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EDITED BY

G. H. BOURNE J. F. DANIELLI

ASSISTANT EDITOR
K. W. JEON

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Intestinal Cell Membranes

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I. Introduction

A major unsolved area in membrane biology is the control of membrane synthesis. Of particular interest are studies of the functional domains of the plasmalemma, since the separation of these domains contributes to the maintenance of tissue function. For example, the hepatocyte plasmalemma has domains that interact with adjacent hepatocytes, other areas that serve specific functions of transport from and secretion into the sinusoids, and yet another domain with different transport and secretory functions forming the canaliculus.

The plasmalemma of the small intestinal epithelial cell (enterocyte) also has well-defined histologic domains that appear to be functionally different. Studies of the intestine, however, are complicated by its being a continually differentiating tissue with a relatively rapid turnover of cells, which are moved or pushed up a villus structure and released into the lumen (Quastler and Sherman, 1959). Thus, in studies of enterocyte plasmalemmal synthesis one must not only consid-

er the easily defined functional domains of microvillus, lateral, and basal areas of the plasmalemma, but also how these domains change during differentiation of the cell as it progresses up the villus. Although cells in tissue culture have been used to study membrane synthesis and to separate functional plasmalemmal domains, such studies do not permit extrapolation to an organized tissue. The intestine's accessibility and the available methods make possible the study of this organized tissue undergoing continual, rapid differentiation.

This article will first discuss the particular properties of the intestine that are important to studies of the plasmalemma of the enterocyte. This will be followed by a review of methods for preparing enterocyte membranes, as well as their particular advantages and problems. The properties and characteristics that distinguish the isolated membrane preparations will be discussed. The rather limited number of studies of enterocyte membrane synthesis will be compared to those with cells in tissue culture or other tissues. Finally, suggestions for future investigation will be made.

II. Special Features of the Small Intestine

This section will note the relevant special characteristics of tissue and cellular organization that affect enterocyte membrane properties and synthesis.

A. Characteristics of Tissue Organization

1. *Aboral Differences in Function and Organization*

Most workers in the field are aware of the functional differences between the duodenum, jejunum, and ileum: the duodenum is the site of pancreaticobiliary secretion and the most responsive to vitamin D (Walling *et al.*, 1974); the jejunum is the area where most fat is absorbed, where intestinal-mediated terminal hydrolysis and absorption of di- and tripeptides and disaccharides occur, and where there are active transport mechanisms for the absorption of amino acids and monosaccharides; and the ileum is where one finds specific mechanisms for absorption of vitamin B_{12} (cobalamin) (Levine *et al.*, 1984) and bile salts (Wilson and Treanor, 1981; Heubi *et al.*, 1982; Kramer *et al.*, 1983), and where bacterial content in the lumen is highest.

Similarly, duodenal–ileal differences and gradients have been described for enzymes, receptors, and transport systems. Many of these are related to membrane function and synthesis, an obvious example being the different distributions of various microvillus membrane-associated enzyme activities (Garvey *et al.*, 1976; Miura *et al.*, 1983; Shields *et al.*, 1984). There are sharp aboral gradients of alkaline phosphatase and γ-glutamyl transpeptidase activities, with

the highest activities in the duodenum. Sucrase activity is highest in the proximal jejunum (Fig. 1) (Garvey *et al.*, 1976). The receptor for intrinsic factor–cobalamin, as would be expected, has been localized to the ileum (Levine *et al.*, 1984).

Transport of nutrients from the intestinal lumen is a particularly important function of the microvillus portion of the enterocyte; it too shows significant differences along the length of the intestine. For example, Crane and Mandelstam (1960) showed that glucose accumulated at higher concentrations in the jejunum, whereas galactose was higher in the ileum. In each case the difference was 2–2.5 times more. Batt and Peters (1976) measured *in vivo* absorption of galactose by the rat intestine and found that the absorptive capacity was 4–5 times greater in the jejunum as compared with the ileum. They also claim differences in K_m (J = 53, I = 20 nM) and V_{max} (J = 30, I = 6.4 μmol/hour/cm). Hopfer *et al.* (1976), using microvillus membrane vesicles, showed that D-glucose transport was 2–3 times slower in the ileum. Similarly, Kessler *et al.* (1978b) showed significant differences in the kinetics of uptake for D-glucose by microvillus membrane vesicles prepared from rabbit jejunum and ileum (K_m: J = 185, I = 145 μM; V_{max}: J = 1.45, I = 0.45 nmol/mg of protein/second). It is interesting to note that physiologists have mainly used the ileum for their studies

