Fatty Acid-Binding Protein in Small Intestine

IDENTIFICATION, ISOLATION, AND EVIDENCE FOR ITS ROLE IN CELLULAR FATTY ACID TRANSPORT

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ABSTRACT A soluble fatty acid-binding protein (FABP), mol wt ~ 12,000 is present in intestinal mucosa and other tissues that utilize fatty acids, including liver, myocardium, adipose, and kidney. This protein binds long chain fatty acids both in vivo and in vitro.

FABP was isolated from rat intestine by gel filtration and isoelectric focusing. It showed a reaction of complete immunochemical identity with proteins in the 12,000 mol wt fatty acid-binding fractions of liver, myocardium, and adipose tissue supernates. (The presence of immunochemically nonidentical 12,000 mol wt FABP in these tissues is not excluded.) By quantitative radial immunodiffusion, supernatant FABP concentration in mucosa from proximal and middle thirds of jejuno-ileum significantly exceeded that in distal third, duodenum, and liver, expressed as micrograms per milligram soluble protein, micrograms per gram DNA, and micrograms per gram tissue. FABP concentration in villi was approximately three times greater than in crypts. Small quantities of FABP were present in washed nuclei-cell membrane, mitochondrial and microsomal fractions. However, the amount of FABP solubilized per milligram membrane protein was similar for all particulate fractions, and total membrane-associated FABP was only about 16% of supernatant FABP. Intestinal FABP concentration was significantly greater in animals maintained on high fat diets than on low fat; saturated and unsaturated fat diets did not differ greatly in this regard.

The preponderance of FABP in villi from proximal and middle intestine, its ability to bind fatty acids in vivo as well as in vitro, and its response to changes in dietary fat intake support the concept that this protein participates in cellular fatty acid transport during fat absorption. Identical or closely related 12,000 mol wt proteins may serve similar functions in other tissues.

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INTRODUCTION

In recent studies, we described a fatty acid binding-protein (FABP)[1] in cytosol of intestinal mucosa (1, 2). The existence of this protein was postulated in order to explain differences observed earlier between saturated and unsaturated long chain fatty acids in regard to their intestinal absorption and esterification (3); these differences could not be accounted for by existing concepts of intestinal fat absorption. It was proposed that FABP might participate in the translocation of fatty acids from the plasma (microvillus) membrane to the endoplasmic reticulum through the cytosol, an aqueous medium in which long chain fatty acids would be expected to exhibit only limited solubility.

In the initial studies of FABP, however, it became evident that similar or identical proteins were present in many organs other than intestine and that these organs, including liver, heart, adipose, and kidney, shared with intestinal mucosa the capacity to take up and metabolize significant quantities of long chain fatty acids. Furthermore, FABP exhibited slight binding to sulfobromophthalein (BSP), and this observation, together with its elution characteristics during gel filtration (2), suggested that FABP might be related to the "Z" protein, described earlier as the lesser of two hepatic cytoplasmic organic anion-binding proteins (4).

The cellular uptake and metabolism of fatty acids have received considerable attention and have been studied in a number of systems (5–9). Hitherto, how-

[1] *Abbreviations used in this paper:* BSP, sulfobromophthalein; FABP, fatty acid-binding protein; PCMB, *p*-chloromercuribenzoate; PCMPS, *p*-chloromercuriphenylsulfonate.

ever, no specific protein has been shown to participate in the utilization of fatty acids before their activation to acylcoenzyme A derivatives in the endoplasmic reticulum. For this reason, and in view of the widespread tissue distribution of FABP, it seemed possible that this protein (or a group of closely related proteins) might play a more general role in intracellular fatty acid transport or utilization, transcending its relationship to intestinal fat absorption. We felt that this concept would be strengthened, however, if it could be shown for the intestine that FABP exhibited binding to fatty acids in vivo and that mucosal FABP concentrations were responsive to changes in dietary fat intake.

In this report, we present evidence regarding the identification of FABP and its physiological function in the intestine. The isolation of intestinal FABP is described, as is the specific immunochemical assay employed to measure concentrations of FABP in intestinal mucosa and to document its response to changes in dietary fat intake. Evidence concerning its possible relationship to Z is also presented. Portions of these studies have been reported in abstract form (1, 10, 11).

METHODS

Materials. ^{14}C- or ^{3}H-labeled oleic and palmitic acids (> 95% radiochemically pure) were obtained from New England Nuclear, Boston, Mass. Other materials were obtained from the indicated sources: Sephadex: Pharmacia Fine Chemicals, Inc., Piscataway, N. J.; acrylamide monomer, *N,N*-methylene-bisacrylamide, *N,N,N',N'*-tetramethyl ethylenediamine and riboflavin: Eastman Organic Chemicals Div., Eastman Kodak Co., Rochester, N. Y.; ammonium persulfate: Matheson, Coleman, and Bell, East Rutherford, N. J.; trypsin and chymotrypsinogen A: Worthington Biochemical Corp., Freehold, N. J.; ribonuclease A (bovine pancreas), napthal blue-black (Buffalo Black) stain, *p*-chloromercuriphenyl sulfonic acid, monosodium salt, and cytochrome C (horse heart): Sigma Chemical Co., St. Louis, Mo.; pronase and *p*-chloromercuribenzoic acid, sodium salt: Calbiochem, San Diego, Calif.; albumin (crystalline bovine): Mann Research Labs, Inc., New York.

Animals and diets. Male Sprague-Dawley rats, 300–350 g, were maintained ad lib on standard laboratory chow,[2] except as specified. Diets high in fat, carbohydrate, or medium-chain triglyceride were prepared by adding to the chow sufficient corn oil, sucrose, or medium-chain triglyceride oil[3] to provide 40% of total calories. In experiments comparing unsaturated and saturated fat, safflower oil or lard was added to the chow. Animals were not fasted before sacrifice in these studies.

For study of fatty acid binding to FABP in vivo, tracer quantities of labeled palmitate were administered intraduodenally ([^{3}H]palmitate in 1.0 ml 10 mM taurocholate, followed by 0.5 ml saline). After 2 min, the animal was sacrificed, and mucosal crypts and villi were prepared as indicated below.

