G. P. Kuiper, Ed. (Univ. of Chicago Press, Chicago, 1954), p. 371.
21. E. R. Lippincott, R. V. Eck, M. O. Dayhoff, C. Sagan, *Astrophys. J.* **147**, 753 (1967).
22. J. L. Bada and S. L. Miller, *Science* **159**, 423 (1968).
23. P. H. Abelson, *Proc. Nat. Acad. Sci. U.S.A.* **55**, 1365 (1966).
24. W. E. McGovern, *J. Atmos. Sci.* **26**, 623 (1969).
25. C. Sagan, in I. S. Shklovskii and C. Sagan, *Intelligent Life in the Universe* (Holden-Day, San Francisco, 1966), pp. 222–223.
26. Donn *et al.* (*15*) unaccountably concluded that the amount of gas-phase NH_3 in equilibrium with seawater would yield an insignificant greenhouse effect.
27. J. Bada, in *Proceedings of the 5th Conference on the Origins of Life, Belmont, Md., April 1971*, L. Margulis, Ed. (Gordon & Breach, New York, in press).
28. M. G. Rutten, *The Geological Aspects of the Origin of Life on Earth* (Elsevier, Amsterdam, 1962). But R. T. Cannon [*Nature* **205**, 586 (1965)] has described occasional oxidized red beds more than 2 aeons old, which suggests at least local oxidizing conditions in this epoch.
29. P. Ramdohr, *Abh. Deut. Akad. Wiss. Berlin Kl. Chem. Geol. Biol.* **3**, 35 (1958); D. R. Derry, *Bull. Geol. Soc. Amer.* **70**, 1587 (1959).
30. E. C. Perry, J. Monster, T. Reimer, *Science* **171**, 1015 (1971).
31. L. van Valen, *Science* **171**, 439 (1971).
32. See also J. M. Olson, *ibid.* **168**, 438 (1970).
33. See also L. V. Berkner and L. C. Marshall, *J. Atmos. Sci.* **22**, 225 (1965).
34. See, for example, A. L. Lehninger, *Biochemistry* (Worth, New York, 1970), pp. 560–561.
35. N. H. Horowitz, *Proc. Nat. Acad. Sci. U.S.A.* **31**, 153 (1945).
36. R. Y. Stanier, M. Doudoroff, E. A. Adelberg, *The Microbial World* (Prentice-Hall, New York, ed. 2, 1963).
37. C. Sagan, in *International Dictionary of Geophysics*, S. K. Runcorn, Ed. (Pergamon, London, 1967), p. 97; C. Barth, A. Stewart, C. Hord, A. Lane, *Icarus*, in press.
38. See, for example, W. Vishniac, K. C. Atwood, R. M. Bock, H. Gaffron, T. H. Jukes, A. D. McLaren, C. Sagan, H. Spinrad, in *Biology and the Exploration of Mars*, C. S. Pittendrigh, W. Vishniac, J. P. T. Pearman, Eds. (Publication No. 1296, National Academy of Sciences–National Research Council, Washington, D.C., 1966); J. Lederberg and C. Sagan, *Proc. Nat. Acad. Sci. U.S.A.* **48**, 1473 (1962); C. Sagan, "Life," in *Encyclopaedia Britannica* (Benton, Chicago, 1970); *Icarus* **15**, 511 (1971).
39. B. C. Murray, L. A. Soderblom, R. P. Sharp, J. A. Cutts, *J. Geophys. Res.* **76**, 313 (1971); C. R. Chapman, J. B. Pollack, C. Sagan, *Astron. J.* **74**, 1039 (1969); A. Dollfus, R. Fryer, C. Titulaer, *C. R. H. Acad. Sci. Ser. B* **270**, 424 (1970); C. Sagan and J. Veverka, *Icarus* **14**, 222 (1971); W. K. Hartmann, *ibid.* **15**, 410 (1971).
40. C. Sagan, Technical Report TR 32-34, Jet Propulsion Laboratory, Pasadena (1960); A. P. Ingersoll, *J. Atmos. Sci.* **26**, 1191 (1969); S. I. Rasool and C. deBerg, *Nature* **226**, 1037 (1970).
41. J. B. Pollack, *Icarus* **14**, 295 (1971).
42. We are grateful to E. E. Salpeter, J. W. Schopf, M. Schwarzschild, and J. Bahcall for stimulating conversations; to B. N. Khare for the laboratory infrared spectra; to L. D. G. Young for the calculated H_2 spectra; and to J. Katz for a comprehensive discussion of the reliability of current models of main-sequence solar evolution. This research was supported by the Atmospheric Science Section, National Science Foundation, under grant GA 23945.
* Present address: Department of Physics, Mansfield State College, Mansfield, Pennsylvania 16993.

