two fully viable clones with mixed components suggests that appropriate procedures will probably be found to assemble most combinations of nuclei, cytoplasm, and membranes.

There are many known examples of the partial use of reassembly methods in amoebae. For instance when nuclei from cells lethally damaged by x-ray or nitrogen mustard (*6*) are placed in unirradiated cytoplasm, the nuclei are irreversibly damaged, whereas nuclei from cells lethally damaged by actinomycin D readily recover (*7*). Injection of cytoplasm (*3*) and of cytoplasmic homogenate, either fresh or lyophilized (*3*), can result in a modification of strain-specific characters in the host progeny.

The success of our reassembly experiments means that we now have the technical ability to assemble amoebae which contain any desired combination of components and thus have an excellent test system. This system can be used to test the condition of particular cell components; for example, an organelle from an amoeba which has been prevented from undergoing division for some time can be used in a reassembled cell, the other components of which are from cells undergoing normal, logarithmic growth. Also, the viability of cell components, for example, nuclei or mitochondria isolated by standard procedures (*8*), can be examined. In this way the viability and compatibility of cell organelles from diverse sources can be determined.

K. W. JEON, I. J. LORCH
J. F. DANIELLI

Center for Theoretical Biology and *School of Pharmacy, State University of New York at Buffalo, Amherst 14226*

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Intestinal Enzymes: Indicators of Proliferation and Differentiation in the Jejunum

Abstract. *Some intestinal enzymes were assayed which were related to: (i) cellular proliferation, for example, aspartate carbamoyltransferase, thymidine kinase, uridine kinase, and dihydroorotase; (ii) cellular differentiation, for example, lactase, invertase, maltase, alkaline phosphatase, and dipeptidase; and (iii) lysosomes, for example, beta-glucuronidase, acid beta-galactosidase, and acid phosphatase. These enzymatic determinations can be used to distinguish the crypt from the villus during healthy or diseased states.*

The small intestine is a complex organ composed of basic functional units comprised of crypts containing dividing cells and of villi made up of highly differentiated cells.

The cells of the crypt can incorporate radioactive thymidine into DNA (*1*). After division, the cells migrate from the crypts along the length of the villi and complete their lifespan within 36 to 48 hours (*1*, *2*) by extrusion from the tips of the villi into the intestinal lumen. The cells of the crypt are nondifferentiated whereas the epithelial cells of the villus are highly specialized for the primary intestinal functions of absorption and digestion.

The crypt and the villus differ morphologically. A number of enzymatic activities have been shown to be predominantly located in villi (*3*, *4*), while synthesis of DNA (*1*), of RNA (*5*), and of cholesterol (*6*) occurs in crypts. We report that these two special zones of the intestine can be identified readily under normal and abnormal conditions by analysis of certain enzymes responsible for specific cellular functions.

We used the technique of Van Genderen and Engel (*3*), as modified by Dahlqvist and Nordström (*4*), which permits sequential biochemical analyses from villus to crypt in the intestine of the rat. The tissue was cut transversely with a cryostat into 40 to 60 slices, each approximately 12 μ thick. These slices were used either for biochemical determinations or for morphologic examination.

The cells of the crypt are arranged in tubules about a central lumen and have a glandular appearance (Fig. 1). The histological appearance of the crypt is different from that of the villus which has columnar epithelial cells arranged around an inner core of lamina propria. The junctional zone is composed of cells from the crypt and the villus.

Three groups of enzymes were studied. Protein was determined according to Lowry *et al.* (*7*). The ratios (crypt/villus) of enzymatic activity were calculated from the highest activity of the enzyme observed in the crypt and the villus. In the first group—composed of enzymes associated with pyrimidine and pyrimidine nucleotide biosynthesis—all of the ratios exceeded unity. Thymidine kinase (*8*) had a high ratio of 50, in contrast to 2.3 for aspartate carbamoyltransferase (*9*), 2.5 for dihydroorotase (*10*), and 1.7 for uridine kinase (*11*).

The second group of enzymes, which are associated with specific intestinal functions, had ratios less than 0.5 as follows: maltase (*12*) was 0.17, lactase

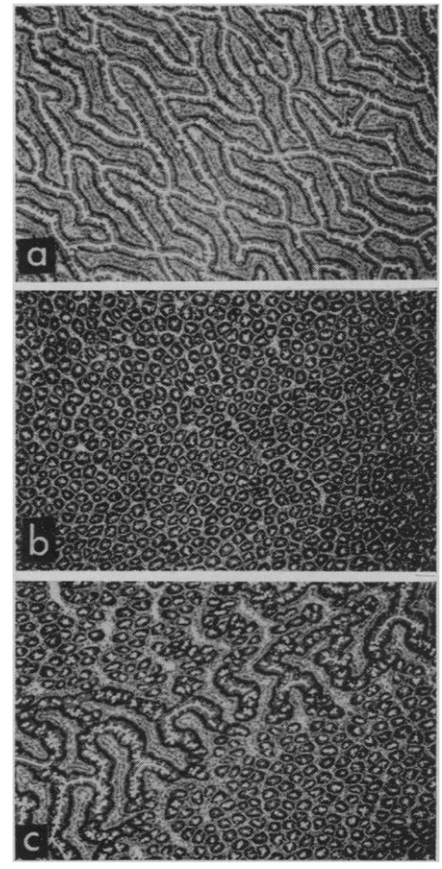

Fig. 1. Cryostat sections (4 μ thick) of villus (a), crypt (b), and junctional zone (c). Tissues were stained with toluidine and methylene blue. Magnification ×100.

