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DNAsynthesis and cell renewal in small and large intestines of mouse*

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Abstract

DNA synthesis and cell renewal of mouse intestinal epithelium were studied with radioautography after injection of thymidine-H^3 to know the difference of the mode of epithelial cell generation relating to the different frequency of cancer developement in several parts of small and large intestines. Succinic dehydrogensase activity was also observed by histochemical method. Renewal time of the intestinal epithelium of mouse is about three days throughout the intestine with somewhat longer time in rectum and anus, and relatively shorter one in ileum compared to the other parts of the intestine. Daily regenerating rate was low in large intestine, especially in rectum and anus. Strong activity of succinic dehydrogenase appeared in the bottom of crypt and seems to be correlated to the active cell division. Epithelial cells in large intestine move very slowly upward and few of them seem to move to the opposite side or stay long time at one place. Intermitotic time is about 27 hours in small intestine and about 40 hours in large intestine. These suggest some relations between the mode of the epthelial cell renewal and cancer development. Because in human the frequency of cancer development is very high in large intestine, rectum and anus, and the epithelial renewal of these areas is supposed to be delayed similarly as in mice.

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DNA SYNTHESIS AND CELL RENEWAL IN SMALL AND LARGE INTESTINES OF MOUSE

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As is well known that the small intestinal epithelial cells are incessantly fading at the top of villi and being renewed by the cells coming from the bottom of villi keeping a "steady state system". This has been established by the recent works by using radioisotopes of thymidine. QUASTLER and SHERMAN (1959)[1] reviewed the cell kinetics of intestinal epithelium of mouse by using radioisotopes. In 1948 LEBLOND already estimated the renewal time of the epithelium of small intestine of rat to be 1.4～1.6 days calculating from the mitotic rate of the epithelium. Later in 1958, LEBLOND and his associates[2,3] made precise observations on the mouse intestine by using thymidine-C^{14} and -H^3, revealing the renewal time in several areas of the mouse intestine ; 48 hours in duodenum, 72 hours in jejunum, ileum and colon. BERTALANFFY (1960)[4] estimated the renewal time of rat intestinal epithelium from mitotic rate, which was observed by using corchicine and he found that it was 1.3 days in jejunum, 10 days in colon, 6.2 days in rectum, and 4.3 days in anal epithelium. In 1961 OEHLERT and BÜCHNER[5] observed the renewal time of the intestinal epithelium of mouse to be 48 hours in duodenum, less than 48 hours in jejunum, and 72 hours in colon. Quite recently, KASAHARA (1966)[6] made similar observations on the mouse epithelium and calculated it to be as 43 hours in duodenum and jejunum, and 46 hours in ileum. In other words, the renewal time of the intestinal epithelium of mouse is found to be 40～48 hours in duodenum, 43～72 hours in iejunum, 46～72 hours in ileum and 72 hours in colon, showing that the shorter the renewal time is in duodenum, the longer is it in jeiunum, ileum and colon.

It is generally known that in human beings the rate of cancer development in the intestine is higher in rectum and colon and very low in small intestine. Concerning this, ONOE (1964)[7] suggested a close correlation between the renewal time of intestinal epithelium or regenerating process and the cancer formation. With his view as an incentive a series of study was conducted on the renewal

time of mouse intestinal epithelium by injecting thyminide-H^3, and the results of the study revealed the delayed renewal time of the epithelium of rectum and anus and low regenerating rates in large intestine where the cancer develops at higher rates, suggesting some relations between the modes of the epithelial cell renewal and cancer development.

MATERIALS AND METHODS

Adult Strong-A mice weighing about 30 g were injected intravenously with 1 μc per 1gm of thymidine-H^3 (specific activity 14.8 c/mM, Japanese Radiochemical Center). The animals were killed 1/2, 2, 4, 8, 24, 32, 40, 48, 72, 96 and 120 hours after injection. Specimens were obtained from duodenum near pylorus, jejunum near Treiz's ligament, ileum near coecum, coecum, transverse colon, rectum and anus near transitional part to squamous epithelium. Tissues were fixed in 10 per cent neutral formalin for one day and embedded in paraffin and then sectioned 4μ thick. Autoradiographs were prepared by the stripping film technique with Kodak AR-10 stripping film for three weeks at 5°C, thereafter stained with hematoxilin and eosin for observation (D-19-B solution was used for development).

