The Distribution of Lipid in Colonic Mucosa* (34051)

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(Introduced by J. B. Kirsner)

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The distribution of stainable lipid in the mucosa of the gastrointestinal tract of man has not been completely described. Characteristic patterns have been demonstrated in the jejunum in health and disease (1–3), but little is known about the pattern of fat distribution in the colon of man (4). This study demonstrates the localization of stainable lipid in normal rectal mucosa and an altered pattern of distribution in patients with steatorrhea.

Materials and Methods. Ninety-two pieces of colonic mucosa were obtained from 79 subjects. All but six specimens were obtained by biopsy of the lower rectum at sigmoidoscopy; four specimens were obtained at operation and two from colostomy stomas. The control group (Group I) consisted of 32 biopsies obtained from 13 normal subjects and 19 patients with a variety of nonspecific gastrointestinal diseases. Twenty-seven biopsies were obtained from 23 patients with ulcerative colitis (Group II) and 20 biopsies from 12 patients with granulomatous colitis (Group III). Thirteen biopsies were obtained from 12 subjects with steatorrhea (Group IV) of varying etiology.

Experimental Procedures. Oleic acid emulsions were prepared by mixing 50 ml of 0.4 *N* phosphate buffer (pH 6.3), 50 ml of oleic acid (Matheson, Coleman and Bell) and 250 mg of sodium taurocholate (Sigma Company) in a Waring Blendor for 10 min. Two patients with "double-barrel" colostomies had a defunctionalized rectal segment which allowed access via the distal colostomy. One hundred milliliters of the oleic acid emulsion was infused into this colonic segment via the colostomy. Biopsies of the rectal mucosa were obtained prior to, and 2 hr after, the introduction of the emulsion.

Cecostomies were performed in the five mongrel dogs weighing between 15 and 22 kg. One hundred milliliters of the oleic acid emulsion was introduced on ten occasions into the colon of these dogs through the cecostomy with a catheter. During two additional studies, the oleic acid was omitted and only the sodium taurocholate solution was infused. Suction biopsies of the rectum of these dogs were then obtained at varying intervals. Colonic tissue from six control dogs was obtained immediately prior to sacrifice.

Histological Techniques. Each specimen from the human subjects was cut in half, and one piece was immediately frozen in liquid nitrogen and stored at —70°. Cryostat sections were cut at 4 μ and stained by the oil red O method (5). The other half was placed in 10% buffered formol saline; paraffin sections were cut and stained with hematoxylin and eosin. The entire specimen from each dog was immediately frozen in liquid nitrogen, stored at —70°, later sectioned at 4μ with a cryostat and stained with oil red O.

All specimens were coded and reviewed without knowledge of the clinical diagnosis or experimental procedures. The presence of fat was graded on a 0–4+ scale in each of three locations: (1) in the supranuclear area of the surface epithelial cell; (2) in the subnuclear area of the surface epithelial cell, and (3) in the lamina propria where it was

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TABLE I. Distribution of Fat in Colonic Mucosa.

Group[a]		Surface epithelium		
		Supra-nuclear	Sub-nuclear	Lamina propria
Control (32)	0	32	14	5
	1+	0	9	11
	2+	0	9	13
	3+	0	0	3
	4+	0	0	0
Ulcerative colitis (27)	0	27	12	3
	1+	0	5	4
	2+	0	7	18
	3+	0	3	2
	4+	0	0	0
Granulomatous colitis (20)	0	18	8	3
	1+	2	2	4
	2+	0	7	6
	3+	0	1	4
	4+	0	2	3
Steatorrhea (13)	0	6	2	1
	1+	0	2	2
	2+	1	4	8
	3+	2	3	2
	4+	4	2	0

[a] Figures in parentheses represent number of biopsy specimens studied.

found either free in lymphatics or within macrophages.