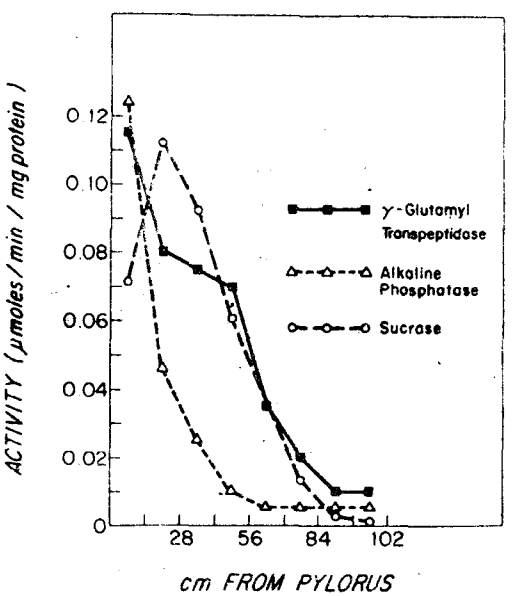

FIG. 1. Activity of brush border enzymes along the length of the rat small bowel. From Garvey *et al.* (1976).

of glucose and galactose fluxes (Naftalin and Curran, 1974), despite the ileum having lower rates of transport.

Cell metabolism also differs among the regions of the small intestine and this may affect the content and physical properties of subcellular membranes isolated from the intestinal epithelial cell. The sites of cholesterol synthesis, i.e., HMG–CoA reductase activity, differs from that of cholesterol esterification (Stange and Dietschy, 1983a,b; Stange *et al.*, 1983). Cholesterol synthesis, as measured by [^{3}H]water incorporation into digitonin-precipitable sterols, is as much as four times higher in the ileum than in the jejunum, whereas acyl-CoA/cholesterol acyltransferase-specific activity differed little between the jejunum and the ileum (Stange *et al.*, 1983). An intestinal phospholipase has been found to be three times higher in the ileum than in the proximal jejunum (Mansbach *et al.*, 1982). There are significant differences in the levels of glycosyltransferase activities among the three regions of the intestine (Kim *et al.*, 1975). Galactosyltransferase activity is 3–10 times higher in the terminal ileum than in either the duodenum or the jejunum (Fig. 2) (Weiser *et al.*, 1985).[1] Increased breakdown of substrates, however, does not sufficiently account for the decreased activity in the jejunum. What this might suggest as to differences in membrane glycoprotein and mucin synthesis is unclear.

Altmann (1975) demonstrated profound differences in the way cycloheximide affects the different areas of the intestine. A major effect of relatively large doses of cycloheximide (15 mg/kg) was to cause premature exfoliation of cells and atrophy of villi. The duodeno–jejunal areas were much more reduced than the ileal areas.

It is possible that some, but not all, of these intestinal regional differences are secondary to "environmental" factors of the intestine, i.e., the effects of pancreaticobiliary secretions in the duodenum–jejunum and the presence of bacteria (and their glycosidases, etc.) in the ileum. Bile acids have been shown to release enzymes from the rat jejunum (Bossmann and Haschen, 1983). Alpers and Isselbacher (1967) pointed out that pancreatic ribonuclease activity, even though relatively low in intestinal preparations, significantly affected protein synthesis measurements. Similarly, Sugano *et al.* (1977) showed that one could not accurately measure HMG–CoA reductase activity in the intestine without including a trypsin inhibitor, a finding, compatible with the earlier observation of Woodley and Kenny (1969), that pancreatic protease activities persisted in microvillus

[1]Lau and Carlson (1981) have noted increased breakdown of UDPgalactose by intestinal homogenates and indicated that preincubation with EDTA to inhibit pyrophosphatase activity was necessary before galactosyltransferase activity could be accurately measured. More recently, Byrd *et al.* (1985) have purified intestinal nucleotide pyrophosphatase activity and shown it to be highest in jejunal microvillus membrane. We have also found increased breakdown of UDPgalactose by jejunal homogenates due to the combined action of nucleotide pyrophosphatase and alkaline phosphatase activities (Weiser *et al.*, 1986).