Tissue preparation. Tissue homogenates and the 105,000-*g* supernatant fraction were prepared in 0.154 M KCl-0.01 M phosphate buffer, pH 7.4, as described previously (2). Mucosal scrapings were homogenized in 3 or 4 vol of buffer in a Potter-Elvehjem homogenizer and subjected to preparative ultracentrifugation for 2 h at 105,000 *g*. Preparation of jejunal crypts and villi were obtained by differential scraping (12). Subcellular particulate fractions, including cell membrane-brush border-nuclei, mitochondria, and microsomes were obtained by homogenization and preparative ultracentrifugation in 0.25 M sucrose-0.01 M phosphate buffer, pH 7.4 (13); fractions were washed twice after initial separation by resuspension and centrifugation. Protein in washed particulate fractions was partially solubilized by three methods: (*a*) 4 M KCl, 16 h, 20°C (14); (*b*) 50% glycerol, 30 min, 20°C (14); (*c*) sonication, 1,800 watt-s, 4°C.

Gel filtration of supernates was performed as described (2). FABP was partially purified (as the 12,000 mol wt fraction) by at least two passages through Sephadex G-50. Fractions containing FABP were identified by the presence of radioactive fatty acid and by analytical polyacrylamide gel electrophoresis; fractions were concentrated when appropriate in an Amicon ultrafiltration cell (Amicon Corp., Lexington, Mass.) by using a UM-2 membrane.

Electrophoresis and isoelectric focusing. Polyacrylamide gel electrophoresis (15) employed 3.5% concentrating and 7% separating gels, buffered with 0.5 M Tris in 0.38 M glycine, pH 8.6. Electrophoretic runs were carried out at 20°C, 3 mA/gel, for 70–90 min. Gels were fixed and stained in 7% acetic acid containing 1% Buffalo Black and were destained electrophoretically.

Polyacrylamide gel electrophoresis in sodium dodecyl sulfate was performed according to the method of Shapiro and Maizel (16). FABP, purified by Sephadex G-50 chromatography and isoelectric focusing, was applied to 10% gels run at 8 mA/gel for 2–3 h. Gels were fixed and stained in a solution of Coomassie Brilliant Blue in methanol-acetic acid-water, 45:45:10. The relative mobility of FABP was compared with that of protein standards to determine molecular weight (17).

Isoelectric focusing was performed according to the method of Vesterberg and Svensson (18), using an LKB apparatus containing a 1% (0.4–1.2%) solution of carrier ampholytes, pH 4–6 (Ampholine, LKB Produktes, Stockholm) in a discontinuous 2–46.7% sucrose gradient. The column was allowed to stabilize for 40–42 h.

Studies of ligand binding by FABP. The effect of various physical, chemical, and enzymatic agents on the binding of oleic acid by partially purified hepatic and jejunal FABP was determined by means of an assay involving the use of a Sephadex G-25 column, 0.9×21 cm, as previously described (2). Radioactivity appearing with FABP in the void volume was used to calculate nanomoles bound.

Immunochemical techniques. Rabbits were immunized against approximately 75 μg of purified jejunal FABP in complete Freund's adjuvant. Specificity of antiserum and immunochemical identity of antigens in tissue supernates was assessed by Ouchterlony's technique of double immunodiffusion. Concentration of FABP in tissue fractions was measured by the technique of Mancini, Vaerman, Carbonara, and Heremans, using quantitative radial immunodiffusion (19).

Miscellaneous analytical methods. Protein concentration in column eluates was measured as absorbance at 280 nm. In quantitative studies of binding (G-25 column), protein was measured by the method of Lowry, Rosebrough, Farr, and Randall (20), and BSP was quantitated by measuring

[2] Feedstuffs Processing Co., San Francisco, Calif.

[3] Mead Johnson and Co., Evansville, Ind.

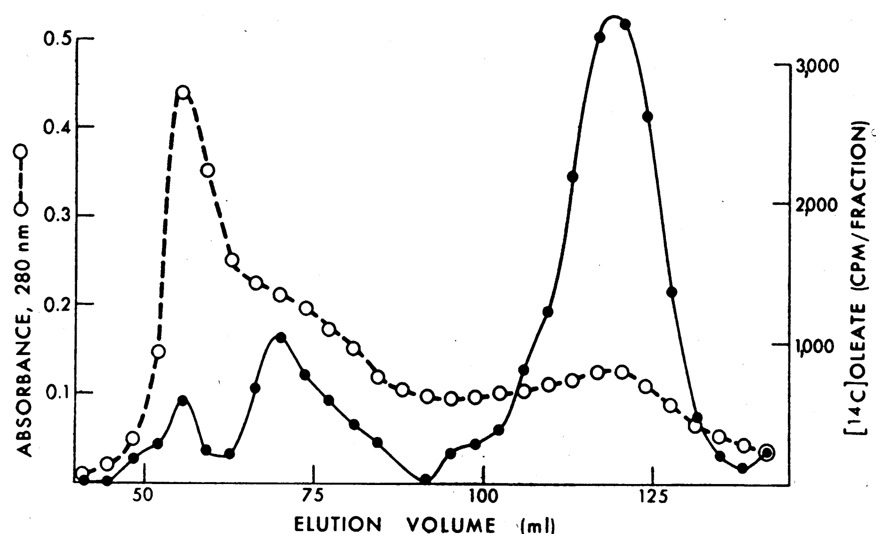

FIGURE 1 Gel filtration of rat jejunal 105,000-*g* supernate with [^{14}C]oleic acid. [^{14}C]oleic acid (71 nmol in 50 μl methyl ethyl ketone) was added to 19 mg supernatant protein in 2 ml KCl-phosphate buffer, pH 7.4, and chromatographed on Sephadex G-75 (2.6 × 32 cm, 4°C, 25 ml/h 3.6 ml fractions).

absorbance at 580 nm after alkalinization of samples. DNA was measured in whole homogenates by the method of Kissane and Robins (21) as modified by Hinegardner (22). Aqueous samples were assayed for radioactivity in a Packard Liquid Scintillation Spectrometer (Packard Instruments Co., Inc., Downers Grove, Ill.) by using a 10% solution of Biosolv BBS 3 (Beckman Instruments Inc., Fullerton, Calif.) in Liquifluor (New England Nuclear) and toluene.

Statistical methods. Significance of differences among experimental groups was determined by paired or unpaired *t* tests or by analysis of variance. Binding plots were analyzed by linear regression (23).