Results. Human. Table I summarizes the distribution of fat in the surface epithelium and the lamina propria. Fat was found frequently in the subnuclear portion of the surface epithelium and in the lamina propria in the control and all disease groups (Fig. 1). A pattern of distribution in these two locations could not be related to the presence of any specific disease. When specimens of mucosa from the right and left portions of the colon were obtained from two patients at subtotal colectomy, the pattern of fat distribution was similar in the right and left colon.

Heavy (2+ or more) fat staining in the supranuclear portion of the surface epithelium was found in seven patients (Fig. 2). All seven of these subjects had significant steatorrhea. The difference in supranuclear fat staining between patients with steatorrhea and either normal controls or patients with colitis was highly significant ($p<0.0001$) when analyzed by Wilcoxon's sum-of-the-ranks test. In only two other subjects was there any fat (1+) in the supranuclear portion of the surface epithelium of the rectal mucosa; both of these patients had granulomatous colitis with associated small intestinal involvement and low serum carotene levels. Fat was not found in the supranuclear portion of the surface epithelium in any other subject.

In subject D.L., in whom steatorrhea was secondary to massive small intestinal resection, a biopsy specimen of mucosa was obtained proximal to a temporary colostomy and another from the defunctionalized rectum. The proximal specimen contained 4+ fat in the supranuclear portion of the surface epithelium while that from the defunctionalized rectum contained none. In two other patients, mucosa from a defunctionalized rectum was examined before and after perfusion of the lipid emulsion through the distal colostomy. Although supranuclear lipid was not identified prior to infusion, 2 hr later lipid was noted in both patients in the supranuclear portion of the surface epithelium.

Dogs. Lipid was never demonstrated in 16 specimens of colonic mucosa from the five dogs obtained at varying intervals prior to fat administration, nor was fat identified in the surface epithelium in any of the material from the six control dogs.

Biopsy specimens were obtained from the rectum 2 hr after administration of the oleic acid emulsion ten times in the five dogs. On each occasion lipid was identified in the supranuclear portion of the surface epithelium. The location of lipid was identical to that observed in the mucosa obtained from patients with steatorrhea. In five specimens obtained 24 hr after the introduction of the oleic acid mucosal lipid was no longer present; nor was mucosal lipid demonstrated 2 hr after the introduction of the sodium taurocholate solution.

Discussion. The present study demonstrates a similar pattern of lipid distribution in the colonic mucosa of normals and of patients without steatorrhea. In these patients stainable lipid is found in the lamina propria and in the subnuclear portion of the surface

