DEMONSTRATION OF GLYCERIDE SYNTHESIS BY BRUSH BORDERS OF INTESTINAL EPITHELIAL CELLS

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Most of the dietary triglycerides are presented for transport across the mucosal cell in the form of fatty acids and monoglycerides. Subsequent to their entry into the cell, these lipid moieties are reesterified to triglycerides by enzymes which to date have been found to be primarily localized to the microsomes of the absorbing cell (Senior, 1964; Isselbacher, 1965).

Most electron microscopic studies of the intestine during fat absorption have shown lipid droplets in between the outermost projections of the epithelial cells, i.e. the microvilli. It has been suggested that the lipids penetrate the mucosal cells after passing between these microvilli, perhaps by the process of pinocytosis (Palay and Karlin, 1959). It is noteworthy that as soon as lipid can be observed within the cell itself, it is seen as membrane enclosed droplets deep to the terminal web.

Since microvilli and terminal web areas of intestinal cells can be isolated as an organelle known as the brush border (Miller and Crane, 1961), we were anxious to determine if some of the initial reactions in glyceride synthesis might indeed occur at this site. Previously only hydrolytic enzymes (disaccharidases, lipases) have been demonstrated in the microvilli (Miller and Crane, 1961; Senior and Isselbacher, 1963). The purpose of this report is to present evidence that brush border preparations, obtained in a much purer manner than heretofore described, are capable of incorporating C^{14}-palmitate into higher glycerides and that some of the enzymes involved in this esterification are associated with the microvilli of the mucosal cells.

MATERIALS AND METHODS

Rat intestinal mucosal scrapings and microsomes were obtained as previously described (Senior and Isselbacher, 1962). Brush borders were prepared by the method of Miller and Crane (1961). They were then washed twice in 2.5mM EDTA, pH 7.4, and resuspended in the same solution. This preparation (Fraction I) was used in the initial studies.

Further purification was achieved by suspending Fraction I in 100mM saline for 30 minutes at 4°. The resulting dense white sediment, which consisted of villous remnants, cellular and nuclear debris, was removed by passage through glass wool (Harrison and Webster, 1964). The brush borders present in the non-adsorbed fraction were again suspended in 2.5mM EDTA, pH 7.4, and this more purified preparation (Fraction II) was used in all experiments unless otherwise noted. The details of this method will be described more fully elsewhere.

Fraction II was essentially free of particulate debris and cytoplasmic tags by phase contrast and electron microscopy. Cytochrome oxidase activity was absent. Purification was monitored by using alkaline phosphatase and invertase as reference enzymes. Specific activities of these enzymes were always 19-25 times greater than in the original homogenate.

Palmitate-1-C^{14} was obtained from Applied Science Laboratories, State College, Penna. Chromatographically pure 2-monopalmitin and 1,2 diolein were gifts of Dr. F. H. Mattson. Isolation of lipid fractions and radioactivity measurements were performed as described previously (Scheig and Isselbacher, 1965). Thiokinase and monoglyceride acylase were determined by the methods of Senior and Isselbacher (1960,1962). Diglyceride acylase was assayed radiochemically (Ailhaud *et al*, 1964) with palmityl-1-C^{14} CoA and 1,2 diolein and spectrophotometrically with unlabeled substrates using the Ellman (1959) reagent to measure CoA release.

RESULTS

The data in Table 1 indicate that lipid synthesis can be demonstrated in vitro by brush borders of intestinal mucosa. Palmitate was incorporated into the major glycerides including phospholipid. This incorporation occurred even without the addition of any acceptor; in fact the addition of α-glycerophosphate to the system did not appreciably enhance the fatty acid incorporation. However, the addition of 0.5 μmole monopalmitin caused a doubling of the esterification with the major increase being in the diglyceride fraction. Consistent with earlier observation on intestinal microsomes (Dawson and Isselbacher, 1960), when CoA and ATP were omitted negligible amounts of label were incorporated.