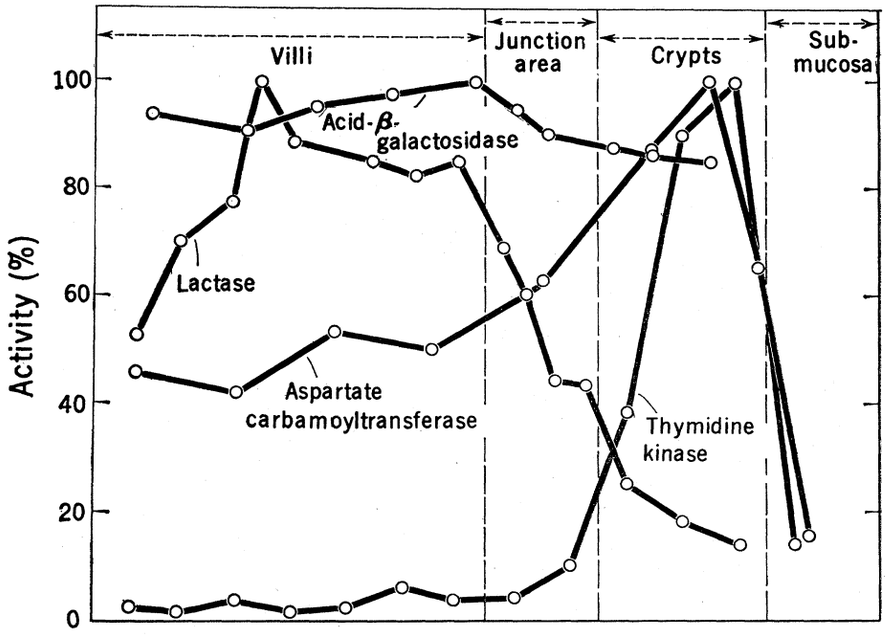

Fig. 2. Distribution of enzymes in the functional unit of intestine. The highest specific activity for each enzyme was arbitrarily set at 100 percent to obtain relative activity. Activity units were, for lactase, micromoles of glucose released per gram of protein per 10 minutes; for aspartate carbamoyltransferase, micromoles of carbamyl aspartate formed per milligram of protein per 10 minutes; for acid β-galactosidase, micromoles of *o*-nitrophenylgalactoside hydrolyzed per milligram of protein per 10 minutes; and for thymidine kinase, nanomoles of phosphorylated thymidine formed per gram of protein per minute. Each value represents four or six continuous cryostat sections combined as needed for the enzyme assay.

(*13*) was 0.25, invertase (*13*) was 0.11, dipeptidase (*14*) was 0.43, and alkaline phosphatase (*15*) was 0.49. The low activity ratios of maltase, invertase, and lactase indicated an activity five to ten times greater in cells of the villus than in those of the crypt. Thus, certain enzymes can be designated "crypt" enzymes and other enzymes can be called "villus" enzymes.

The third group of enzymes are present in the lysosomes, which are located in many cells with minimum digestive activity. The activity ratios of these enzymes were near unity. Acid β-galactosidase (*13*) was 0.86, β-glucuronidase (*16*) was 0.88, and acid phosphatase (*17*) was 0.83. There was no difference between the enzyme activities in cells of crypt and cells of villus.

The pattern of activities of thymidine kinase, aspartate carbamoyltransferase, lactase, and β-galactosidase are shown in Fig. 2. These data were plotted so that the highest specific activity for each enzyme was arbitrarily adjusted to 100 percent; all other activities were calculated relatively. This permitted presentation of all enzymatic activities on the same ordinates.

Each enzyme demonstrates a different pattern according to its cellular distribution and biochemical properties. The activity of thymidine kinase and aspartate carbamoyltransferase was very low in the submucosa, which was not in a proliferating state. Activity of thymidine kinase is relegated primarily to the crypts and the shape of its pattern indicates that this enzyme has a short half-life. The activity of aspartate carbamoyltransferase, although decreasing, persists throughout the extent of the functional unit. The shape of the pattern for aspartate carbamoyltransferase is consistent with a half-life greater than 12 to 24 hours. Activity of lactase is very low in the crypts, reaches a maximum in the mid-villus, and diminishes as the tip of the villus is approached. The pattern for this enzyme portrays a situation where synthesis of the protein exceeds the rate of degradation throughout most of the cellular passage. The exact locus for initiation of synthesis of these enzymes of differentiation is probably in close proximity to the top of the crypt (*18*). The shape of the curve for the lysosomal enzyme is not characteristic of either crypt or villus.

We have used this biochemical information in the investigation of gastrointestinal disease after administration of colchicine. Low oral doses (0.4 mg/100 g) of the drug were administered to young rats (*11*). Although there were no morphologic abnormalities detected, activity of the enzymes having specific digestive functions were considerably diminished, but those of the crypt were unaffected. When a high dose of drug (0.1 mg/100 g) was administered subcutaneously the villi were blunted and there was a decrease in mitotic activity in crypts associated with a decrease in activities of enzymes in both villus and crypt.

These results indicate that: (i) the intestine is a superb organ for the study of development because it demonstrates constant proliferation and differentiation throughout the life of the organism; (ii) measurement of enzymes having specific functions can be utilized to distinguish proliferation from differentiation; and (iii) the locus of intestinal malfunction can be determined with a combination of biochemical and morphological methods.

R. FORTIN-MAGANA,* R. HURWITZ
J. J. HERBST, N. KRETCHMER
Lt. J. P. Kennedy, Jr., Laboratories for Molecular Medicine, Department of Pediatrics, Division of Developmental Biology, Stanford University School of Medicine, Stanford, California

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* Present address, Departmento de Pediatria, Universidad de El Salvador, Facultad de Medicina, San Salvador, El Salvador. Supported by a fellowship from PHS training grant HD-00049.

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