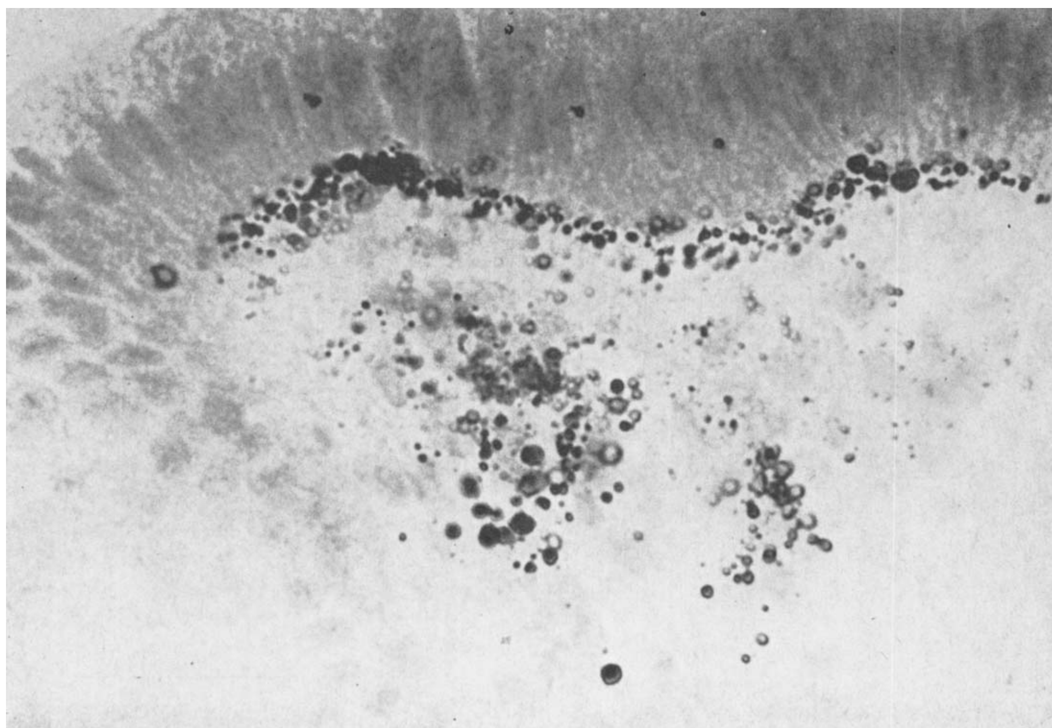

FIG. 1. Rectal biopsy specimen from a normal control subject. The black dots represent lipid scattered in the lamina propria and the subnuclear portion of the surface epithelium. This distribution was found in rectal mucosa from both normal subjects and patients without steatorrhea; oil red O stain; ×450.

epithelium. Neither a quantitative nor a qualitative distribution pattern is apparent. Deposition of lipid in the basal part of the surface epithelium and the adjacent lamina propria was recently reported in the rectal mucosa of four normal subjects (4). No other information about either presence or distribution of rectal lipid is available.

The origin of the lipid found in normal subjects and patients without steatorrhea is not known. It may represent absorbed dietary lipid, lipid transported from within the mucosa outward toward the luman or structural lipid deposited in these locations. However, the identification of the same distribution of lipid in three biopsies from defunctionalized colons strongly suggests that the lipid did not originate from colonic luminal contents.

Much more information is available about the localization of stainable lipid in jejunal mucosa. The normal fasting jejunal mucosa demonstrates stainable lipid in the lamina propria (1, 2).

An altered pattern is observed in the lipid distribution in the jejunal mucosa in various diseases. Schenk *et al.* have stated that in celiac sprue, lipid is seen within the surface epithelial cell in a supranuclear location (1, 2). In tropical sprue, these same investigators demonstrated the presence of lipid in the area of the thickened basement membrane (2). They concluded that the distribution of lipid in fasting jejunal mucosa is so characteristic for celiac sprue and tropical sprue that these two diseases may be distinguished on this basis alone.

In abetalipoproteinemia, a defect in lipid absorption exists. The failure to form chylomicrons prevents the removal of lipid from the epithelial cells. In fasting jejunal mucosal biopsies in abetalipoproteinemia, massive amounts of lipid are demonstrated in the supranuclear portion of the surface epitheli-

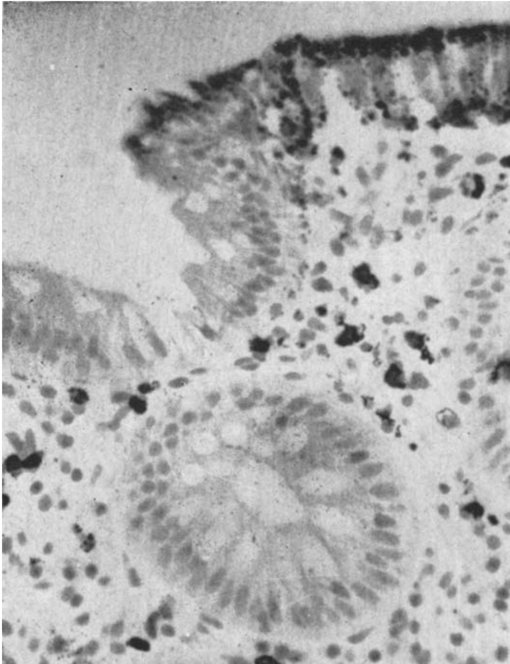

FIG. 2. Colonic mucosa from patient who had steatorrhea. Lipid (black) is seen in lamina propria and in both supranuclear and subnuclear portions of surface epithelium. The presence of lipid in a supranuclear location was seen only in patients with steatorrhea; oil red O stain; ×150.

um (3). This lipid presumably represents dietary lipid which has been absorbed but is unable to be transported away from the cell.