17 December 1971; revised 21 April 1972

A Binding Protein for Fatty Acids in Cytosol of Intestinal Mucosa, Liver, Myocardium, and Other Tissues

Abstract. *A protein of molecular weight ~ 12,000 which binds long-chain fatty acids and certain other lipids has been identified in cytosol of intestinal mucosa, liver, myocardium, adipose tissue, and kidney. Binding is noncovalent and is greater for unsaturated than for saturated and medium-chain fatty acids. This protein appears to be identical with the smaller of two previously described cytoplasmic anion-binding proteins. Binding of long-chain fatty acids by this protein is greater than that of other anions tested, including sulfobromophthalein, and does not depend on negative charge alone. The presence of this binding protein may explain previously observed differences in intestinal absorption among fatty acids, and the protein may participate in the utilization of long-chain fatty acids by many mammalian tissues.*

Translocation of fatty acids from cell surface to endoplasmic reticulum and mitochondria is fundamental to the intestinal absorption of lipids and to the utilization of free fatty acids in plasma by liver, muscle, and other tissues. However, although long-chain fatty acids are at best poorly soluble in aqueous media, a mechanism to account for the apparent facility with which they traverse the cytosol (aqueous cytoplasm) has not been identified.

In studies of the intestinal absorption of long-chain fatty acids (*1, 2*), we observed that although saturated and unsaturated fatty acids were taken up by everted jejunal sacs at equal rates, unsaturated fatty acids were esterified more rapidly. However, our studies and those of others (*3*) indicated that these results could not be explained by corresponding differences in the activation of fatty acids by microsomal fatty acid–coenzyme A (CoA) ligase (*4*). Accordingly, we postulated (*2*) that apparent differences in rates of esterification might be due to different rates of translocation of fatty acids from the microvillus membrane to the site of their activation in the endoplasmic reticulum. As a result, we discovered a binding protein for long-chain fatty acids in the cytosol of jejunal mucosa and of other mammalian tissues (*5*).

Male Sprague-Dawley rats—fasting if intestine was to be studied, otherwise nonfasting—were killed by decapitation. The proximal half of the small intestine, distal to the ligament of Treitz, was removed and flushed with 40 ml of 0.01*M* phosphate buffer in 0.154*M* KCl (*p*H 7.4, 4°C). Mucosa was extruded, weighed, homogenized in three volumes of buffer, and centrifuged at 105,000*g* for 2 hours. The supernatant, exclusive of floating fat, was used for gel filtration. The liver was perfused in situ through the portal vein with cold buffer before homogenization and ultracentrifugation as just described. Appropriate ligands (Figs. 1 and 2) were added in vitro to 105,000*g* supernatants, and the mixture was subjected immediately to gel filtration on Sephadex G-75. Protein concentration in the column effluent was measured as absorbance at 280 nm; radioactivity was determined by liquid scintillation spectrometry; sulfobromophthalein (BSP) was measured as absorbance at 580 nm after alkalinization.

Sephadex G-75 chromatography of rat jejunal supernatant with [^{14}C]oleate showed association of radioactivity with a protein of low molecular weight, which we have designated "fatty acid binding protein" (FABP) (Fig. 1). Variable radioactivity was also associated with macromolecules (including lipoproteins) in the excluded (void) volume, and with residual albumin in the tissue homogenate. By lipid extraction (*6*) and thin-layer chromatography of the FABP peak, more than 95 percent of ^{14}C was recovered as free fatty acid, a result indicating that binding was noncovalent and not the result of prior conversion to fatty acyl–CoA or other derivatives. A FABP with virtually identical elution characteristics was demonstrated in liver supernatant.

An estimation of the molecular weight of FABP was obtained by comparing its relative elution volume (V_e/V_o) with that of proteins of known molecular weight on Sephadex G-75. Both jejunal and hepatic FABP were consistently eluted in a volume ($V_e/V_o = 2.10 \pm .02$) slightly greater than that of cytochrome c (horse heart, Sigma, molecular weight 12,400, $V_e/V_o = 2.08 \pm .02$); this indicates a molecular weight of about 12,000. This value must be regarded as an approximation, however, because elution characteristics of proteins on gel filtration show a

slightly better correlation with Stokes' radius than with molecular weight (*7*).

