IN VITRO STUDY OF HUMAN RECTAL EPITHELIAL CELLS. I. ATYPICAL ZONE OF H³ THYMIDINE INCORPORATION IN MUCOSA OF MULTIPLE POLYPOSIS

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Research Article

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IN VITRO STUDY OF HUMAN RECTAL EPITHELIAL CELLS.
1. ATYPICAL ZONE OF H³ THYMIDINE INCORPORATION IN MUCOSA OF MULTIPLE POLYPOSIS

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(Submitted for publication January 15, 1963; accepted August 16, 1963)

The use of labeled precursors that incorporate within the cells of the gastrointestinal tract has made it possible to study cell migration, the lifespan of cells, and other parameters describing the DNA, RNA, and protein metabolism of these cells. Numerous isotopic investigations relating to cell function in the gastrointestinal tract have been carried out in animals (1, 2), but relatively few have been performed in human subjects (3–5). Isotopic studies with precursors such as H³ thymidine, which is incorporated solely into DNA (6), have been limited to selected preterminal patients, particularly since the carcinogenic and mutagenic nature of H³ thymidine has been established (7–9). It was therefore considered advantageous to devise a technique that would maintain normal and diseased tissues outside the body for isotopic studies of this type.

This paper reports the successful use of an in vitro technique that allows the study of thymidine incorporation into deoxyribonucleic acid (DNA) of epithelial cells in normal and abnormal rectal tissue. With this procedure, it has been possible not only to locate cells within the rectal crypts that incorporate thymidine, but also to show a difference in the pattern of incorporation of these cells when normal rectal crypts are compared with those found in multiple polyposis.

MATERIAL AND METHODS

Sixteen patients were biopsied for studies of normal mucosa. Ambulatory hospital patients were selected from the Second (Cornell) Medical Division. The majority were convalescing from respiratory illnesses or alcoholism.

Food was withheld from the patients for 12 hours, and they were then given a saline enema 2 hours before the biopsy procedure. The patients were placed in the left lateral decubitus position. An 8-inch proctoscope was introduced. No abnormalities were seen in the proctoscopic appearance of the rectal mucosa in the normal subjects. Biopsies were taken from the rectal valves with a Turell angulated biopsy forceps or Wood’s suction biopsy tube. Sharp cutting edges provided specimens that survived best in tissue culture, since ragged edged specimens tended to flake.

Hemostasis was achieved with gentle pressure. No complications were encountered. One specimen from each patient was preserved in 10% formalin, and the others were placed in tissue culture medium.

Biopsy specimens were removed from the forceps and first placed in normal saline at room temperature for transport to the tissue culture room located adjacent to the proctoscopy room. Within 1 to 2 minutes the tissue specimen was removed to a second normal saline solution to be washed and rinsed. It was then placed in a test tube containing Eagle’s basic salt solution with 10% human serum and approximately 1 μc of H³ thymidine (SA, 1.9 c per mmole) per ml of media. Any changes in pH were controlled by the addition of 5% sodium bicarbonate, and the growth of any contaminant bacteria was successfully checked by the addition of streptomycin and penicillin (500 U each per ml). The tube was then gassed with 95% O₂ and 5% CO₂ placed in a Dubnoff shaker at 37° C, and allowed to incubate with gentle agitation. After a specified time interval, the tissue was removed from its nutrient medium and fixed in neutral formalin. It was then dehydrated and embedded, and 3 to 4-μ sections were cut. Slides containing the sections were deparaffinized and coated with NTB (Kodak) emulsion under standard conditions (10). The coated slides were stored in light-proof, black, plastic boxes in the refrigerator for an exposure time of approximately 2 weeks. The slides were then developed by standard photographic technique and stained with basic fuchsin.

Longitudinal sections through entire rectal crypts were examined microscopically and scored in detail for the number of labeled cells present and their relative cell position in the crypt, the number of labeled and unlabeled mitoses, and the total number of cells per crypt.