The present investigation demonstrates significant amounts of stainable lipid in the supranuclear portion of the surface epithelium only in seven patients, all of whom have steatorrhea. The origin or fate of this stainable lipid is not known. But evidence exists which suggests that this lipid is derived from the colonic luminal content. The hypothesis that this lipid is derived from colonic luminal content is supported by: (1) the demonstration of lipid in the supranuclear portion only in patients with steatorrhea, *i.e.*, patients with increased fat in their fecal content; (2) the occurrence of lipid in the supranuclear portion in a colostomy biopsy specimen of patient D.L., but not in the mucosa from his defunctionalized rectum; (3) the appearance of lipid in the supranuclear portion of the surface epithelium in isolated loops of human colon after infusion of an oleic acid emulsion; and (4) the consistent appearance of lipid in the supranuclear portion of the surface epithelialium in the canine rectal mucosa after infusion of an oleic acid emulsion.

Other data exist that indicate that the colon may absorb lipids, primarily short-chain fatty acids. Octanoate has been absorbed from the rat and dog colon (6, 7) and hexanoate has disappeared from the colon of man during perfusion studies (8). Several lipid-soluble substances have been demonstrated to be absorbed by the colon of man. Furthermore, it has been suggested that the colonic absorption of medium-chain triglycerides may account in part for part of the decrease in fecal fat excretion in patients with short bowels when medium-chain triglycerides have been given (9).

This study, then, suggests that the origin of lipid in the supranuclear portion of the surface epithelium of the rectal mucosa of patients with steatorrhea is from fecal content and that lipid may be taken up by the colonic epithelium whenever increased amounts of fat are presented to it. Nothing is known, however, about the fate of this lipid. Further experiments to demonstrate more precisely both the origin and fate of this lipid are planned.

Summary. A description of the distribution of stainable lipid in the colonic mucosa of man is presented. In normal individuals and patients without steatorrhea, stainable lipid is observed in the lamina propria and the subnuclear portion of the surface epithelium. Only in patients with steatorrhea is lipid found in the supranuclear portion of the surface epithelium. This observation and an identical distribution of lipid (*i.e.*, supranuclear part of the surface cell) in segments of colon perfused with fat in both man and dog suggests that the lipid observed in the supranuclear area may originate from colonic luminal lipid.

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1. Samloff, I. M., Davis, J. J., and Schenk, E. A., Gastroenterology **48,** 155 (1965).

2. Schenk, E. A., Samloff, I. M., and Klipstein, F. A., Am. J. Pathol. **47,** 765 (1965).

3. Isselbacher, U. J., Scheig, R., Plotkin, G. R., and Caulfield, J. B., Medicine **43,** 347 (1964).

4. Melnyk, C. S., Braucher, R. E., and Kirsner, J. B., Gastroenterology **52,** 985 (1967).

5. Bancroft, J. D., "An Introduction to Histochemical Technique," 268 pp., Butterworth, London (1967).

6. Valdivieso, V. D. and Schwabe, A. D., Am. J. Digest Diseases **11,** 474 (1966).

7. Pihl, E. G., Glotzer, D. J., and Patterson, J. F., J. Appl. Physiol. **21,** 1059 (1966).

8. Levitan, R. and Patterson, J. F., Gastroenterology **50,** 856 (abstr.) (1966).

9. Fordtran, J. S., Gastroenterology **50,** 899 (1966); comment on New Engl. J. Med. **274,** 72 (1966).

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