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Separation of Proliferative and Mature Cells in Stomach, Jejunum and Colon of Rat[1] (38201)

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In a previous report (1) a method was described for separation of mature and immature cells from villi and crypts of small intestine using a tissue-planing apparatus. Cutting of villi and crypts was accomplished by sliding a micrometer-mounted blade horizontally across the mucosa that had been opened and stretched out on a lucite block. The method enabled analysis of changes in activity and heat stability of enzymes active in the intermediary metabolism of nucleic acids, as cells of rat jejunum (1, 2) and colon of man (3) underwent differentiation and migration.

In this study, the tissue-planing method has been modified and extended to the stomach and distal colon of the rat. The modifications enable mature well differentiated cells lining the surface of the stomach and the distal colon to be separated from the immature cells in the deeper layers of the mucosa. Using the cell separation procedure, data in the rat have shown a decrease in activity of thymidine kinase as cells of stomach, jejunum and colon underwent differentiation and migrated to the surface of the mucosa.

Materials and Methods. Experimental procedure. Male CFE rats (Carworth Farms) weighing 150–200 g and fed laboratory chow ad libitum were killed by cervical dislocation. From each animal the upper 6 cm of jejunum or terminal 6 cm of colon was removed.

In separating successive layers of epithelial cells from villi and crypts of jejunum the tissue-planing technique previously described was used (1). This consisted of mounting a segment of jejunum on the lucite block, opening it lengthwise allowing the villi to protrude upwards, and sliding a micrometer-mounted razor blade horizontally across the tissue to cut off the villus layers. In addition, in the present study the side bars of the apparatus were fitted with steel tension springs to provide a self-propelled, more uniform forward thrust and shock absorption of the cutting edge of the instrument.

For separation of cells in descending colon and stomach, new tissue supporting bases were made. To eliminate longitudinal folds in the descending colon a 0.5 × 1.5 × 5.0 cm Teflon mounting block was used over which the slightly stretched upward facing mucosa was attached. A steel mesh sleeve with 3 ml radiating spines surrounded the mounting block to hold the stretched mucosa and eliminate the folds (Fig. 1A). The descending colon was successfully stretched in this manner, but not the upper colon where diagonal folds were still present. In stomach, to eliminate the gastric rugae, a circular shaped teflon mounting block 2 cm in diameter and 1.4 cm in height, and fitted with a similar sleeve was devised (Fig. 1B). The stomachs which were slit along their lesser curvatures were attached to the block by stretching across the attachment spines.

Based on preliminary histological studies, the following scraping levels were chosen. In the colon, 2 successive 50 μm steps were followed by a glass slide scraping of the remaining mucosa, separating surface lining from middle and lower half of the crypts. In stomach, three successive 75 μm steps down from the surface were followed by a

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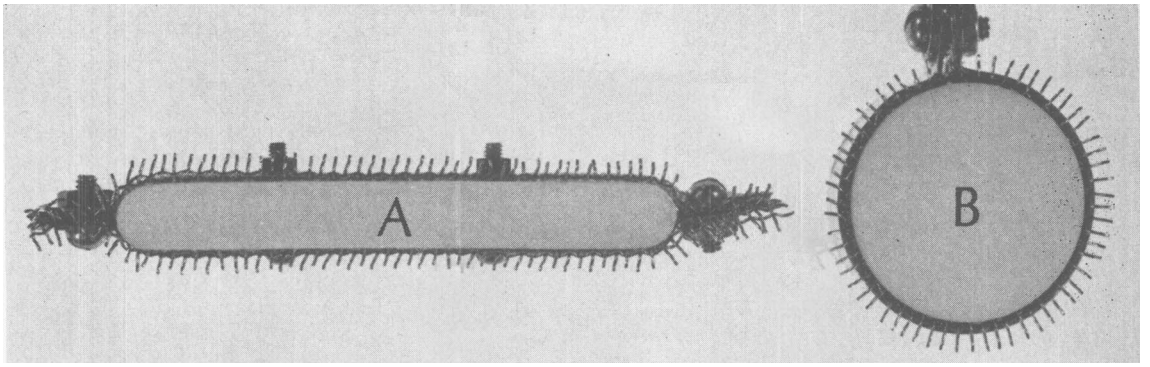