The behavior of jejunal and hepatic FABP on gel filtration was similar to that reported for the smaller of two cytoplasmic anion-binding proteins, designated "Z" by Levi *et al.* (*8*), and also described by Grodsky *et al.* (*9*), who estimated its molecular weight to be about 12,000. Because fatty acids are, at physiological *p*H, organic anions of major qualitative and quantitative biological significance, we explored the possibility that FABP might be related to this previously described anion-binding protein.

Liver supernatant was subjected to gel filtration on Sephadex G-75 with both [^{14}C]oleate and BSP (Fig. 2). Elution characteristics of FABP and Z were identical. Moreover, binding of [^{14}C]-oleate to this fraction was about threefold greater than that of BSP, even though approximately equimolar quantities of the two were applied. [^{14}C]Oleate showed insignificant binding to "Y" protein (*8*), more recently designated "ligandin" (*10*), although a small amount was bound to residual albumin in the liver supernatant.

For more direct comparison of binding affinities, FABP was partially purified from jejunal supernatant by two consecutive passages through Sephadex G-50. After polyacrylamide gel electrophoresis of this material, radioactive fatty acid was associated with a single protein band. Partially purified FABP was added to each of various radioactive ligands, and percentage of binding was determined by gel filtration (Table 1). Binding of unsaturated long-chain fatty acids exceeded that of saturated and medium-chain fatty acids and of BSP. In other experiments, binding of fatty acid and BSP was competitive, a result that further supports the concept that FABP and Z are the same protein. Moreover, methyl palmitate and hexadecanol (uncharged, long-chain lipids) were bound to a greater extent than were medium-chain fatty acids; this indicates that negative charge was neither necessary nor sufficient for binding. Rather, binding appeared to be related to length and saturation of fatty acid chains; this suggests that the interaction may be steric (*11*) or hydrophobic, or both. In addition, of the three neutral lipids tested, methyl palmitate was bound to a greater extent than hexadecanol and cholesterol, consistent with the possibility that hydrogen bonding (at the oxygen function) may also be involved.

Table 1. Binding of ligands to FABP. Partially purified FABP (1.0 ml, 0.5 mg of protein) was added to ^{14}C-labeled ligand (25 nmole, except that 10.9 and 14.7 nmole were used for oleate) and applied to Sephadex G-25 (0.9 by 21 cm, 4°C, 12 ml/hour). Lipids were at least 95 percent radiochemically pure as judged by thin-layer chromatography. Radioactivity eluted with protein in the void volume was used to calculate nanomoles bound. Values shown are results of individual experiments.

Ligand	Bound (%)
Fatty acids (anions)	
Linoleate (18:2)	46.7, 40.7
Oleate (18:1)	60.8, 59.9
Stearate (18:0)	4.4
Palmitate (16:0)	12.3, 16.5
Laurate (12:0)	0.7
Octanoate (8:0)	0.01, 0.04
Neutral lipids	
Methyl palmitate	3.1, 2.4
Hexadecanol	0.9
Cholesterol	0.07
BSP	7.0, 6.8

Although quantitative interpretations of binding in this system must be limited, differences in binding of ligands to the Sephadex itself cannot explain our results. Thus, laurate and octanoate applied to G-25 columns in the absence of FABP showed no binding to the column. In contrast, both oleate and BSP were completely bound, and could not be eluted even in five bed volumes; palmitate was only partially bound. Therefore, binding to FABP was not simply due to lack of binding to Sephadex, but rather reflected intrinsic binding affinity between ligand and FABP. Experiments to determine binding affinity and stoichiometry more precisely are needed.