Table 1

Incorporation of palmitic acid-1-C^{14} into glycerides by rat and hamster brush border preparations

Conditions	Phospholipid and Monoglyceride	Diglyceride	Triglyceride	Total
		%		
Rat				
Complete system	4.4	1.8	6.0	12.2
Plus α-glycerophosphate	7.8	2.8	4.9	15.5
Plus 2-monopalmitin	1.0	30.5	7.4	38.9
Without CoA, ATP	0.8	0.0	1.7	2.5
Hamster				
Complete system	3.2	0.8	13.3	17.3
Plus 2-monopalmitin	0.5	14.5	20.2	35.2

The complete system contained 20 μmoles each of KF, $MgCl_2$, ATP and GSH, 1.0 μmole Coenzyme A, 300 μmoles Tris pH 7.4, 20mμmoles palmitic acid 1-C^{14} (specific activity 8.5 μc/μM) plus 0.5 mg. protein in a final volume of 2.5 ml. Incubations were for 60 minutes at 37°C. 1.0 μmole of α-glycerophosphate or 0.5 μmole 2-monopalmitin was added as noted. The values shown include corrections for simultaneous zero time controls. Rat brush borders were purified 19 fold with respect to alkaline phosphatase; hamster brush borders were purified 23 fold with respect to invertase.

Since Johnston and Borgström (1964) suggested that hamster brush borders (isolated by the method of Miller and Crane, 1961) were not capable of glyceride synthesis, we examined hamster brush borders prepared by the present techniques. It will be noted (Table 1) that C^{14}-palmitate was incorporated as actively by hamster as by rat brush borders. However, with the hamster preparation there was predominantly triglyceride formation, while rat preparations yielded mostly diglyceride. A similar species difference of *in vitro* glyceride synthesis has been noted by Johnston *et al* (1965).

Intestinal microsomes are normally very active in lipid esterification and the possibility existed that the synthetic activity of the brush border preparations was solely due to microsomal contamination. However, the most pure brush border fraction showed not a decrease in palmitate incorporation but actually a slight increase above the less pure preparation (Table 2). Had significant contamination occurred by particles such as microsomes, their removal should have resulted in a lower incorporating activity.

Table 2

Palmitic acid-1-C^{14} incorporation into glycerides by rat brush border preparations at different stages of purification

Preparation	Alkaline Phosphatase	C^{14}-palmitate incorporation
	units/mg protein	μmoles/mg protein
Homogenate	13	16.8
Brush Borders		
Fraction I	108	7.9
Fraction II	265	9.4

Conditions of incubation were similar to those described in Table 1. A unit of alkaline phosphatase activity represented 1 μmole of p-nitrophenyl-phosphate hydrolyzed per 30 minutes.

Further evidence for the role of brush borders in lipid synthesis was obtained by specific enzymatic studies. Palmitate thiokinase, mono-

glyceride and diglyceride acylase activities could readily be demonstrated in rat brush border preparations. The specific activity of thiokinase and monoglyceride acylase in the brush borders was 1/5 to 1/3 (respectively) that of the microsomes. However, using two separate assays, diglyceride acylase specific activity was consistently greater in the brush border preparations (Fraction II) compared to microsomes.

DISCUSSION

The results of the present experiments provide evidence that the brush borders of intestinal epithelial cells possess enzymes capable of incorporating long chain fatty acids into neutral glycerides and phospholipids. To our knowledge this is the first demonstration of synthetic activity in the outermost structure of the mucosal cells. It seems unlikely that this activity was due to particulate contamination in view of the purity of preparation used, the enhanced C^{14}-palmitate incorporation with increasing purification and the fact that a component of the esterifying system (diglyceride acylase) was more active in brush borders than in microsomes.

The major question raised by the above experiments is what the overall functional significance of this enzymatic localization may be. It is possible that microvilli may be involved in the initial esterification of long chain fatty acids and monoglycerides as they enter the mucosal cell. Consistent with such a postulate would be reports which describe lipid-like particles not just between but also within microvilli during fat absorption (Lacy and Taylor, 1962; Rostgaard and Barnett, 1964).

It is also conceivable that the brush border enzymes may be involved in maintaining the structural lipids of the microvilli. The fact that the C^{14}-palmitate incorporation required no exogenous acceptor would suggest that endogenous acceptor sites were present in the membrane glycerides but studies on the exact lipid composition of the purified microvilli will be needed to elucidate this. It is of interest that in preliminary experiments

we have been unable to demonstrate in the microvilli any de novo synthesis of fatty acids from acetyl-CoA or malonyl-CoA.

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