Specimens for succinic dehydrogenase staining were prepared as follows. Small and large intestines two hours after the injection were cut at 15μ in a cryostat kept at -20°C without fixation, then incubatcd with the following medium for 30 minutes at 37°C; M/5 sodium succinate 5 ml, M/10 phosphate buffer pH 7.6, 5 ml, nitro BT 5 mg/3 ml, 6 ml, aq. dist. 10 ml.

RESULTS

Table 1 shows labeled index, situation, and density of labeled cells at each sacrifice time after injection calculated from neighboring five crypts. The labeling index is the number of labeled cell per all epithelial cells of a crypt and villi.

At 30 minutes after injection radioautographic reactions were observed only in crypts near the bottom. In the epithelial cells of villi or on the surface of large intestine such reactions were absent. Labeled cells were more abundunt in the crypt cells of small intestine than in those of large intestine. Grain counts were 15~30 per nucleus in the majority.

At two hours after the injection situation and labeled index hardly differed from that at 30 minutes but at four hours labeled cells moved a little toward the luminal side in the crypt.

At eight hours these labeled nuclei disseminated in the crypt and strongly labeled cells reached the upper end of the crypt in small intestine. On the other

Table 1 Position and Per Cent of Labeled Cells

	1/2hr	2 hrs	4 hrs	8 hrs	24 hrs	32 hrs	40 hrs	2 days	3 days	4 days
Duodenum	9.1	9.5	12.0	22.2	28.1	32.2	57.2	82.3	61.3	50.2
Jejunum	9.0	13.2	10.5	20.2	45.6	44.9	55.9	75.6	58.0	25.9
Ileum	11.3	12.5	9.5	26.1	35.2	63.7	66.8	50.5	58.9	29.9
Coecum	17.2	12.1	11.8	10.7	18.1	57.7	42.2	56.7	50.8	42.9
Colon	7.1	5.4	10.1	7.2	14.2	54.9	38.0	58.3	29.1	34.8
Rectum	7.6	4.8	12.2	10.9	10.4	28.7	37.0	60.3	33.3	28.3
Anus	12.5	7.5	8.6	8.3	18.9	32.0	24.3	55.8	46.2	31.2

hand, in large intestine the dissemination was not so marked but several labeled cells were found far from the base of crypt.

At 24 hours the migration of labeled cells were prominent. In small intestine, especially in ileum, strongly labeled cells reached about two thirds from the top of the villi. Duodenum and jejunum showed a similar tendency. In large intestine the migration of labeled cells upward luminal side was observed but not so marked as seen in small intestine. As a whole, grain counts in the crypt cells of small intestine decreased. Occasionally strongly labeled cells remained in the base of crypt.

At 32 hours the labeled cells reached higher place of villi than at 24 hours in small intestine and at this time labeled cells markedly increased in large intestine and strongly reacting nuclei were seen at about three fourths of crypt's height in coecum and colon but in rectum and anus the upper half of the crypt cells was not yet labeled. At 40 hours, these migrations of labeled cells toward the lumen and diminution of grain counts in the crypt cells became more prominent.

At 48 hours the strongly labeled cells reached the top of villous and surface epithelium both in small and large intestines. In small intestine these strongly labeled cells were disseminated in the upper two thirds of the villi. Also in large intestine the labeled cells reached the top of the surface but certain cells with strongly labeled nucleus were sometimes in the crypt far from the luminal

side.

At 72 hours these strongly radioactive nuclei were observed in the upper one third of the villi or less than in one third of duodenum and jejunum. In ileum the grain counts in the upper side of villous epithelium were less than