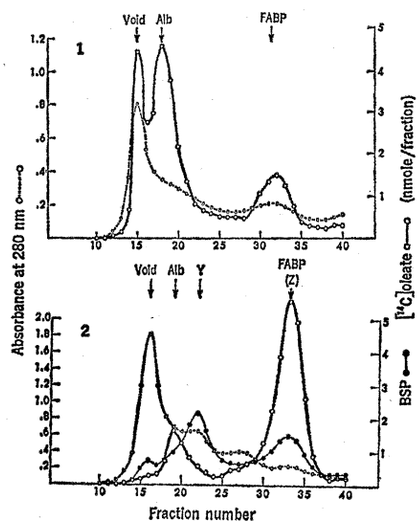

Fig. 1. Sephadex G-75 chromatography of [^{14}C]oleate with rat jejunal 105,000*g* supernatant (*Alb*, albumin). Jejunal supernatant (2 ml, 31.1 mg of protein), added to 226 nmole of [^{14}C]oleate in 10 μl of methyl ethyl ketone, was applied to Sephadex G-75 (2.6 by 32 cm, 4°C, 25 ml/hour, 3.6-ml fractions). Fig. 2. Sephadex G-75 chromatography of [^{14}C]-oleate and BSP with rat liver 105,000*g* supernatant. Liver supernatant (2 ml, 44.4 mg of protein) was added to 145 nmole of [^{14}C]oleate in 10 μl of methyl ethyl ketone and 125 nmole of BSP and applied to Sephadex G-75 (2.6 by 32 cm, 4°C, 25 ml/hour, 3.6-ml fractions).

A FABP with chromatographic and electrophoretic properties similar to those of the protein in rat jejunum and liver was isolated from human jejunal mucosa by gel filtration and ion exchange chromatography. FABP was also identified in human liver and myocardium, and in rat myocardium, adipose tissue, and kidney. It was not detected in rat or human plasma, nor in human erythrocytes.

A role for this protein in cytoplasmic fatty acid transport is consistent with certain hitherto unexplained findings concerning the Z protein. Whereas Y protein was present only in low amounts in fetal liver but increased substantially after birth, Z protein was present in fetus and adult in similar concentrations (*12*). Moreover, unlike Y protein, Z did not respond to the inductive effects of phenobarbital (*13*), a result further suggesting that its physiological role differed from that of Y. Our results, as well as other evidence indicating that the fetus utilizes free fatty acids in plasma (*14*), suggest that at all stages of life the primary function of this peptide (FABP or Z) is related to the cytoplasmic transport of long-chain fatty acids. Accordingly, if tissue FABP concentrations vary in response to physiological or pathological phenomena, changes either in the availability of fatty acids (within the intestinal lumen or in plasma) or in the fatty acid requirements of specific tissues might be among the determining factors.

Although our studies deal with the presence of FABP in cytosol, the possibility that it may also be associated with the plasma membrane, thereby affecting fatty acid uptake, is not excluded. This seems unlikely, however, because we found similar rates of jejunal uptake of fatty acids with differing affinities for FABP (*1*, *2*). It is not clear whether FABP fulfills only a transport function, or whether it also

regulates or modifies the intracellular metabolism of fatty acids. In jejunal mucosa, relative binding of long-chain and medium-chain fatty acids to FABP correlates well with their propensity for esterification. This aspect of the physiological role of FABP warrants further investigation.

We conclude that in the intestine, FABP may account for differences in the absorption of fatty acids. It may also participate in the cellular utilization of fatty acids, and possibly certain lipid-soluble drugs and toxins, by other epithelial and nonepithelial mammalian tissues.

ROBERT K. OCKNER
JOAN A. MANNING
RUTH B. POPPENHAUSEN
WILLIAM K. L. HO
Department of Medicine, University of California School of Medicine, San Francisco 94122

References and Notes

1. R. K. Ockner, J. P. Pittman, J. L. Yager, *J. Clin. Invest.* **50**, 71a (1971).
2. ———, *Gastroenterology* **62**, 981 (1972).
3. D. N. Brindley and G. Hubscher, *Biochim. Biophys. Acta* **125**, 92 (1966).
4. J. B. Rodgers, E. M. Riley, G. D. Drummey, K. J. Isselbacher, *Gastroenterology* **53**, 547 (1967).
5. R. Ockner, *J. Clin. Invest.* **51**, 70A (1972).
6. J. Folch, M. Lees, G. M. Sloane Stanley, *J. Biol. Chem.* **226**, 497 (1957).
7. P. Andrews, *Methods Biochem. Anal.* **18**, 1 (1970).
8. A. J. Levi, Z. Gatmaitan, I. M. Arias, *J. Clin. Invest.* **48**, 156 (1969).
9. G. M. Grodsky, H. J. Kolb, R. E. Fanska, C. Nemechek, *Metabolism* **19**, 246 (1970).
10. G. Litwak, B. Ketterer, I. M. Arias, *Nature* **234**, 466 (1971).
11. N. Spritz and M. A. Mishkel, *J. Clin. Invest.* **48**, 78 (1969).
12. A. J. Levi, Z. Gatmaitan, I. M. Arias, *N. Engl. J. Med.* **283**, 1136 (1970).
13. H. Reyes, A. J. Levi, Z. Gatmaitan, I. M. Arias, *J. Clin. Invest.* **50**, 2242 (1971).
14. C. M. Van Duyne, R. J. Havel, J. M. Felts, *Amer. J. Obstet. Gynecol.* **84**, 1069 (1962); O. W. Portman, R. E. Behrman, P. Soltys, *Amer. J. Physiol.* **216**, 143 (1969).
15. Supported in part by NIH grants AM-13328 and AM-14795 and research career development award AM-36586 to R.K.O.