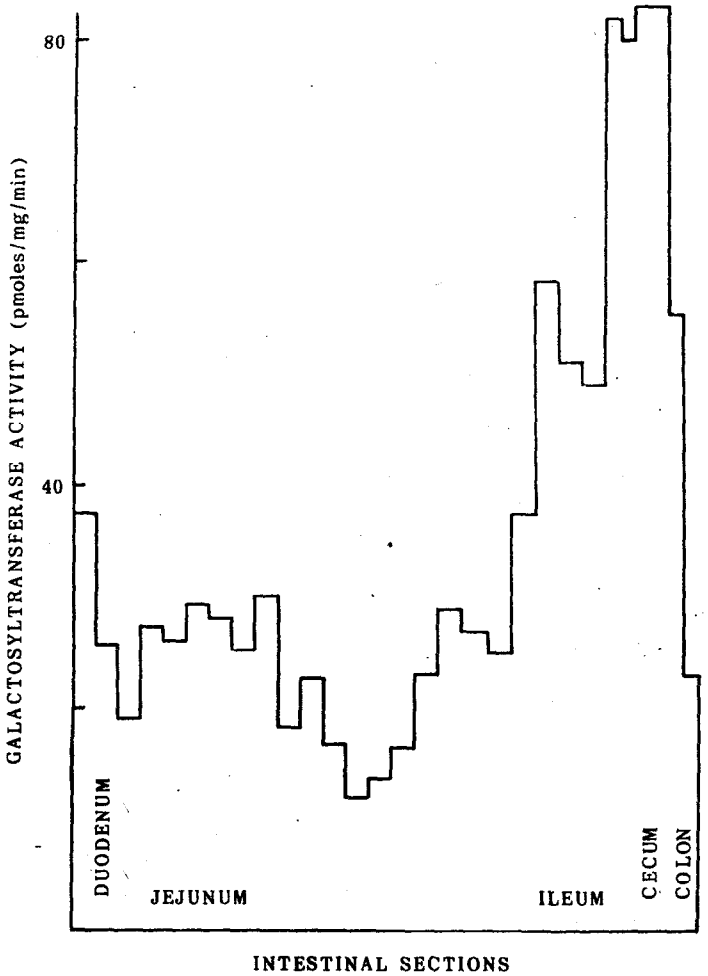

FIG. 2. Aboral gradient of galactosyltransferase activities. The data shown are for Gal:GlcNAc galactosyltransferase activity, using ovalbumin as acceptor. Gal:GalNAc galactosyltransferase activity, using ovine submaxillary mucin as acceptor, had a similar gradient (unpublished observations). From Weiser *et al.* (1986).

preparations. Alpers and Tedesco (1975) have suggested that pancreatic proteases may be important to normal intestinal microvillus membrane protein turnover. Furthermore, we have found significant phospholipase activity in homogenates of rat mucosal scrapings but not in those of isolated cells (Table I). The implications for the isolation and characterization of intestinal membranes are apparent but infrequently considered.

TABLE I
PHOSPHOLIPASE AND FATTY ACID DIFFERENCES DUE TO THE METHOD OF INTESTINAL CELL PREPARATION[a]

Intestinal cell preparation	Phospholipase A activity of homogenates (pmol/minute/mg protein)	Nonesterified fatty acid content of purified membrane (nmol/mg protein)	
		Golgi	Lateral–basal
Scrapings	122 ± 5	1374 ± 120	825 ± 103
Isolated cells	23 ± 5	69 ± 33	<50

[a]Data from Walters *et al.* (1984).

