method (6) and the slide placed in 100% ethanol. The slides to be autoradiographed were passed through an alcohol series to water or stored in 70% alcohol until needed. Slides not autoradiographed were simply removed from the alcohol and made permanent with "Euparal."

Crypts from both rats and mice can be easily dissected by this method and villi of mouse ileum are small and easy to remove, but rat villi are large and difficult to separate and only epithelial cell samples have been obtained from them. This technique has been adapted for epithelium in several other sites in the alimentary canal, in the cornea, bladder epidermis, etc. Generally a 10 minute acid hydrolysis time is sufficient for Feulgen stained tissues other than those in the alimentary tract.

The autoradiographs were made by the "dipping" technique using the Ilford K5 liquid emulsion. Slides were exposed 7 days in light tight black plastic boxes at 4° C and developed in Kodak D 19b for 6 minutes and fixed. Figure 1 shows a squash of a whole crypt. The labeled cells are plainly recognizable. The number of grains over each cell is proportional

to the amount of tritium in that cell (7).

Tissue sections used in this study were prepared by standard techniques using Bouin's fixative and hematoxylin and eosin stains. The intestine was sectioned so that the crypts appeared in cross section for cell column counts and lengthwise for cell row counts. Figure 2.

RESULTS AND DISCUSSION

The total number of cells per crypt found after 5 days of irradiation decreases with increasing dose rate. Table I and Figure 3 illustrate these data. Crypt counts of rats receiving the same treatment are found to differ by amounts which are statistically significant at the five per cent level. The number of labeled cells and the number of mitotic figures per crypt also decreases with increasing dose rate, but relatively less than the total number of cells so that the percentage of labeled cells and the percentage of cells in mitosis (mitotic index) increases slightly with dose. If the number of mitoses per crypt is divided by the number of labeled cells per crypt, a ratio is found that should remain constant if the relative duration of DNA synthesis to mitosis is constant. These values of this ratio are given also in Table I; they are constant within the limits of error. These data together with previous findings on the duration of the nuclear cycle in the crypt at 415 rads per day suggest that the cycle is little altered under continuous irradiation, although an alteration of G_1 cannot be excluded. We have not checked the generation time at the two lower doses. If the generation time is constant, we can deduce that: (1) the over-all cell production per crypt is reduced

in the irradiated animal, (ii) if the villus transit time is also unchanged, which would seem to be the case (1), then the villus population would decrease and indeed, the villi are found to be shortened in row counts of sectioned material and (iii) the increase in percentage of cells labeled at the higher doses indicates that maturing cells spend less time than normal in the crypt, unless $\bar{G}_1$ is shortened.

In previous work (1, 3) the number of cell rows along the length of the crypt was found to be the same in irradiated and control animals. This led the authors to assume that the crypt size was unaffected. The data here presented on the basis of crypt squashes show that there is a reduction in crypt cell population. Counts have also been made, from cross sections of crypts, of the number of cell columns (Fig. 2a) and Table II shows that the number of cell columns per crypt is reduced after 5 days exposure. Furthermore, contrary to the previous work, the number of cell rows along the length of the crypt was also found to be slightly reduced. An estimate of crypt cell population is given by the product of row count and column count. This agrees reasonably well with the value obtained from crypt squashes insofar as the percentage reduction from the control value for each of the dose rates is approximately the same for both methods (see Tables I and II). The actual cell population as determined by the product of row count and column count is consistently lower than that found on direct counts on squashes. The crypt, in preparation for squashing, breaks off on a point higher than that considered as the crypt boundary on sections. We cannot explain why the results of the row counts of crypt cells were not the same in earlier work and in this experiment. In spite of this apparent

difference in response we are certain that the number of crypt cells is reduced by irradiation, as shown by the more accurate whole crypt technique. The data from the current experiments illustrate that under chronic irradiation at these levels of dose rate there is a change in the crypt shape, with the cross section of the crypt suffering a greater reduction than the crypt length. In the earlier studies the crypt itself showed no reduction.

A technique for the study of the entire villus cell population in the rat has not yet been developed. Quastler et al. (1) reported from data taken on sections that a decrease in total numbers of cell rows on the villus occurs during irradiation.