17 February 1972; revised 14 April 1972

Retina: Ultrastructural Alterations Produced by Extremely Low Levels of Coherent Radiation

Abstract. *The effect of low levels of coherent radiation on the eye is not fully established, but is generally presumed to be noninjurious. Irradiation of the retina with a Q-switched ruby laser emitting low amounts of energy (0.1 percent probability of creating damage) consistently produces ultrastructural alterations in rods and cones. Outer segments of these cells are broken and disorganized and their lamellae are in disarray 1 day after such irradiation.*

The potential biologic hazards of lasers have long been recognized, and the ocular hazards of these devices emitting visible radiation have been of particular concern. This is so because the inherent focusing and high transmittance of the eye concentrate energy density striking the retina at least 100,000 times as compared to that striking the cornea. To minimize this hazard, intensive efforts have been made to define damage thresholds by the construction of dose-response curves. Such curves have been statistically derived by plotting the energy incident on the cornea against the presence or absence of retinal lesions visible with the ophthalmoscope (*1*). Damage thresholds have then been derived from these curves (*1*); the lowest point on the curve at which damage was ever observed in our laboratory approximates the 15 percent probability of damage level (*2*). This derivation has been based on the tacit assumption that areas of the retina subjected to small doses of irradiation and not bearing visible lesions are free of damage (*1*). Two observations support this assumption: the study of serial histologic sections through such irradiated areas discloses only a few more lesions than are seen with the ophthalmoscope (*3*), and damage thresholds vary widely not only between eyes but between different areas of the same eye as well (*2*). However, the assumption remains unverified, since it has not been subjected to the critical test of ultrastructural analysis. We now have evidence, as a result of such a study, that extremely low levels of coherent radiation can produce ultrastructural alterations in the exposed retinas.

To explore this question, we irradiated, with a Q-switched ruby laser, both retinas from two rhesus monkeys and studied the ultrastructure of the irradiated areas. The laser, whose wavelength was 6943 Å, produced a retinal irradiance diameter of 1000 μm. The total energy incident on the cornea (70 μjoule) had been demonstrated statistically to have a probability of creating ophthalmoscopically visible damage once in a thousand times (0.1 percent probability). None of the irradiated areas ever displayed ophthalmoscopically visible alterations. Twenty-four hours later, we took samples both of the irradiated areas from all four eyes and of directly adjacent areas. We used an artifactual optical marker that denoted the exact lased area on fundus photographs (*4*). By noting the location of the marker in relation to retinal vessels, we were easily able to sample the otherwise undetectable irradiated areas. Samples were then prepared for electron microscopy (*4*). On each experimental sample, we made both thick and thin sections at at least two levels.

In general, histologic observation of the samples was unrewarding. With one exception, all samples—control and experimental—showed no alterations and maintained the usual retinal histologic pattern. The exception, an experimental sample, had very subtle changes in pigment epithelium, including swelling and decrease of pigment granules and discharge of granules into the subretinal space; barely perceptible swelling and disarray of outer segments of both rods and cones were also noted.

By contrast, ultrastructural examination of experimental samples disclosed marked pathologic changes in the retina. Outer segments of both rods and cones were disorganized, shrunken, separated from one another, retracted from pigment epithelium, broken into ball-like segments, and often in complete disarray (Fig. 1a). The usually orderly lamellae were disorganized, separated, retracted from the edge of the cells, and often segmentally absent (Fig. 1b). Disorganized lamellae formed bizarre fingerprint-like whorls (Fig. 1b) and on occasion ran parallel to the cell axis rather than at the usual right angle (Fig. 1, c and d). In extreme cases, limiting membranes appeared to be dissolved (Fig. 1e). All of the lamellar changes were much more prominent proximally—near the junction with inner segments. Inner segments had mitochondrial swelling and vesicle formation. The changes occurred in rods and cones with equal frequency. Pigment epithelium had reactive changes of condensation and thickening of microvilli, loss of pigment granules, condensation of smooth endoplasmic reticulum, and increase of lysosomes (Fig. 1a). The one sample displaying histologic alterations had the above-mentioned changes plus limited focal necrosis of pigment epi-