RESULTS

Gel filtration chromatography of 105,000-g supernate with [^{14}C]oleic acid. After an overnight fast, rat jejunal mucosa was scraped, and the soluble fraction was prepared as described. 2 ml (19 mg of protein) were added to 71 nmol of [^{14}C]oleic acid (1.55 × 10^5 cpm) in 50 μl of methyl ethyl ketone and immediately applied to Sephadex G-75 (2.6 × 32 cm). Effluent fractions (3.6 ml) were analyzed for protein and for radioactivity. The results are shown in Fig. 1. It can be seen that there is a significant peak of radioactivity associated with a low molecular weight fraction (FABP, elution volume 120 ml). Of the [^{14}C]oleic acid applied to the column, 14.6 nmol (20.6%) was recovered in 1.26 bed volumes (214 ml); of this, 6.9 nmol (47% of recovered) was associated with the FABP peak. The unrecovered fatty acid is assumed to have bound to the Sephadex. On extraction and thin layer chromatography, more than 95% of the FABP-associated radioactivity was recovered as noncovalently bound free fatty acid. Smaller amounts of fatty acid appeared in the void volume (56 ml) and in association with a fraction corresponding in elution volume (70 ml) to albumin. Unbound fatty acid and fatty acid applied in the absence of the 105,000-*g* supernate eluted from the column substantially later (~ 190 ml).

A similar experiment was carried out with 105,000-*g* supernate prepared from liver of a nonfasting rat (45.8 mg protein, 80 nmol [^{14}C]oleic acid, 1.74 × 10^5 cpm). The results are shown in Fig. 2. Again a significant peak of fatty acid is associated with a low molecular weight fraction, but virtually none is bound to other proteins, except for a small amount associated with a fraction corresponding to an apparent mol wt of approximately 46,000 (as determined by relative elution volume).

Binding of fatty acid to a similar FABP fraction was observed after gel filtration with preparations of rat myocardium, kidney, and adipose tissue, as well as with human jejunum, stomach, ileum, liver, and myocardium. When the [^{14}C]fatty acid was subjected to gel filtration chromatography with rat or human plasma, radioactivity was bound only to albumin, and there was no detectable association with a low molecular weight fraction corresponding in its elution characteristics to FABP. A FABP fraction was not detectable in rat or human erythrocytes. Thus, the widespread tissue distribution of FABP could not be attributed to contamination by a substance present in blood.

The relative elution volume (Ve/Vo) of rat jejunal, hepatic, and myocardial FABP were determined on Sephadex G-75 and compared with those of standard

proteins (24). Ve/Vo for jejunum, liver, and myocardium were essentially identical (2.1) and corresponded to an apparent mol wt of 12,100. This value agreed closely with the mol wt of 12,400, as determined for purified FABP by polyacrylamide gel electrophoresis in sodium dodecyl sulfate (see below).

In vivo binding of fatty acid to FABP. In order to further examine the physiological significance of intestinal FABP, studies were carried out in which labelled fatty acid was administered in vivo. [^{3}H]palmitate was administered in a single dose via an intraduodenal tube; after 2 min, villus and crypt preparations were obtained, and the supernate from each was subjected to gel filtration. As shown in Table I, only a very small percentage of total administered radioactivity was recovered in the supernatant fraction, and significantly more was associated with villi than with crypts. Of the villi supernatant radioactivity, however, 20% was associated with FABP, whereas only 9% of that in crypts was bound. In both cases, over 80% of FABP-associated radioactivity was shown by thin layer chromatography to be fatty acid. Most of the other supernatant radioactivity (chiefly triglyceride and phospholipid) eluted in or near the void volume, presumably associated with lipoproteins and albumin.

Isolation of intestinal FABP. The 12,000 mol wt fraction of rat jejunal supernate was subjected to isoelectric focusing as described in Methods. After the column had stabilized (at least 40 h), pH was measured on consecutive fractions, and absorbance at 280 nm was determined. The results are shown in Fig. 3. Two major and three minor protein peaks were recognized. To determine which of the peaks represented FABP, fractions from each were subjected to Sephadex G-50 chromatography to remove ampholytes. Approximately equal amounts of each protein were then subjected individually to column chromatography on Sephadex G-25 with [^{3}H]palmitate in methyl ethyl ketone; protein in the column eluate was assayed for radioactivity. As shown in Fig. 3, all peaks showed some degree of binding, but the pH 5.55 peak was far greater than the others. (Although not shown in this experiment, the pH 5.68 fraction exhibited binding but was found on polyacrylamide gel electrophoresis to contain the major peptides present in the starting material.) It was concluded, therefore, that the pH 5.55 fraction represented FABP.

The 5.55 peak was subjected to polyacrylamide gel electrophoresis and compared with the original 12,000 mol wt fraction from which it was isolated. As shown in Fig. 4, a high degree of purification was achieved by the combination of gel filtration and isoelectric focusing described above. By linear gel scanning photodensitometry the isolated FABP was determined to be at least 90% pure.

Purified FABP showed a single band on polyacrylamide gel electrophoresis in sodium dodecyl sulfate. The relative mobility of FABP compared with that of standard proteins indicated a molecular weight identical to that of cytochrome C (mol wt 12,400), a value which

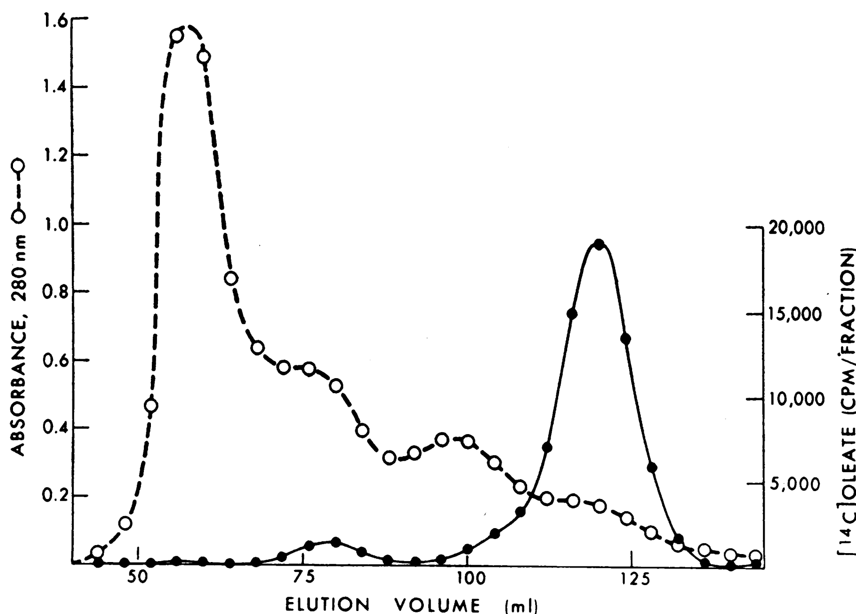

FIGURE 2 Gel filtration chromatography of rat liver 105,000-*g* supernate with [^{14}C]oleic acid. [^{14}C]oleic acid (80 nmol) and 45.8 mg supernatant protein were subjected to Sephadex G-75 chromatography, as in Fig. 1.