2. *Gradients of Differentiation and Their Relationship to Membrane Synthesis*

The rat intestinal epithelium renews itself every 36–72 hours. As diagrammed in Fig. 3, the zone of active mitosis is at the bottom half of the crypt and the zone of extrusion of the "excess" cells is at the villus tip. In between are areas of differentiating and differentiated cells. The zone of differentiating cells extends from the upper crypt to the lower villus areas. Cells from different areas may be obtained by planing devices (Imondi *et al.*, 1969; Fortin-Magana *et al.*, 1970) or by sequential release in EDTA or citrate buffers (Webster and Harrison, 1969; Weiser, 1973a; Miura *et al.*, 1983); the data derived from these different methods are similar and are summarized in Figs. 4 and 5 and Table II. The data indicate that the level of enzymatic activities depends on the position the cell occupies along the crypt–villus axis. Most of the enzyme activities in the crypt area probably reflect mitotically active undifferentiated cells and the gradient of enzyme activities in the villus area most likely signifies progressive differentiation. Many of the enzyme activities tested are associated with development of the microvillus structure. However, no explanation has been sought as to why disaccharidase activities reach a plateau near the villus–crypt transition zone, whereas alkaline phosphatase activity continues to increase until the cell is extruded at the villus tip.

Active transport of monosaccharides and amino acids appears to be a property of the microvillus membrane of the differentiated villus cell (Garvey *et al.*, 1976). In contrast, active ion and water secretion has been attributed to the crypt zone, particularly hormone-induced chloride secretion (Field *et al.*, 1986). Most of the evidence for crypt involvement in chloride secretion is based on Ussing chamber experiments. It is difficult to explain the data on a cellular basis, however, if one views the crypt area as a zone of relatively undifferentiated cells. Four explanations might be considered: (1) Undifferentiated or differentiating cells can perform hormone-responsive secretory functions. (2) There are special

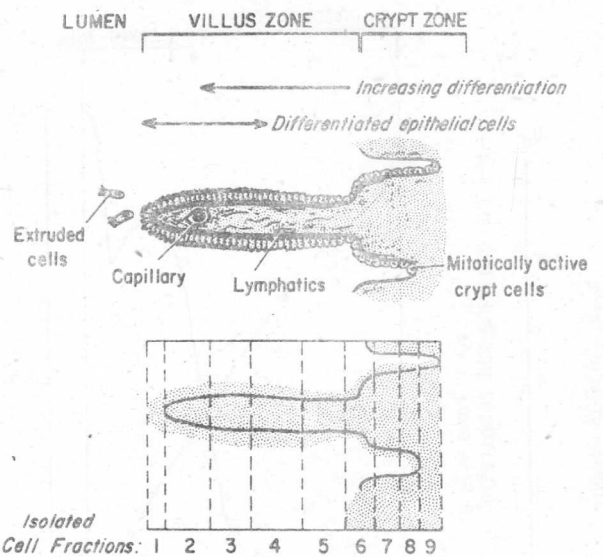

FIG. 3. Diagram of an intestinal crypt–villus unit and an idealized representation of isolated cell fractions that are obtained using the method described by Weiser (1973a).

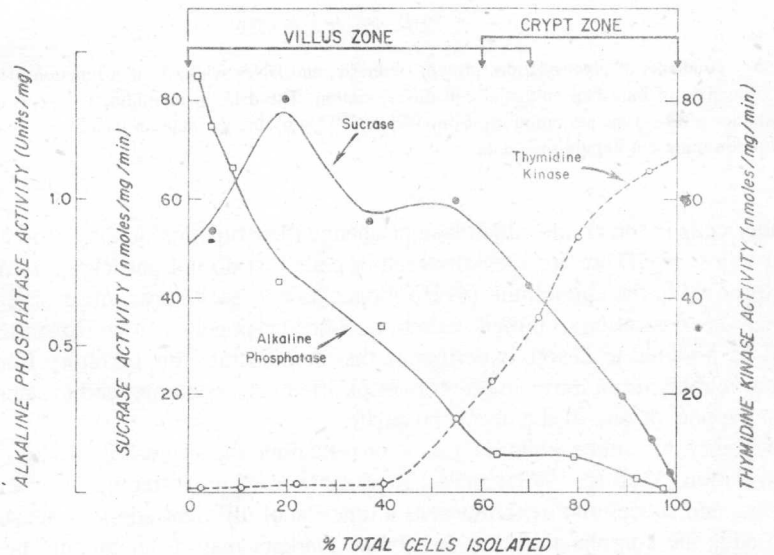

FIG. 4. Enzyme activity gradients as a function of the crypt–villus axis of intestinal epithelial cell differentiation. From Weiser (1973a).

