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The Influence of Rat Age on Crypt Cell Kinetics after Partial Resection of the Small Intestine[1] (40099)

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Any investigator assumes *a priori* that data derived from studies utilizing different ages of animals cannot be rigorously compared. Several papers dealing with the compensatory response to intestinal resection have utilized animals of quite different age and weight and rarely has the stock been the same (see Table I). This study was designed to test the premise that the number of crypts per small intestine varies with animal weight and that indigenous crypt number will influence some features of the compensatory response of the crypt and villus cell population normally seen after intestinal resection (1–3).

A reduction in the total villus and crypt cell population by surgical removal of a portion of the intestine is soon followed by compensatory responses, the most prominent being alteration in values for several parameters of mucosal cell kinetics (2, 3).

The compensatory response to partial resection was assessed by the determination of (a) proliferative activity per crypt, (b) proliferative activity per milligram of tissue, (c) the number of crypts per milligram of tissue, and (d) the amount of tritiated thymidine (^{3}HTdR) incorporated per epithelial cell nucleus. The values obtained from the above measurements were compared with values obtained from control animals and those with varying degrees of intestinal resection.

Materials and Methods. Male juvenile (70 ± 10 g) and adult (350 ± 30 g) rats of the Holtzman and Sprague Dawley stocks were used. Two stocks of animals were used because not enough animals of one stock could be obtained at the time of the experiment.

The animals underwent transection, resection of the small intestine, or a simple laparotomy. The procedures were the same as described elsewhere (2). The mid-portion of the combined ileum and jejunum was transected or resected as noted below and in Table II.

Four groups, i.e., adults and juveniles of both stocks, were studied. In each of the four groups, 5° of resection were performed (0, 10, 50, or 80% resection or simple transection) with five animals per category.

The animals were housed in a room with constant temperature and a 12-hr light–dark cycle in wire bottom cages in groups of 6–8. For the first 24 hr postoperative, the animals were allowed 5% sucrose. Ten grams of laboratory chow per animal were allowed during the second 24-hr postoperative period. Laboratory chow and tap water *ad libitum* were available beginning the third postoperative day.

Fourteen days after a surgical procedure, [which is 2 days after the compensatory response reaches a plateau (1)], the animals were injected via the femoral vein with ^{3}HTdR, 6.0 Ci/m*M*, 1 μCi per g of body weight. One hr after ^{3}HTdR injection two 1 cm pieces of ileum were removed from an area 5 cm proximal to the ileocecal junction, fixed in Carnoy's solution for 24 hr, then stored in 70% ethyl alcohol.

Tissue was hydrolyzed in 1 N HCl and stained with the Feulgen reaction. Fifty crypts were isolated by microdissection in triplicate, placed in a counting vial to which a liquid scintillation mixture of toluene, PPO (5 g/liter) and DMPOPOP (0.3 g/liter) was added (1). After being dark adapted for 12 hr, the vials were counted to 10,000 counts in a Beckman liquid scintillation spectrometer, and dpm obtained by entering the data into the "CBLIQUID" computer program (CBLIQUID program obtained from C. Bischof and F. Kirchner, Radiation Research Laboratory, University of Iowa, Iowa City, IA) which calculated dpm from cpm and external standard channels ratio data.

Since one purpose of this investigation was

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TABLE I. CHARACTERISTICS OF LABORATORY RATS USED BY REPRESENTATIVE INVESTIGATORS IN THE STUDY OF INTESTINAL EPITHELIAL CELL KINETICS.

Investigator	Stock	Sex	Original weight	Interval between resection and sampling
Loran & Crocker (5)	Sprague-Dawley	Male	300–350 g	2 months
Dowling & Booth (3)	Wistar-Firth	Female	150–220 g	6 months
Clarke (4)	Albino Wistar	Male	143–183 g	No resection
Hanson & Osborne (2)	Holtzman	Male	250–300 g	2 months
Tutton (6)	Holtzman	Male	"adult"	No resection
Rijke *et al.* (7)	Glaxo-Wistar	Male	250 g	2 months

TABLE II. COMPARISON OF PARAMETERS MEASURED FOR SIGNIFICANT INFLUENCES OF AGE, STOCK OF RAT, AND PERCENTAGE OF INTESTINE RESECTED.[a]

	Influences					
	Stock	Age	Percent resection			
Parameters measured	(Sprague–Dawley vs Holtzman)	(70 g vs 250 g)	T	10	50	80
dpm/Crypt	>0.500	<0.001	>0.500	<0.500	<0.100	<0.050
dpm/mg	<0.500	<0.001	>0.500	>0.500	>0.500	<0.500
Crypts/mg	<0.100	>0.030	>0.500	>0.500	<0.100	<0.010
LN/Crypt	<0.300	<0.400	<0.200	<0.200	<0.020	<0.050
dpm/LN	<0.500	<0.001	<0.400	<0.200	>0.500	>0.500