TABLE I

Binding of [³H]Palmitate to Intestinal Supernatant FABP After Intraluminal Administration In Vivo

	[³H]Supernatant	Binding to FABP	FABP-³H as fatty acid
	cpm/ml	*% of supernate cpm*	*%*
Villi	5,400	20.2	82.2
Crypts	3,168	8.7	82.1

After an overnight fast, 1.05×10^6 cpm[³H]palmitate in 1.0 ml 10 mM taurocholate was administered via an indwelling duodenal catheter. After 2 min, the animal was sacrificed, the intestine flushed with ice-cold isotonic saline, and preparations of villi and crypts were obtained from the proximal half, as described in Methods. Supernatant fractions were then subjected to G-50 chromatography. "Binding" was equated with radioactivity eluting with the FABP fraction. Recovery of isotope applied with supernate to column was 100% and 83.4% for villi and crypt preparations, respectively.

agreed closely with that of 12,100 as determined by Sephadex G-75 chromatography.

Immunochemical identification and quantitation. A rabbit antiserum against purified FABP was prepared as described in Methods. As shown in Fig. 5, the single precipitin arc formed between the antiserum and FABP show a reaction of complete identity with that formed against the 12,000 mol wt fatty acid-binding fractions of jejunal, liver, and adipose tissue supernates. Although no reaction is seen against the 12,000 mol wt fraction of myocardium in this preparation, it was demonstrated in other experiments. We have not observed a reaction against the kidney preparation.

These findings indicate that jejunal FABP shares immunochemical identity with proteins in the fatty acid-binding 12,000 mol wt fractions of other tissues. The possibility that other closely related but immunochemically distinct 12,000 mol wt fatty acid-binding proteins are present as well in these tissues is not excluded, however.

The antiserum produced in rabbits was used in the development of a quantitative assay for FABP, employing the technique of Mancini et al. (Methods). As shown in Fig. 6, the procedure was highly reproducible and permitted accurate quantitation of as little as 100–125 ng. With this assay, therefore, it was possible to measure tissue FABP concentration.

Anatomic and subcellular distribution of intestinal FABP. Mucosa from segments of the small intestine was obtained from nonfasting rats. The 105,000-*g* supernate was prepared, and samples were assayed for FABP concentration by radial immunodiffusion and for total protein concentration. The results are shown in Table II. Expressed as micrograms per milligram soluble protein, micrograms per milligram DNA, or micrograms per gram tissue, the proximal and middle thirds of the jejuno-ileum contained significantly greater quantities of supernatant FABP than did either distal third or duodenum. In addition, FABP concentrations in all segments of rat small intestine greatly exceeded that of immunochemically identical FABP in liver

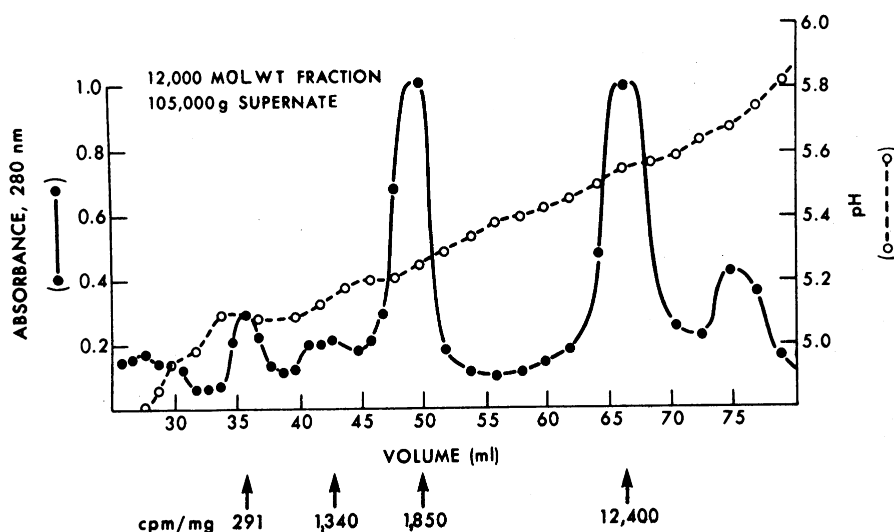

FIGURE 3 Isolation of FABP from intestinal mucosa by isoelectric focusing. The 12,000 mol wt fraction from rat jejunal supernate was subjected to isoelectric focusing in a discontinuous sucrose gradient as described in Methods. The pH and absorbance at 280 nm was determined on successive fractions eluted from the column. Protein peaks were subjected to G-50 chromatography to remove ampholytes, and binding of [³H]palmitate (cpm/mg protein) was determined by subsequent G-25 chromatography (Methods).

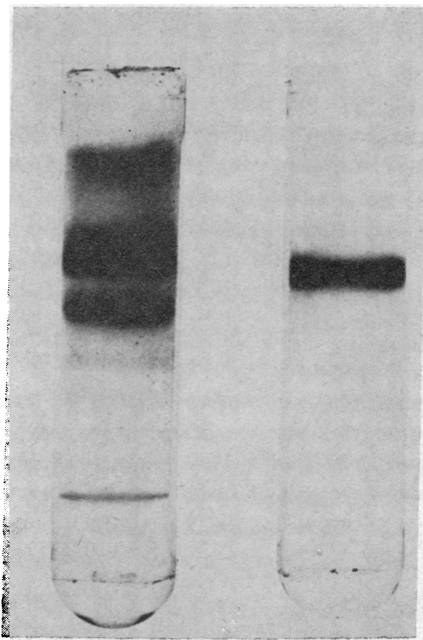

FIGURE 4 Disc gel electrophoresis of intestinal FABP. The partially purified 12,000 mol wt fraction is compared with FABP isolated by subsequent isoelectric focusing. FABP was determined to be at least 90% pure by linear gel scanning photodensitometry.

supernate. Because the binding of fatty acid by hepatic 12,000 mol wt fraction is grossly similar to that of intestine, however, (compare Figs. 1 and 2) it seems likely that most hepatic FABP is not identical to the intestinal protein.

Although FABP is highly soluble and abundant in the 105,000-*g* supernate, its lipid-binding properties suggested that it might also be associated with membranous components of the cell. Accordingly, plasma membrane-nuclei, mitochondria, and microsomes were prepared and subjected to solubilizing procedures as described in Methods, in order to quantitative FABP in these fractions.