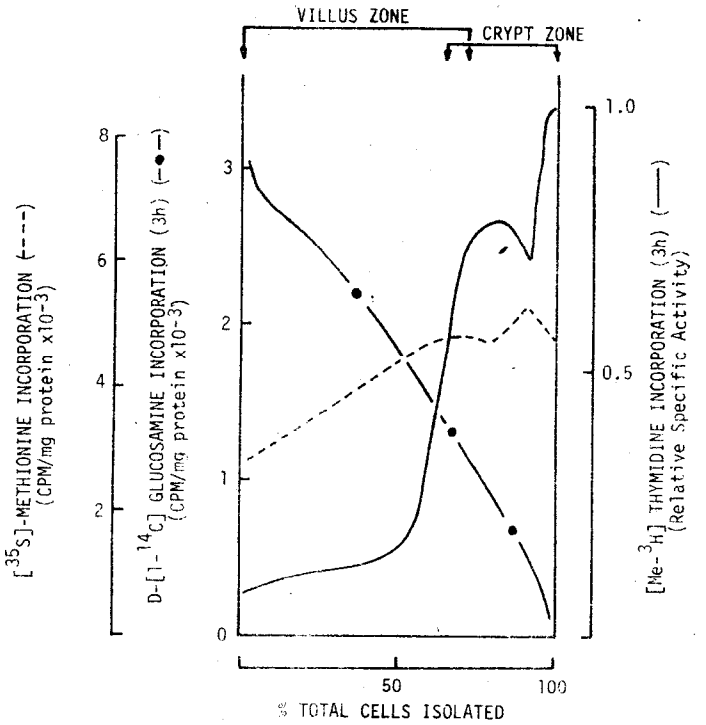

FIG. 5. Gradients of glycosylation, protein synthesis, and DNA synthesis as a function of the crypt–villus axis of intestinal epithelial cell differentiation. The data on thymidine (————) and glucosamine (—●—) incorporation are from Weiser (1973a,b) and the data on methionine (- -.-) incorporation represent unpublished data.

secretory cells in the crypts which lose or change their function as they progress up the villus. (3) There are special secretory cells that do not participate in the rapid turnover of the epithelium. (4) Hormones do not reach upper villus cells in sufficient concentration to initiate secretion. Since crypt cells can be isolated, it should be possible to design experiments that demonstrate this secretory function, particularly as an intrinsic property of purified plasmalemma, and to obtain data in support of one of the above postulates.

The ability to isolate cells as fractions reflecting the crypt–villus axis of differentiation (Weiser, 1973a) makes it possible to study differences in plasmalemma and subcellular membranes as a function of differentiation. However, it also adds the complication that membrane markers may differ among these cells; the plasmalemma of the differentiated villus cells is quite different from that of the undifferentiated crypt cell or of the goblet cell. Thus, alkaline phos-

TABLE II

VILLUS–CRYPT GRADIENT LOCALIZATION OF PEAK CONCENTRATIONS OR ACTIVITIES[a]

Villus tip	Lower villus/upper crypt	Crypt
	Protein synthesis	
Alkaline phosphatase		
5'-Nucleotidase		
Na^+,K^+-ATPase		
Guanylate cyclase		
		Adenylate cyclase
		Thymidine kinase
Adenosine deaminase		
Thymidylate phosphatase		
		S-Adenosylmethionine decarboxylase
Ornithine decarboxylase		
		Phospholipase
Nucleotide–sugar pyrophosphatase		
		Galactosyltransferase
Sialyltransferase		
		N-Acetylglucosaminlytransferase I
	N-Acetylglucosaminlytransferase II	
	Dolichyl-p-mannosyltransferase	
Sucrase and other disaccharidases		
Aminopeptidases and dipeptidyl aminopeptidase		
Enterokinase		
Nonspecific esterase		
		Cholesterol synthesis
	Acyl-CoAcholesterol acyltransferase	
Active transport processes (CHO and AA)		
Fat absorption		
CaBP		
ATP-dependent Ca^{2+} uptake		
		Chloride secretion
Intrinsic factor–cobalamin receptor (ileum)		
Glucosylceramide		
Hematoside		
		Trihexosylceramide

(*continued*)