Two to 18 biopsy specimens were obtained from each patient, and a total of 131 biopsies was studied. Tissue was removed after 1, 3, 6, 7, 12, and 19 hours of incubation. Normal subjects were studied at only one time-interval.

Subjects with multiple polyposis. Two subjects with multiple polyposis were seen. Patient 1 was seen on two
occasions 6 months apart, and in all, 32 small, pink, 1-to-
2-mm polyps were removed for tissue-culture purposes.
This tissue was examined after 1, 3, and 20 hours of in-
cubation. Patient 2 was studied on one occasion, and 15
1-to 2-mm polyps were removed. The tissue was ex-
amined after 1, 3, and 21 hours of incubation.

Patient 1, a 30-year-old white male, was first examined
in 1949 at the age of 17 and found to have multiple polyps
with no unusual pigmentation present. Sigmoidoscopy
revealed polyposis throughout the entire rectum, and no
normal rectal tissue was visible. Biopsy of the sigmoid
colon showed adenomata with cellular atypism but no
evidence of malignancy. A barium enema revealed in-
volve ment of the entire colon.

In 1951, a subtotal colectomy and an ileoprostostomy
were performed. The patient's course since then has
been characterized by the continued formation of polyps
of all sizes that are periodically removed by snare and
electrocoagulation.

Family history revealed that his mother is living and
well, but that his father died of a disease believed to be
cancer of the stomach. He has five brothers and one
sister who are living and well. Three of the patient's
brothers were seen in the clinic. Barium enemas and
proctoscopy examinations of all three have been negative.
The rest of the family has not consented to be examined.

The patient has subsequently married and has a 2-year-
old daughter who is entirely well and has not been exam-
ined.

Patient 2, a white female, age 37, was seen in 1961
when a diagnosis of cancer of the descending colon was
established. Later in 1961, a resection of the carcinoma
with anastomosis of the left colon and sigmoid was per-
formed. She was seen again in 1962 when recurrence of
the carcinoma was proved at a laparotomy. In addi-
tion, during the latter admission, a proctoscopic examina-
tion revealed more than 15 small (2 mm in diameter),
isolated polyps in the rectum and sigmoid.

Family history disclosed that the patient's mother had
died at age 46 of primary cancer of the rectum and metas-
tases. The patient's only son has not been exam-
ined to date.

In both cases described here, cellular atypism was seen
on pathological examination, and in Patient 1 polyps were
distributed throughout the entire colon. These are
features that are present in familial polyposis. Al-
though the term "familial polyposis" has been used to
describe cases of this type (11, 12), the hereditary pat-
tern of the disease is not clearly borne out in either of the
present cases, probably because the examination of
family members is incomplete.
TABLE I

<table>
<thead>
<tr>
<th>Incubation time</th>
<th>Patients scored</th>
<th>Total cells observed</th>
<th>Labeled cells</th>
<th>No. mitoses</th>
<th>Cells/crypt</th>
<th>Labeled cells</th>
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<tr>
<td>hrs</td>
<td>no.</td>
<td>no.</td>
<td>no.</td>
<td>no.</td>
<td>%</td>
<td>mean no. %</td>
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<td>1</td>
<td>1</td>
<td>9</td>
<td>994</td>
<td>10</td>
<td>8</td>
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<tr>
<td></td>
<td>5</td>
<td>16.5</td>
<td>2,402</td>
<td>145</td>
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<tr>
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<td>4</td>
<td>15</td>
<td>1,654</td>
<td>203</td>
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<td>0.3</td>
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<tr>
<td>12</td>
<td>2</td>
<td>18</td>
<td>1,165</td>
<td>98</td>
<td>6</td>
<td>0.5</td>
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<tr>
<td>19</td>
<td>4</td>
<td>12</td>
<td>1,162</td>
<td>204</td>
<td>3</td>
<td>0.3</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1</td>
<td>8</td>
<td></td>
<td>1,278</td>
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<td>763</td>
<td>78</td>
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<tr>
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<td></td>
<td>439</td>
<td>87</td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1</td>
<td>9</td>
<td></td>
<td>1,147</td>
<td>17</td>
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</tr>
<tr>
<td>20</td>
<td>9</td>
<td></td>
<td>623</td>
<td>131</td>
<td>8</td>
<td>1.28</td>
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<td></td>
<td></td>
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<tr>
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<td>12</td>
<td></td>
<td>1,315</td>
<td>286</td>
<td>5</td>
<td>0.4</td>
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</table>