FIG. 1. Attachments used on the planing apparatus. (A) Photograph of the new mounting block for rat colon; (B) Photograph of new mounting block for rat stomach.

glass slide scraping of all the remaining mucosa. This enabled separation of surface epithelium from cells of the pits, the pit gland junction and the fraction containing most of the gastric glandular elements. After planing, the scrapings were removed from the razor blade and placed in a chilled glass microhomogenizer for enzyme studies, or were immediately deep frozen for studies of thymidine-methyl-^{3}H(TdR^{3}H) incorporation into DNA. In stomach and colon scrapings from the individual layers of 6 animals were pooled in order to obtain sufficient material for enzyme assays. However, in jejunum material from a single animal was used. Measurements of enzyme activity were repeated 4–6 times.

Enzyme Assay. Tissues used in enzyme assays were homogenized in ice cold 0.01 *M* Tris–HCl (pH 7.4) and adjusted to a volume of 1–1.5 ml. The homogenates were centrifuged in the cold at 15,000*g* for 20 min.

ATP: thymidine 5′ phosphotransferase (EC 2.7.1.21) (thymidine kinase): the enzyme was assayed as previously described using the reaction mixture of Behki and Morgan (4) and the DEAE paper method of Breitman (5). Radioactivity not washed from the paper was measured in a scintillation spectrometer.

Incorporation of thymidine-methyl-^{3}H-(TdR^{3}H) into DNA. Rats were injected intraperitoneally with 0.5 ml containing 100 μCi of TdR^{3}H in saline (S.A. 20 Ci/mmol). They were sacrificed in groups of three at 1 hr, 24 hr, and 72 hr after injection. Tissues were obtained by the planing technique described above, pooled from three animals for the gastric and colonic determinations and taken from one rat for jejunal assays. They were homogenized in 1.5 ml of 0.01 *M* Tris–HCl (pH 8.0), washed twice with ice cold 0.5 *N* perchloric acid and once each with 70%, 95% and 100% ethanol, ethanol–ether and ether. The dried pellet was then subjected to acid hydrolysis and assayed for DNA (6) and for incorporated tritium using a liquid scintillation spectrometer.

Results and Discussion. Incorporation of thymidine-methyl-^{3}H into DNA. The results of the incorporation study are shown in Fig. 2. In each organ thymidine incorporation one hour after injection was greatest in cells in the area below the surface of the mucosa occupied by proliferating cells. Cells labeled with TdR^{3}H then moved toward the surface of the mucosa where the amount of label increased at 24 and 72 hrs.

Thymidine kinase activity. Enzyme activities in mucosa of jejunum, colon and stomach are shown in Fig. 3. In jejunum, thymidine kinase activity fell markedly as cells migrated from crypt to villus as in previous studies (1, 7), and in colon and stomach TdR kinase activity also was observed to decrease in migrating and differentiating cells.