[a] All entries are probabilities of means being equal.

to determine whether dpm/labeled nucleus (LN) was constant throughout the various age and treatment groups, individual Feulgen stained crypts were "squashed" to spread the cells in a monolayer, dipped in Kodak NTB nuclear track emulsion, and exposed for a period of 7 days. The slides were then developed and 10 crypts per animal scored for the number of labeled cell nuclei. The values for dpm/crypt were then divided by the values for LN/crypt to obtain the values of dpm/LN. If the values of dpm/LN were constant, the dpm per piece of tissue was proportional to the number of proliferative cells in the piece of tissue.

After 24 hr in fixative, a small piece of tissue (approx. 30 mg) was removed, dried, weighed, and solubilized in 0.5 cc Soluene (Packard Instrument Co., Downers Grove, IL). Then 15 cc of the liquid scintillation counting mixture was added, the samples assayed for radioactivity as noted above, and the data converted to dpm/mg.

Values of dpm/mg tissue were divided by the values for dpm/crypt (utilizing comparable tissue) to yield crypts/mg values.

Standard error of the means were calculated for all data, and an Analysis of Variance used to test for differences within treatment groups and between treatment means.

Results. After appropriate statistical comparisons of the data for the variables examined, no significant differences ($P > 0.05$) due to rat stock were seen in the response of the crypt epithelium to resection (see Table II and Figs. 1–4).

The values of dpm/crypt, crypts/mg, and LN/crypt were increased significantly ($P < 0.05$) in the 80% resected group (Table II and Figs. 1, 3 and 4).

Age was also a factor which influenced some of the parameters. The values for dpm/crypt, dpm/mg, crypts/mg, and dpm/LN were consistently higher for the juveniles of both stocks (Figs. 1, 2, 3, and 5).

Discussion. Holtzman rats were used in this study because the response of this stock to the stimulus of intestinal resection is marked and has been well documented in this laboratory (1, 2). The Sprague–Dawley rat was chosen because it was the only other stock in which enough large animals could be obtained in a reasonable length of time. Since supply was a problem, it was fortunate that the cell kinetics from both stocks were the same.

No differences were noted in response to resection which could be attributed to the stocks of rats used (Table II and Figs. 1–4). This is not surprising when one considers that the Holtzman stock originally derived from the Sprague–Dawley stock some years ago.

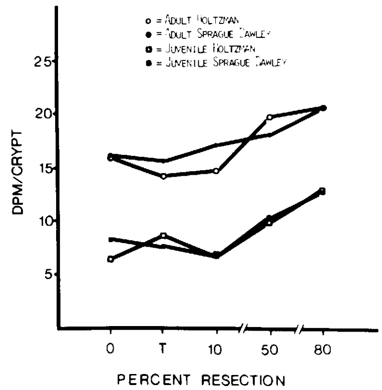

FIG. 1. The influence of percentage of intestine resected on the proliferative activity per crypt.

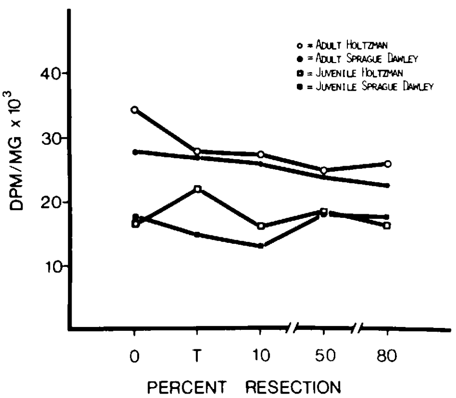

FIG. 2. The influence of percentage of intestine resected on the proliferative activity per milligram of tissue.

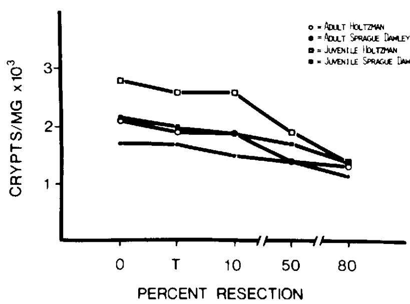

FIG. 3. The influence of the percentage of intestine resected on the number of crypts per milligram of tissue.