As shown in Table III, the amounts of FABP solubilized from all fractions were virtually identical, regardless of the treatment employed or the degree of solubilization of total protein. Solubilized FABP constituted only a small percentage of the total solubilized protein. Furthermore, if the values are corrected to micrograms soluble (or solubilized) FABP per gram tissue for each subcellular fraction, the amount present in the 105,000-*g* supernate is approximately six times

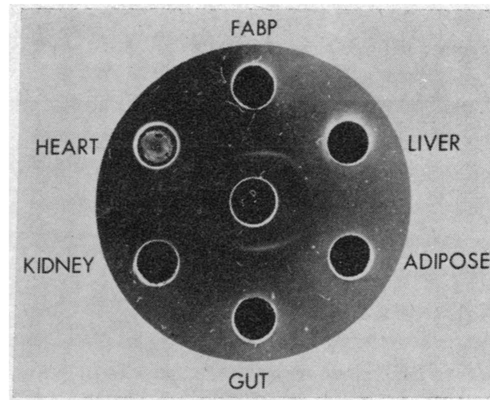

FIGURE 5 Immunochemical comparison of purified FABP with 12,000 mol wt fatty acid-binding fractions of liver, adipose, intestine, kidney, and myocardium. Rabbit antiserum to purified FABP is in the center well. A reaction of complete identity among FABP and the 12,000 mol wt fractions of liver, adipose tissue, and intestine is demonstrated. No definite reaction was demonstrable in this preparation with either heart or kidney, but was seen with heart in other experiments.

that in the combined particulate fractions. The recovery of FABP from particulate fractions probably is not complete, and therefore, they may in fact account for a somewhat greater proportion of the total cell

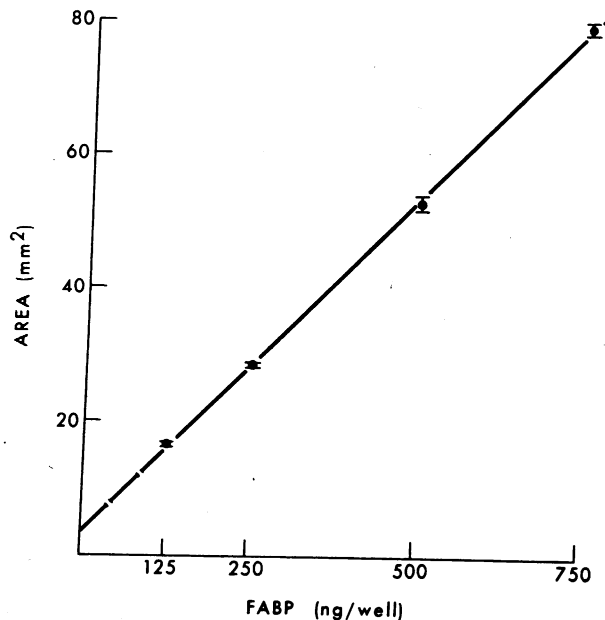

FIGURE 6 Quantitative radial immunodiffusion assay for FABP. Employing the technique of Mancini et al., standard purified FABP was used to determine the reproducibility of the assay. Indicated data points represent mean±SE for five determinations; line calculated by linear regression analysis.

Table II
Concentration of Supernatant FABP in Rat Intestinal Mucosa and Liver

Tissue	FABP		
	Soluble protein	DNA	Tissue
	μg/mg	*μg/mg*	*μg/g*
Duodenum (n = 4)	5.4±0.1	—	220±13
Jejuno-ileum (n = 6)			
Proximal	14.9±1.7	164±9	794±81
Middle $\frac{1}{3}$	15.9±0.8	139±7	828±49
Distal $\frac{1}{3}$	7.3±0.9	45.2±3.7	348±42
Analysis of variance	$F = 15.48$	$F = 84.01$	$F = 19.93$
	$P \ll 0.01$	$P \ll 0.01$	$P \ll 0.01$
Liver (n = 4)	0.69±0.05	—	59.9±3.7

FABP and total protein concentration were measured in the 105,000-*g* supernate prepared from intestinal mucosa and liver as described in Methods. DNA was measured in whole homogenates. Mean±SE.

FABP; it seems clear, however, that the soluble fraction accounts for the vast majority.

The fact that the amounts of FABP associated with the various particulate fractions were so similar further suggests that the association of this protein with membranes is nonspecific and constitutes additional evidence (2) against the possibility that FABP might be a specific brush border membrane protein involved in fatty acid uptake.

Intestinal localization of FABP was further examined by comparing its concentration in homogenates from villi and crypts from proximal intestine. FABP concentration in villi (Table IV) significantly exceeded that in the crypts and in the whole mucosa, regardless of how the results were expressed. This difference in FABP concentration between villi and crypts is consistent with an absorptive function for this protein and probably at least partially accounts for the differences in in vivo binding shown in Table I.

It is of interest that a 3-day fast caused a significant increase in mucosal FABP relative to fed controls (Table IV), so that by all three methods of comparison (milligram protein, milligram DNA, gram tissue), its concentration resembled that of the villi. Mucosal weight after a 3-day fast fell by an average of 31.5%. Thus, total mucosal FABP remained essentially unchanged, while tissue weight, DNA, and soluble protein declined, indicating a relative "sparing" of FABP during starvation.

Effect of diet on intestinal FABP levels. The findings thus far presented are consistent with the hypothesis (1, 2) that FABP might participate in the intracellular transport or metabolism of fatty acids. We felt that this concept would be strengthened, however, if it could be shown that intestinal FABP levels responded appropriately to changes in intestinal fatty acid transport. Accordingly, four groups of rats (mean weights, 207–214 g) were maintained on control and substituted (high fat, low fat, high medium-chain triglyceride) diets for 3 wk, as described in Methods, at which time they were sacrificed; supernatant FABP was measured in proximal, middle, and distal intestine. Final weights in all four groups of rats were similar (mean weights, 317–330 g). Total mucosal weights were slightly, but not significantly, greater in the high fat and low fat groups than in controls (4.44±0.17 g and 4.42±0.15 g, vs. 4.32±0.25 g, respectively). The results expressed as micrograms FABP per milligram soluble protein and per gram tissue, are shown in Table V.