RESULTS

Survival of the entire biopsy specimen with little or no lysis was found in nearly all specimens of the normal and polypoid tissue examined after short incubation times. Survival was determined by retention of normal morphology of the tissue and the continued appearance of mitotic figures. After 19 hours, however, 50% of the specimens demonstrated some degree of histological tissue deterioration. The remaining specimens appeared intact, and the rectal crypts remained well-defined and without cellular necrosis. Figure 1 is a low-power photomicrograph of an entire biopsy specimen that demonstrates normal mucosa without

![Figure 1](image-url)

**Fig. 3. Rate of appearance of epithelial cells labeled with tritiated thymidine in the normal rectal tissue and in the multiple polyposis specimens.**
evidence of tissue degeneration. The appearance of one crypt from this biopsy after 19 hours of incubation can be seen under higher magnification in Figure 2.

Table I provides the data obtained from the analysis of the tissue culture material. The number of crypts scored ranged from 7.5 to 18, and the mean number of cells in each crypt ranged from 64.7 to 145.5 in the normal subjects and from 54.8 to 159.7 in the polyposis specimens. The percentage of labeled cells ranged from 1% at 1 hour to 18% at 19 hours in the normal mucosa, and from 0.5% to 2% at 1 hour and from 19% to 22% at 20 to 21 hours in the polyp tissue.

Figure 3 shows the percentage of labeled epithelial cells of the normal rectal tissue and polyposis specimens plotted against time. There is a gradual increase in the number of labeled cells observed with increasing time in tissue culture. The rate of appearance of newly labeled cells was similar in both the normal and polyp tissue, and close agreement occurred both at the short and long incubation periods.

<table>
<thead>
<tr>
<th>Incubation time</th>
<th>Lower crypt</th>
<th>Middle crypt</th>
<th>Upper crypt</th>
</tr>
</thead>
<tbody>
<tr>
<td>hrs</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>70</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>56</td>
<td>33</td>
<td>11</td>
</tr>
<tr>
<td>6-7</td>
<td>54</td>
<td>33</td>
<td>13</td>
</tr>
<tr>
<td>12</td>
<td>54</td>
<td>29</td>
<td>17</td>
</tr>
<tr>
<td>19</td>
<td>42</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td>Multiple polyposis (exp't 1a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>16.5</td>
<td>16.5</td>
<td>67</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>33</td>
<td>52</td>
</tr>
<tr>
<td>20</td>
<td>23</td>
<td>37</td>
<td>40</td>
</tr>
<tr>
<td>Multiple polyposis (exp't 1b)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>18</td>
<td>76</td>
</tr>
<tr>
<td>3</td>
<td>17</td>
<td>26</td>
<td>57</td>
</tr>
<tr>
<td>20</td>
<td>21</td>
<td>30</td>
<td>49</td>
</tr>
<tr>
<td>Multiple polyposis (exp't 2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>19</td>
<td>11</td>
<td>70</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>2</td>
<td>96</td>
</tr>
<tr>
<td>21</td>
<td>42</td>
<td>36</td>
<td>22</td>
</tr>
</tbody>
</table>

* Percentage labeling based on individual crypt size.

Mitosis at the various intervals studied was infrequent in the normal specimens, occurring in less than 1% of the total number of cells observed. Labeled mitoses both in normal and polyp tissue were seen in specimens incubated for 19 hours (Figure 4). Mitoses were usually seen in the lower two-thirds of the crypts in normal rectal mucosa, but in the polyp tissue they were occasionally seen in the upper third of the crypt (Figure 4).