As shown in Fig. 4, dpm/labeled nucleus increased with animal age. This finding is very important since an experimenter using animals of different ages in studies involving the uptake of labeled thymidine, should take into account that cells synthesizing DNA do not incorporate the labeled precursor independent of age. It should also be noted from Table II that the degree of resection had no significant ($P < 0.05$) effect on the uptake of ^{3}HTdR per labeled cell nucleus. This is in agreement with the literature (1, 2).

Table II also shows that LN/crypt do not change with age, while dpm/crypt, dpm/mg, and crypts/mg do change. Therefore there must have been an increase in the uptake of ^{3}HTdR per intestinal epithelial cell nucleus in the older rats, in agreement with our findings of increased dpm/labeled nucleus with age.

Since there is no increase in chromosome material per nucleus with age, one possible explanation of the observed increase in uptake of ^{3}HTdR by the intestinal epithelial cells of adults is that there is more ^{3}HTdR available per cell nucleus due to fewer cells synthesizing DNA, i.e., the juvenile animals were in a period of rapid growth in which most tissues of the body were proliferating at

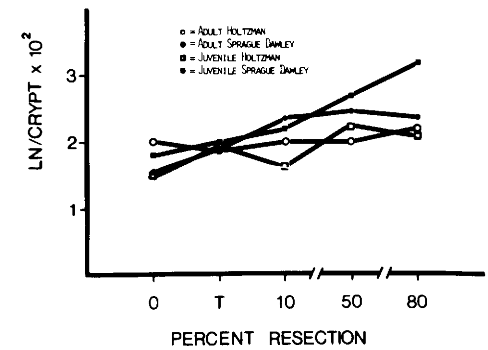

FIG. 4. The influence of the percentage of intestine resected on the number of labeled nuclei per crypt.

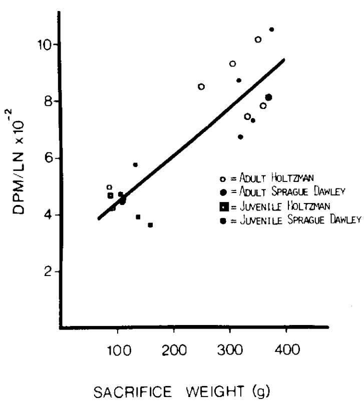

FIG. 5. The influence of animal weight on the uptake of labeled DNA precursor per labeled nucleus.

a higher rate than the adults and thus had more cells capable of incorporating the DNA precursor. Since the ^{3}HTdR was given on a per gram of body weight basis, the adults may have received a larger percentage of the DNA precursor on a per cell basis.

The results of the present study agree with those of Hanson, Osborne, and Sharp (1), who found that the magnitude of the response to resection was dependent upon the percentage of intestine removed, with no significantly different response from normal noted when less than 40% of the combined length of the ileum and jejunum was removed, and with a 70% resection causing a maximum response. The juvenile rats showed a greater percent increase in the dpm/crypt than did the corresponding adult members indicating a higher percentage of the juvenile crypt nuclei are in the S phase of the cell cycle.

Clarke (4) noted that while the number of crypts in the rat small intestine increases with age, the number of villi remains the same, while the villi themselves become larger. This implies that the absolute numbers of crypts increase with age to meet the demands of the enlarging villi for cells. This, added to an increased functional demand per unit area caused by massive resection increases the proliferative rate even higher, as was seen in the large percent change in the juveniles of the two stocks. The adults, which have reached a point where the crypt/villus ratio has essentially stabilized (4), showed a smaller percentage increase (Fig. 1).

Since there was a marked increase in the dpm/crypt values for the juveniles of both stocks, and Hanson *et al.* (1) have shown that there is a thickening of all intestinal wall components after resection, with the degree of thickening increasing as the amount of tissue removed is increased, the fact that dpm/mg of intestine did not rise in the juvenile age group is reasonable. This also explains why the values of dpm/mg of intestine fall in the adult age groups of both stocks of animals; the dpm/crypt was not rising in the adults as rapidly as it had in the juveniles and the thickening of the intestinal wall components increased at a greater rate, thus reducing the value of dpm/mg.

The values for crypts/mg were obtained by dividing dpm/mg by dpm/crypt. Again, on the assumption that all intestinal wall components increased in proportion to the degree of resection, it was reasonable for the values of crypts/mg to decrease as the percentage of intestine resected increased. In the juveniles of both rat stocks, the values for crypts/mg probably were consistently higher in young rats (which initially have smaller small intestines than the adults) than in their adult counterparts because the ability of their intestinal mass to increase was greater than the rate of increase of the crypts.

The larger percent decrease in the level of crypts/mg in the juvenile groups seems to be due to the larger increase in thickening of the intestinal wall components.

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