It can be seen that in middle and distal intestine, animals on the high fat diet showed FABP levels

Table III
Distribution of FABP in Cell Fractions of Rat Intestinal Mucosa

Cell fraction	Solubilized FABP in cell fraction			Protein solubilized			FABP		
	KCl	Glycerol	Sonication	KCl	Glycerol	Sonication	KCl	Glycerol	Sonication
	μg/mg total protein			*% total in cell fraction*			*μg/g tissue*		
Nuclei, plasma membranes	5.63	5.63	5.83	30.2	16.6	30.4	64.2	64.2	66.5
Mitochondria	5.63	6.47	6.27	24.2	25.4	23.0	13.0	15.0	14.5
Microsomes	5.63	6.27	5.83	19.8	12.9	16.5	38.4	42.7	39.6
105,000-g supernatant		13.4±0.8			—			734±45	

Particulate fractions were prepared from pooled proximal mucosa, as described in Methods. Samples of resuspended pellets (3 mg protein in 0.5 ml) were subjected to the indicated solubilizing treatments (see Methods); aliquots were assayed for FABP before and after centrifugation (105,000 *g*, 1 h) to remove insoluble protein. (Centrifugation had virtually no effect on measured amounts of FABP solubilized.) FABP per gram tissue for each fraction is derived from the value for FABP per milligram protein in that fraction and milligram protein in the fraction, per gram tissue.

significantly greater than the control and low fat groups. High fat values in these segments were also significantly in excess of those for the medium-chain triglyceride group when expressed per milligram protein but not per gram tissue. Although inspection of the data for proximal intestine shows that the low fat group was lower than all others, no statistically significant differences were detected by analysis of variance.

For additional clarity, some of these data are analyzed in a different manner, as shown in Fig. 7. Here, values for low fat and high fat groups (micrograms per milligram soluble protein) are expressed as a percent of the corresponding control values. On the low fat intake, FABP concentration decreased in the proximal segment but did not change in more distal segments. In contrast, on the high fat intake, FABP in the proximal segment was not affected but *did* increase significantly in middle and distal segments.

These findings may be interpreted as suggesting that FABP concentration in proximal mucosa is already at maximal levels on ordinary chow diet, which contains 13% of calories as fat; additional fat in the diet does not lead to a further increase in supernatant FABP in this segment, but reduction of dietary fat leads to a fall in FABP. In contrast, FABP in middle and distal mucosa is already present at basal concentrations for those segments on ordinary chow and is not further diminished by low fat intake; FABP does increase significantly in response to high fat diet, however, as these more distal portions of the intestine become involved in the absorptive process.

Additional studies were carried out using animals (mean starting weights, 141–152 g), maintained for 6 wk on control and substituted diets, both to examine the effect of a more prolonged feeding period and to compare diets rich in saturated and unsaturated fatty acids. As in the 3-wk studies, final animal weights were similar (mean, 330–352 g), except for the low fat group (mean weight, 303 g). The results are shown in Table VI.

In general, although control values are somewhat higher, the findings are similar to those found after 3 wk. Thus, proximal levels are reduced in the low fat group, while middle and distal levels are elevated in the two high fat groups. The two high fat groups

TABLE IV

Distribution of Supernatant FABP in Mucosa from Proximal Rat Intestine, and Effect of Fasting

	FABP		
	Soluble protein	DNA	Tissue
	μg/mg	μg/mg	μg/g
Fed			
Whole mucosa	13.4±0.8	164±9	734±45
Villi	28.0±2.9*	251±35‡	996±86*
Crypts	8.8±1.4	51.8±10.7	356±58
Fasted, 3 days			
Whole mucosa	27.0±1.9§	246±30‖	1,175±81.1§

FABP, protein, and DNA were determined in preparations of whole proximal mucosa, villi, or crypts, as in Methods and Table II. Mean±SE.
* $P < 0.005$ vs. crypts (paired *t*).
‡ $P < 0.02$ vs. crypts (paired *t*).
§ $P < 0.001$ vs. fed (unpaired *t*).
‖ $P < 0.02$ vs. fed (unpaired *t*).

TABLE V

Effect of 3-Wk Substituted Diets on FABP Concentration in Rat Intestinal Mucosal Supernate

	FABP			FABP		
	Proximal	Middle	Distal	Proximal	Middle	Distal
	μg/mg soluble protein			μg/g tissue		
Control	14.9±1.7	15.9±0.8	7.3±0.9	794±81	828±49	348±42
High fat	14.4±1.6	21.0±2.0*	10.5±0.9*	729±70	963±66‡	432±29
Low fat	9.6±0.6	14.7±1.1	6.8±0.8	528±36	668±61	296±51
MCT‖	13.4±1.9	16.7±1.6	7.4±0.8	766±93	883±72	378±44
Analysis of variance	$F = 2.53$	$F = 3.63$	$F = 3.79$	$F = 2.71$	$F = 3.96$	$F = 1.82$
	NS	$P < 0.05$	$P < 0.05$	NS	$P < 0.05$	NS

Groups of six rats were maintained for 3 wk on control and substituted diets. Concentration of FABP in mucosal 105,000-g supernate was measured by quantitative radial immunodiffusion. Values for indicated segments were compared among the groups by one-way analysis of variance, and significant individual differences by the method of Tukey (Methods).
* $P < 0.05$ vs. all other groups.
‡ $P < 0.05$ vs. control and low fat.
‖ MCT, medium-chain triglyceride.

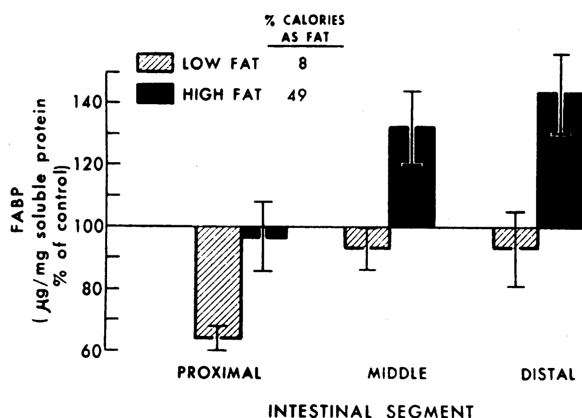

FIGURE 7 Effect of diet on FABP concentration in rat intestinal mucosa. Values shown in Table V for FABP concentration (micrograms per milligram soluble protein) are plotted relative to controls, in order to emphasize the regional differences in response to changes in dietary fat intake.

do not differ from one another except in distal mucosa, where levels in the unsaturated fat group significantly exceed those in the saturated fat group. The reason for the increased distal levels in the low fat group (relative to controls) is not clear.

These 6-wk groups were also compared on the basis of FABP to DNA ratios. The results are shown in Fig. 8 in a manner similar to that in Fig. 7; absolute control values, representing 100%, are those shown in Table II. It can be seen that the results are similar to those when expressed as in Table VI.