The number of mitotic figures present in each polyp specimen varied considerably (Table I). Some polyp specimens were found to have almost twice the number of mitoses present in the normal tissue, with mitotic rates of 1.6% and 1.28% observed and two or more mitotic figures observed in some crypts. Several thousand cells, however, would have to be observed to determine more accurately the mean mitotic frequency of cell division in this slowly dividing tissue.

Seventy per cent of normal, rectal, epithelial cells labeled after 1 hour of incubation were located in the lower portion of the crypts, with no label found in the upper zone and free edge (Table II). Further incubation of normal rectal tissue resulted in an extension of labeled cells to the
upper portion of the crypts and continued heavy labeling of cells in the lower third of the crypts.

In contrast, the polypoid tissue maintained in vitro for 1 hour was found in all experiments to incorporate thymidine actively into epithelial cells along the entire length of the rectal crypts. In addition, 67% (experiment 1a), 76% (experiment 1b), and 70% (experiment 2), respectively, of the labeled cells were located in the upper third of the crypts (Table II). At later periods, labeled cells appeared in greater numbers in the lower portion of the crypts, but the upper third of the crypts continued to have high concentrations of cells that actively incorporated thymidine.

A high-power photomicrograph of the normal, human rectal mucosa after 3 hours in tissue culture medium (Figure 5) demonstrates visually the pattern of thymidine incorporation found in normal tissue. The greatest number of labeled cells is located at the basal portion of the crypt with an occasional labeled cell appearing higher in the crypt column. In contrast, Figure 6 shows a portion of the polypoid tissue also incubated for 3 hours. A reversed pattern of labeling is seen with the greatest number of labeled cells located in the upper third of the crypt and along the luminal border of the crypts.

**DISCUSSION**

An experimental procedure is presented that permits rectal epithelium to be maintained in a viable state for short periods in vitro, in order that actively proliferating cells may be identified both qualitatively and quantitatively. There is good reason to believe that the metabolic activities responsible for thymidine uptake have deteriorated to some extent by 19 to 21 hours in vitro, although the percentage of mitoses seen then is approximately the same or higher than at earlier periods, and the number of cells synthesizing DNA doubled in 20 hours under these conditions. Nevertheless, since 50% of the biopsies lysed by 19 to 21 hours, it is reasonable to postulate some biochemical degeneration in those specimens that survive with
little or no lysis present. The basic difference
shown here between normal and polyp tissue had
manifested itself early, i.e., in 1 to 3 hours, when
the problem of degeneration is not an important
factor in this system.

In the early periods, the question of penetra-
tion or accessibility of the isotope to the lower
zone of the crypts in the polyp tissue could be
raised. Evidence that this factor did not influence
the labeling site in the polyp crypts, however, can
be derived from a number of points. The num-
ber of cells synthesizing DNA at early times in-
creases rapidly in both normal and polyp tissues.
Also, the number of labeled cells per crypt present
in 1 to 3 hours is remarkably similar in both tis-
sues, indicating a population of approximately
the same size is entering the synthesis stage in
both tissues. In fact, in some instances, the num-
ber of labeled cells per crypt was higher in the
polyp tissue than in the normal. In addition, if
accessibility were a factor governing differential
incorporation of H3 thymidine into polyp crypts,
then we might expect no label to occur at the base
of these crypts. Instead we find that 16.5%, 6%, and
19% of the total number of labeled cells
(Table II) present at 1 hour in the polyps are in
the lower third of these crypts. It would appear
then that the isotope was available to all the cells
lining the crypt, but the greatest number of ac-
atively synthesizing cells in the polyp occurred
along the surface closest to the lumen.

The mitotic activity of normal human mucosa
was less than 1%. Other studies in this labora-
tory following in vivo injection of H3 thymidine
have revealed a mitotic frequency of less than 1%
in crypts of the rectum (13). The infrequent ap-
pearance of mitotic figures in our study would also
fit well with the estimate of Cole and McKalen of
a turnover time of about 6 to 8 days (3).