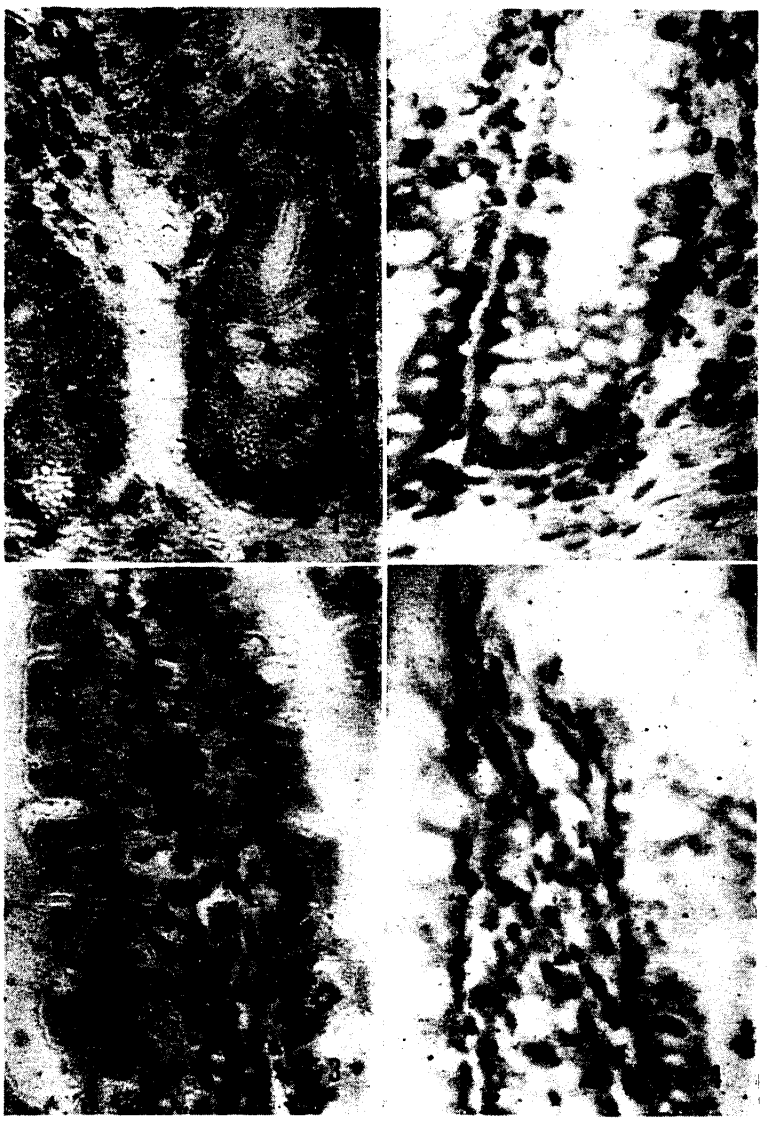

Fig. 1: Jejunum, after 2 hours ×400, Fig. 2: Colon, after 30 minutes ×400, Fig. 3: Ileum, after 32 hours ×400, Fig. 4: Colon, after 48 hours ×400

those in the former two. Grain counts were decreased gradiently down to the crypt in all small intestine and no nucleus in the crypt showed grain counts of over ten. In large intestine the decrease in the number of labeled cells was marked at this time, but occasionally strongly radioactive cells were observed around the base of crypt.

At 96 hours labeled cells were clearly decreased and the grain counts in these nuclei were very small in most region of the crypt of small and large intestines excepting occasional nuclei with many grain counts in large intestine.

At 120 hours unlabeled cells increased and the majority of labeled nuclei had only one or two grain counts.

In addition, succinic dehydrogenase in the intestine of the specimen two hours after the injection was estimated histochemically. The enzyme activity was intense on the surface of villi and the basal side of the crypt (Fig. 5).

Fig. 5 Ileum, succinic dehydrogenase staining ×100

DISCUSSION

Thymidine-H^3 is readily incorporated into DNA in chromosomes soon after the injection where DNA synthesis is taking place. There is no transfer of tritium once chromosomes have been labeled. Therefore, labeled thymidine is an adequate tracer for newly formed deoxyribonucleic acid in radioautographs[8,9].

Radioactive nuclei at 30 minutes and two hours were observed in 20 cells at the bottom of the crypt both in small and large intestines except in several cells far away from the bottom selectively in the crypt. These findings indicate that the DNA synthesis is achieved in these regions. Furthermore, strong succinic dehydrogenase activity seems to be intimately associated with these regions. QUASTLER and SHERMAN[1] reported that the majority of labeled cells

belonged to columnar and goblet cells and did not include the Paneth cells and the silver staining cells, though it was not clear in the present study. Newly formed cells mainly run up along the side of crypt and villi and are protruding into the lumen. However, few cells seem to take the opposite course and are lost from the base of crypt because over eight hours after the injection, when labeled cells migrated upward, strongly reacted cells were occasionally observed in the bottom of crypt especially in large intestine.