Together, these diet studies show that mucosal FABP levels, expressed per milligram protein, milligram DNA, or per gram tissue, are responsive to changes in dietary fat intake. The most consistent effect of high fat intake is an increase in FABP levels in middle and distal intestine; saturated and unsaturated fats do not produce consistently different effects, despite the differences in the affinity with which they bind to FABP (1, 2). Rodgers, Tandon, and Fromm, in studies of the response of intestinal lipid re-esterifying enzyme activities to high fat intake, were also unable to show a difference between saturated and unsaturated fats (25).

Relationship of FABP to Z protein. Because earlier findings (2, 26) suggested that FABP might be identical or closely related to the Z protein, a cytoplasmic anion-binding protein described by Levi, Gatmaitan, and Arias in liver, intestine, and kidney (4, 27), additional studies were carried out to explore this possibility.

In these experiments, the binding characteristics of partially purified rat liver FABP were examined by G-25 column chromatography. The results are shown in Table VII. In three control experiments, an average of 13.6 nmol of fatty acid was bound. Binding of oleic acid was inhibited by prior incubation of FABP with pronase but only minimally with trypsin and ribonuclease; temperatures as high as 60°C for 10 min had no effect. Binding was not affected by the presence of the sulfhydryl inhibitors, *p*-chloromercuribenzoate (PCMB) or *p*-chloromercuriphenyl-sulfonate (PCMPS), indicating that sulfhydryl groups were not essential. The 50% inhibition of binding at pH 6.3 could reflect changes in protein conformation but more likely reflects a change in the degree of ionization of the oleic acid carboxyl group. Thus, hepatic FABP is a heat-stable protein, as shown previously for Z (4). This, together with our previous demonstration that FABP and Z have similar molecular weights and that fatty acid and BSP are competitively inhibitory for binding (2, 10), support the concept that the two proteins are similar or identical.

TABLE VI
Effect of 6-Wk Substituted Diets on FABP Concentration in Rat Intestinal Mucosal Supernate

	FABP					
	Proximal	Middle	Distal	Proximal	Middle	Distal
	μg/mg soluble protein			*μg/g tissue*		
Control	18.2±1.0	18.0±1.2	7.2±0.4	1,033±55	939±51	378±30
Unsaturated fat	17.5±1.7	22.0±1.5*	11.7±0.7*‡	928±79	1,211±85*	630±38*‡
Saturated fat	18.9±1.5	23.0±1.1*	9.3±1.0*	1,034±80	1,255±47*	493±42*
Low fat	11.0±1.3*	15.4±1.5*	9.7±1.1*	576±55*	839±69*	504±53*
Analysis of variance	$F = 7.54$ $P < 0.01$	$F = 7.60$ $P < 0.01$	$F = 5.18$ $P < 0.01$	$F = 10.66$ $P < 0.01$	$F = 10.84$ $P < 0.01$	$F = 6.55$ $P < 0.01$

Experiments were conducted and analyzed as described in Table V, except that groups of six animals were maintained on diets for 6 wk. Mean±SE.
* $P < 0.05$ vs. controls.
‡ $P < 0.05$ vs. saturated fat.

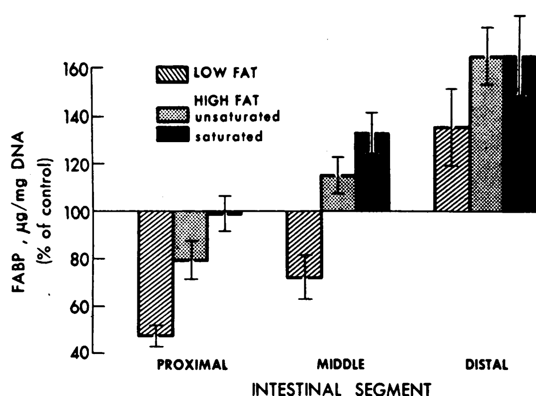

FIGURE 8 Effect of diet on FABP/DNA ratios in rat intestinal mucosa. Supernatant FABP levels as shown in Table VIII were related to mucosal DNA content, per gram tissue. Values for groups on substituted diets (mean ±SE) are expressed as percent of control values for corresponding intestinal segments. By analysis of variance, significant differences existed in all segments at the $P < 0.01$ level. Significant individual differences for each segment: proximal, all groups differed except control and saturated fat; middle, all groups differed; distal, all groups differed from control.

DISCUSSION

The present studies confirm and extend earlier evidence for the existence of a low molecular weight fatty acid binding protein (FABP) in the cytosol of intestinal mucosa and other mammalian tissues and provide additional support for the concept that FABP participates in cellular fatty acid transport. FABP is a heat-stable acidic protein with a mol wt of approximately 12,000; it preferentially binds unsaturated long chain fatty acids and, to a lesser extent, saturated fatty acids. Medium chain fatty acids and another organic anion, BSP, are bound only weakly, as are neutral lipids (1, 2).

The present studies lend support to earlier evidence (1–3, 10) that FABP is closely related or identical to the Z protein, previously described by Levi et al. (4), and for which a role as a cytoplasmic binding protein for organic anions and dyes (such as BSP) was proposed (4, 27, 28). Recently, Mishkin, Stein, Gatmaitan, and Arias (26) also published preliminary evidence concerning the ability of Z to bind fatty acids.

The utilization of fatty acids, including their uptake from extracellular media and their subsequent intracellular metabolism (activation and oxidation, or incorporation into more complex lipids), is a function common to most mammalian cells. Fatty acids usually are derived from plasma where they are bound to albumin. In the special case of the intestine, fatty acids also enter the absorptive cell from the lumen, where they are present in bile salt micelles (29). The mechanism by which fatty acids enter cells remains obscure, although the initial ("uptake") phase of this process is not energy dependent, since it occurs in the absence of oxygen, at low temperature, in the presence of metabolic inhibitors, and even with boiled or dead tissue (5–9, 30). Because uptake is passive, its rate depends on the concentration of free, unbound fatty acid external to the cell, as shown in studies of intestine and other tissues (6, 9, 31). Although it is possible that there are specific receptor sites for fatty acids in the plasma membrane, they have not been demonstrated.