Autoradiographic studies were made on squashed, whole crypts from animals exposed to 84, 176 and 415 rads per day for 5 days. Mean grain counts from incorporated tritiated thymidine in DNA were the same at 84 and 176 rads per day as in the controls. However, at 415 rads per day the number of grains was increased to about twice the control value. Figure 3 shows these results. Though the cell counts in the crypts are reduced at all levels of irradiation the grain count is altered only at the highest dose rate. The hypothesis that the doubled grain count was due to tetraploidy has been disproven by cytophotometry of Feulgen stained cells (8). Several other suggestions that might explain the increased uptake at 415 rads/day are: (i) an increased availability time, (ii) an alteration in the metabolic pathways leading to DNA synthesis, (iii) increased cell permeability, (iv) an increase in tissue concentration of labeled thymidine resulting from a reduction in the number of synthesizing

cells, etc. So far, the evidence supporting any one of these suggestions is small.

SUMMARY

1. Whole crypt squashes allow determinations of the total cell population of crypts, of the number of cells labeled with H^3 -thymidine, and of the number of mitoses.

2. At 415 rads per day, the crypt cell population decreases rapidly and reaches a steady state in two days; it remains approximately constant from then on (as long as observed). It is likely but not established that a steady state has been reached after 5 days at 84 and 176 rads per day; the time course of crypt cell populations has not been followed at these dose rates.

3. The crypt cell population found after 5 days irradiation decreases with increase in dose rate.

4. The "total mitoses:labeled cells" ratio indicates that the relative duration of DNA synthesis or mitosis is not substantially altered under chronic irradiation.

5. The percentage of cells labeled by a single injection of tritiated thymidine is increased at 176 and 415 rads per day. It follows that either the time maturing cells spend in the crypt is shortened, if the generation time remains at control levels; on the other hand, a shortened G_1 period would produce the same effect.

6. H^3 -thymidine incorporation per labeled crypt cell is approximately double that of controls at 415 rads per day. At lower doses there

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is no difference from control levels as far as observed.

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FIGURE LEGENDS

Fig. 1. Autoradiograph of a squashed crypt from rat intestine. Inset shows crypt area magnified in lower photograph.

Fig. 2. Sectioning and counting method used for:

- a. cell columns in crypts
- b. cell rows in crypts.

Fig. 3. Cell population parameters after 5 days continuous irradiation.

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BNL Neg No. 4-146-62 Fig 2a

BNC Neg No. 4-147-62 Fig 2b

CELL POPULATION PARAMETERS AFTER 5 DAYS
CONTINUOUS IRRADIATION. EACH POINT REPRESENTS
THE MEAN OF THE CRYPTS FROM ONE ANIMAL.

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TABLE I

Cell population on crypts of rats continuously irradiated for 5 days (means)

		Dose Rate				Mean Ratio of Std. Error of Mean Mean
		Controls	84 rads/day	176 rads/day	415 rads/day	
Total Cells	No. rats	10	3	4	5	0.10
	No. crypts	51	22	46	17	
	Mean cells per crypt	549	549	473	382	
	Per cent of normal		70	56	45	
Mitoses	No. rats	8	3	4	3	0.20
	No. crypts	40	22	46	11	
	Mean mitoses per crypt	13.8	12.5	7.0	10.3	
	Per cent of normal		91	51	75	
Labeled Cells	No. rats	2	2	2	2	0.04
	No. crypts	8	8	8	4	
	Mean no. labeled cells	182	101	121	103	
	Per cent of normal		56	67	57	0.18
	Mean per cent of cells labeled	21.4	21.3	25.6	27.0	
	Per cent of normal		100	120	126	
	$\frac{\text{No. mitoses}}{\text{No. labeled cells}}$	0.08±.02	0.12±.03	0.06±.03	0.10±.04	

TABLE II

Mean number of cell columns and cell rows in crypts irradiated at several levels of continuous irradiation for 5 days. Two animals per dose level used.

	Controls	84 rads per day	176 rads per day	415 rads per day
Mean number of cell rows 18 samples/animal	28 31	24 26	20 22	20 22
Mean number of cell columns 34 samples/animal	25 24	22 19	17 18	14 14
Product of mean number of rows and columns	723	512	367	294
Per cent of controls	100	71	51	41

END