As indicated in Table 2, grain counts in the bottom cells of the crypts were

Table 2 Mean of Grain Counts of 20 Crypt Cells from the Bottom

	Times after injection									
	1/2	2	4	8	24	32	40	48	72	96 (hrs)
Duodenum	17.5	11.9	17.2	11.6	15.8	6.7	4.8	4.2	3.2	2.7
Jejunum	15.9	17.4	16.2	12.5	9.7	7.8	5.9	3.8	2.9	2.2
Ileum	15.8	16.2	18.8	12.5	13.2	9.7	4.9	3.2	2.7	2.3
Coecum	13.4	13.9	12.8	8.7	12.5	9.5	11.3	4.3	4.4	3.3
Colon	17.7	16.9	13.6	12.5	10.7	11.1	7.6	4.9	5.7	2.4
Rectum	12.7	14.5	15·1	14.4	15.1	6.1	7.4	4.1	5.9	2.8
Anus	13.8	15.0	13.4	14.5	14.5	5.9	10.1	4.7	4.9	2.6

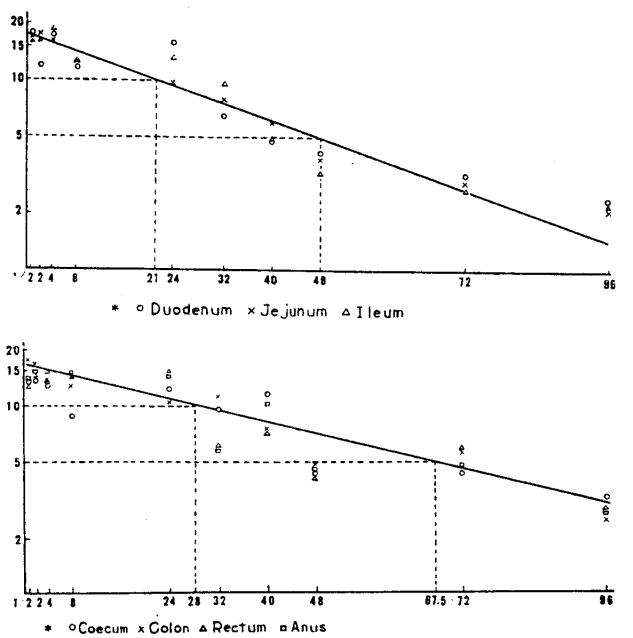

Fig. 6, 7 Mean of Grain Counts of 20 Crypt Cells from the Bottom Plotted Logarithmically Against Time

appearently diminished as the sacrifice time became long after injection. If the daughter cells accept half of DNA with thymidine-H^3 and they part it equally to daughter cells, mean of grain counts in the crypt cells against time should draw a strait line logarithmically (Figs. 6 and 7). Intermitotic time is given from these figures as the time grain counts become a half, it is 27 hours in the small intestine and 39.5 hours in the large intestine.

Renewal time of epithelial cells and daily regenerating rate are shown in Table 3. SHERMAN and QUASTLER[10] reported that the movement of cells along

Table 3 Renewal Time and Daily Regenerating Rate*

	Renewal time	Regenerating rate
Duodenum	72 hours	33
Jejunum	72	31
Ileum	48—72	18—27
Coeeum	72	9
Colon	72	11
Rectum	72—96	7—10
Anus	72—96	6—8

* Daily regenerating rate shows the number of daily faded epithelial cells.

the villi is not caused by the thrust of the cells coming up from the crypt. In the present study there are observed hardly any great differences of renewal time among these regions of the intestine examined since labeling indices differed.

SUMMARY

DNA synthesis and cell renewal of mouse intestinal epithelium were studied with radioautography after injection of thymidine-H^3 to know the difference of the mode of epithelial cell generation relating to the different frequency of cancer developement in several parts of small and large intestines. Succinic dehydrogensase activity was also observed by histochemical method. Renewal time of the intestinal epithelium of mouse is about three days throughout the intestine with somewhat longer time in rectum and anus, and relatively shorter one in ileum compared to the other parts of the intestine. Daily regenerating rate was low in large intestine, especially in rectum and anus. Strong activity of succinic dehydrogenase appeared in the bottom of crypt and seems to be correlated to the active cell division. Epithelial cells in large intestine move very slowly upward and few of them seem to move to the opposite side or stay long time at one place. Intermitotic time is about 27 hours in small intestine and about 40 hours in large intestine. These suggest some relations between the mode of

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