It is evident that the affinity of the plasma membrane for fatty acids is sufficiently great that it competes successfully with albumin and (in the case of the intestine) with the bile salt micelle. Spector, Steinberg, and Tanaka, in studies of Ehrlich ascites tumor cells (6), showed that although fatty acid was initially bound reversibly to the cell membrane after uptake, it could be removed into the extracellular medium only if albumin were present in the latter. Qualitatively similar findings were reported by Mishkin, Yalovsky, and Kessler in studies of fatty acid uptake by everted jejunal sacs (9) and by Shohet, Nathan, and Karnovsky in studies of human erythrocytes (8). These findings are consistent with the concept that the binding of long chain fatty acids to the cell membrane is sufficiently

TABLE VII
Factors Affecting Binding of Oleic Acid by Liver FABP

Conditions	*nmol bound*	*% inhibition*
Control	13.6 (11.3–15.4)	—
Enzyme treatment (3 h, 37°C)		
Trypsin, 5 µg/ml	12.5	8
Pronase, 50 µg/ml	4.0	71
RNase, 20 µg/ml	10.2	25
Temperature		
20°, 10 min	13.5	1
37°, 3 h	14.5	—
50°, 10 min	14.4	—
60°, 10 min	14.3	—
Sulfhydryl inhibitors		
PCMB, 1.0 mM	16.4	—
PCMPS, 2.5 mM	12.5	8
pH 6.3	6.6, 6.9	50

Partially purified rat liver FABP was prepared as described (Methods). [^{14}C]Oleic acid (25 nmol) in 5 µl methyl ethyl ketone was added to 0.52 mg protein in 1.0 ml KCl-phosphate buffer, under the conditions indicated. The mixture was subjected to gel filtration on Sephadex G-25, and nmoles bound was calculated from radioactivity appearing with protein in the void volume.

strong that they will not dissociate from it into an aqueous medium unless a soluble acceptor with a high affinity for fatty acids is present. These observations with mammalian cells are not unexpected in view of the fact that long chain fatty acids partition strongly in favor of the organic phase in heptane-water systems (32). As a corollary, it seems likely that some facilitating mechanism within the cell accounts for the fact that after uptake, fatty acids rapidly leave the plasma membrane (despite their high affinity for it) to enter another aqueous compartment (the cytosol) and subsequently undergo metabolism in the endoplasmic reticulum and mitochondrion.

In view of these considerations, it is plausible that a protein in the cytosol, with a high affinity for fatty acids, subserves the function of facilitating their removal from the inner surface of the plasma membrane. The available evidence concerning FABP, both in our own studies and those of others (2, 3, 26, 27), supports the concept that this protein might fulfill such a role.

It is possible, however, that FABP may serve other functions in addition to, or instead of, the one suggested. For example, in their studies of fatty acid esterification by intestinal microsomes, Hübscher, Clark, Webb, and Sherratt (33) observed that the addition of 105,000-*g* supernate to the incubation significantly enhanced the incorporation of fatty acid into triglyceride. Although these workers subsequently concluded that this enhancement was attributable to soluble phosphatidate phosphohydrolase activity, an effect of other soluble proteins was not excluded (34). Thus, FABP may participate directly (enzymatically) in the activation or esterification process.

FABP might also serve as a component of intestinal lipoproteins, but in preliminary experiments, FABP could not be demonstrated immunochemically, either in whole intestinal lymph or in delipidated lymph lipoproteins (11). A role for FABP in the membrane uptake of fatty acids, as has been proposed recently (35), seems unlikely in view of earlier evidence suggesting the absence of specific fatty acid receptors in cell membranes (36). Also, despite the fact that initial uptake rates for saturated and unsaturated fatty acids by everted gut sacs are virtually identical (3), there are marked differences in the affinity with which they bind to FABP (2). Finally, the present observation that small quantities of FABP are associated with membranes of intestinal mitochondria and microsomes could be construed as evidence for a role in a membrane-associated process (e.g., fatty acid activation or esterification) but equally well could reflect contamination of these fractions by supernatant protein or an association of FABP with membrane lipids.

Although it must be conceded that the precise biochemical function of FABP remains unresolved, it is reasonable to conclude that it plays some role in cellular fatty acid transport. Thus, its preponderance in villi of mucosa from proximal and middle intestine is precisely what would be expected in view of the known anatomical localization of the process of fat absorption (37, 38). Moreover, its response to changes in dietary fat intake parallels the increased ability of distal intestine to absorb fat observed by others under similar conditions (39, 40); in the case of high sucrose intake, the fall in proximal FABP concentration is opposite to the increase in brush border sucrase and maltase activity reported by others (41). The relatively low concentration of FABP in intestinal crypt preparations argues against a major role for this protein in processes that occur primarily in this region of the mucosa, such as fatty acid synthesis (12).

The demonstration that fatty acid binds to intestinal FABP after administration in vivo and that binding is relatively and absolutely greater in villi than in crypts may be interpreted as further evidence in support of our hypothesis. However, the possibility that some binding might have occurred during cell fractionation, rather than in the intact animal, cannot be excluded with certainty.

The mechanism by which intestinal FABP levels respond to changes in dietary fat intake is not known. As noted, other investigators have shown that an increase in the amount of dietary fat presented to distal intestine is associated with an increased capacity of that tissue to absorb fat (39, 40). Unlike a number of "adaptive" responses in the intestine that seem to result from mucosal hyperplasia alone (42), the enzyme adaptation (40) and increased FABP/DNA ratio associated with ingestion of high fat diet is consistent with the concept that there may be an increase in the concentration of these proteins *per cell,* i.e., a functional hypertrophy. (A change in mucosal FABP/DNA ratio could reflect corresponding changes in the relative numbers of absorptive and inflammatory cells in the mucosa, but there is no evidence that this occurs in response to high fat diet.) These observations are consistent with the concept that proteins involved in fat absorption may be substrate inducible, but an indirect, possibly hormonally mediated, effect on their synthesis or degradation is not excluded.

Note added in proof. We have conducted more recent experiments which further define the function of FABP (43, and unpublished observations). Flavaspidic acid-*N*-methyl glucaminate and α-bromopalmitate inhibit the binding of long chain fatty acids to FABP in vitro. In studies with everted jejunal sacs, we have observed that, although these compounds do not affect gut sac uptake of equimolar oleic acid from mixed micelles, they markedly inhibit its

subsequent incorporation into triglyceride. In isolated intestinal microsomes, neither flavaspidic acid nor α-bromopalmitate inhibited fatty acid: coenzyme A ligase or monoglyceride acyltransferase activity in the presence of FABP or albumin (inhibition of the ligase *was* observed when FABP was omitted from the system). The fact that these inhibitors of fatty acid binding to FABP block intestinal incorporation of oleic acid into triglyceride at a point *after* uptake by the microvillus membrane but before activation and esterification in the endoplasmic reticulum supports the proposed function of FABP in cellular fatty acid metabolism. FABP may be of additional importance through its binding of compounds which otherwise could inhibit microsomal enzymes. Such inhibitors include not only exogenous substances (e.g., α-bromopalmitate and flavaspidic acid) but endogenous ones as well (e.g., fatty acid and fatty acyl: coenzyme A).

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