When subjected to analysis by the Wil-

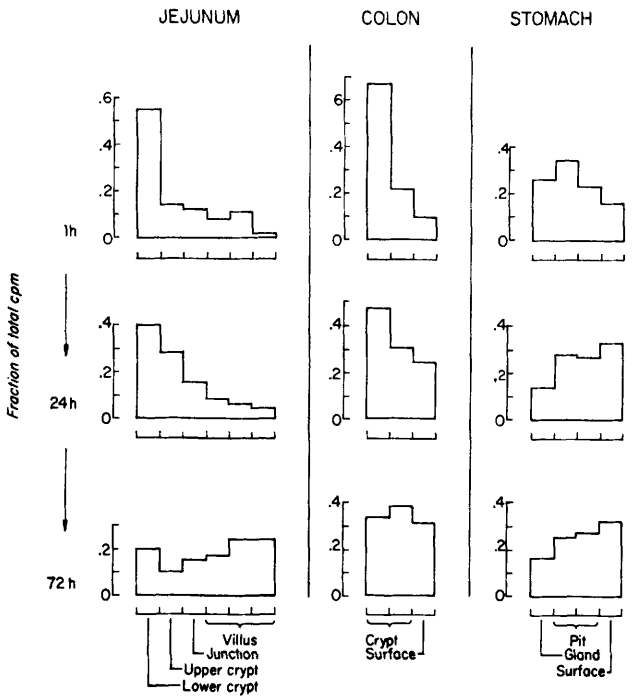

FIG. 2. Distribution of cells labeled with TdR^{3}H in chemically extracted DNA at 1 hr, 24 hr and 72 hr after injecton in rat stomach, jejunum and colon.

coxon 2-sample rank test (8) the comparison of the highest with the lowest enzyme concentrations showed significant differences at the 0.05 level for all thymidine kinase estimations.

The tissue-planing apparatus used in the present study previously had enabled separation of cell layers in small intestine of the rat. However, in the rat the muscular convolutional folds of stomach and colon had prevented the tissues from being stretched on the mounting block used for jejunum. In the present study, by using the new tissue mounting blocks together with the attachment spines it was possible manually to stretch and attach the lower colon and glandular stomach to enable separation of the cell layers. Initial measurements of nucleic acid precursor enzyme activities in colon and stomach and jejunum of rat have now indicated decreasing thymidine kinase activity in differentiating and migrating cells.

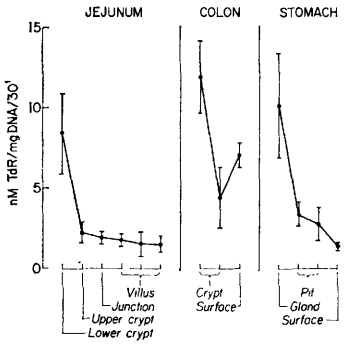

FIG. 3. Distribution of thymidine kinase in layers of stomach, jejunal and colonic mucosa of rat.

A recent study in this laboratory on rat jejunum using the tissue-planing apparatus also has indicated decreasing activity of thymidylate synthetase during migration of cells through the jejunal crypts as cells leave the proliferative cycle [(9), unpublished data]. In other cell systems thymidine kinase and thymidylate synthetase activities characteristically are lower with decreased proliferation (10). In man, similar changes have been observed in cells of the colon (3), but no data are available for stomach.

We have observed neoplastic lesions in the colon of man to have high thymidine kinase activity [(11), unpublished data].

In both their proliferative behavior and their susceptibility to carcinogens, differences are present when the cells of one region of the gastrointestinal tract are compared to another. Epithelial cells of distal colon re-enter the proliferative cycle at a slower rate than proliferating jejunal cells and are retained in the mucosa for a longer time before being extruded (12, 13). Regional differences in the susceptibility of gastrointestinal cells to the potent carcinogens 1,2 dimethylhydrazine (14, 15) and 3,2′ dimethyl-4-aminobiphenyl (16) also have been found. The present method will make possible the separation of proliferating and differentiating gastrointestinal cells in different regions in order to study factors influencing mechanisms of carcinogenesis, and other proliferative and differentiation-specific characteristics of the cells.

Summary. A tissue-planing method has been adapted to separate proliferative and mature cells from stomach, distal colon and jejunum of rat. New mounting blocks have been designed to enable positioning of the tissues for the separation process. Movement of thymidine labeled cells from deeper to superficial layers of mucosa and changes in thymidine kinase activity have been observed in cells separated from stomach, jejunum and colon.

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