The frequency of mitosis in normal rectal epi-
thelium maintained in vitro may have been low
because of the general fasting conditions of the
patients. Fasting has been shown to cause a re-
duced mitotic rate in the rat (14). In addition,
the specific effects on mitotic activity of routine
pharmacologic agents administered to these pa-
tients are not known.

At some of the later incubation times of the
polyposis tissue, there appeared to be a selective
advantage for the survival of shorter crypts, that
is, while the same number of crypts was scored as
at the earlier times, the total number of observed
cells was reduced at later periods. This is seen at
the 20-hour incubation period in both experiments
with the polyposis tissue of Patient 1, whereas in
the normal rectal mucosa and in Patient 2, this se-
lective effect is small.

The earliest time that labeled mitoses were en-
countered in either normal or diseased tissue was
at 19 hours. Thus, the minimal period for a
cell to go from S phase, when thymidine is in-
corporated and DNA synthesized, to the mitotic
phase is 19 hours. Since our sampling does not
cover the period 12 to 19 hours, the G2 or premi-
totic portion of the proliferative cycle may be a
few hours shorter than the figure stated. Al-
though it was not possible to follow this tissue
for several days, one might roughly estimate that
the mean generation time of these cells should be
at least 3 to 4 times this interval of 19 hours (5),
that is, a cell may complete its generation cycle in
rectal tissue kept in vitro approximately once
every 3 to 4 days.

The incorporation of H3 thymidine into the nu-
clei of normal specimens occurred in the same lo-
cation as after in vivo injection of the isotope
(5). The lower portions of the crypts were most
frequently labeled at early incubation times, al-
though 11% of the surface cells were labeled at 3
hours. After 19 hours of incubation, but not af-
ter 3, some cells on the crypt, epithelial cell column
could have undergone division and supplied new,
labeled, surface epithelial cells. In a study in
which H3 thymidine was injected in vivo, labeled,
rectal, crypt epithelial cells were found to reach
the surface in 20 hours (13).

Tissue from the patients with multiple polyposis
showed a pattern quite different from the normal,
with labeled cells present at early incubation pe-
riods located almost exclusively in the upper por-
tion of the crypt and along the free surface of the
mucosa. After 19 hours of incubation, some ni-
tosis, and occasionally a labeled mitosis, was found
in the upper crypt. This pattern of early incor-
poration is abnormal and reveals the presence of
proliferating cells at the upper portion of the
crypts, where mature, well-differentiated cells are
characteristically present. The evidence that these
surface cells in the polyp tissue incorporate thy-
midine shortly after exposure to label is of in-
terest because of the abnormal growth of the polypoid tissue and the malignant degeneration known to take place. This observation may also be related to the differences in staining quality seen in cells near or at the surface of polyps (15).

A possible interpretation of the data presented is that two populations of cells are present, a fast-moving population at the tip and a slower moving one at the base of the crypt. The slower growing cells may replace a subsurface epithelium, and the faster cells may perpetuate the polyposis tissue by continued outgrowth or budding. Further studies similar to the one presented here are continuing with the hope that correlations can be developed to identify the rate and mechanism of growth of polypoid tissue and the probability of the future malignancy of such tissue.

SUMMARY

A method is described for in vitro culture of human rectal mucosa for periods up to 24 hours. The rate of incorporation of H3 thymidine was measured in nuclei of epithelial cells of rectal mucosa from 16 normal subjects and two subjects with multiple polyposis. Quantitative data on the percentage of labeled cells found in various portions of the rectal crypts at specific time intervals after incubation with the isotope revealed that the rate of incorporation in normal and polyposis tissue was similar. An alteration in the pattern of thymidine incorporation into the crypt epithelium of the polyposis tissue was noted, however, indicating a displacement of the zone of active cell proliferation in these crypts.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the advice and encouragement of Dr. Thomas P. Almy and the technical assistance of Mrs. Camille Boyle, Miss Geraldine Kraska, and Mr. Jay Rubin.

One multiple polyposis subject was provided through the kind cooperation of Dr. Michael Deddish and Dr. Maus Stearns, Rectum and Colon Service, Memorial Hospital, New York, N. Y.

REFERENCES