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.

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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-

thelium. Control samples had none of these changes and were uniformly of normal ultrastructure.

To date, we have examined 13 experimental samples and 12 control samples, each of the latter taken about 1 mm from the adjacent irradiated area. Of 13 experimental samples, 12 have displayed the foregoing ultrastructural changes; none of 12 matched control samples have. In 5 of the experimental samples, we saw no changes at the edge of the block, but found profound changes in the center.

These data suggest that very low levels of energy from a ruby laser incident upon the retina can produce ultrastructural alterations in the outer segments of rods and cones there. Pathologic alterations were consistently seen in experimental samples taken from irradiated areas, but not in control samples taken from areas directly adjacent to irradiated areas. Too, altered and unaltered retinas were present in the same sample on several occasions. Finally, the validity of relating the observed changes to the irradiation is supported by the consistent character of the alterations and their obvious pathologic nature. The trivial explanation of the data, that the changes are artifactual, presupposes extreme coincidence and, hence, seems highly unlikely. However, in view of the limited number of eyes sampled, the widespread applicability of the above conclusion is not yet fully established.

The most surprising result of this study is the presence of damage in areas subjected to very small amounts of coherent radiation. Previous studies from our laboratory have shown that such power levels have a statistical probability of producing ophthalmoscopically visible damage once in a thousand times (*2*); in point of fact, we have never observed by ophthalmoscopy or histologic examination any damage under these experimental conditions below the 15 percent probability level (*2*). To our knowledge, production of retinal damage at such low levels of irradiation (0.1 percent) has not been reported.

Two possible pathogenetic bases for the observed damage may be postulated. Light absorbed by pigment granules may be converted to heat and, thus, produce damage to surrounding tissues. Alternatively, light directly absorbed by rods and cones may be directly toxic to these elements. We cannot completely discriminate between these alternatives on the basis of present data. However,

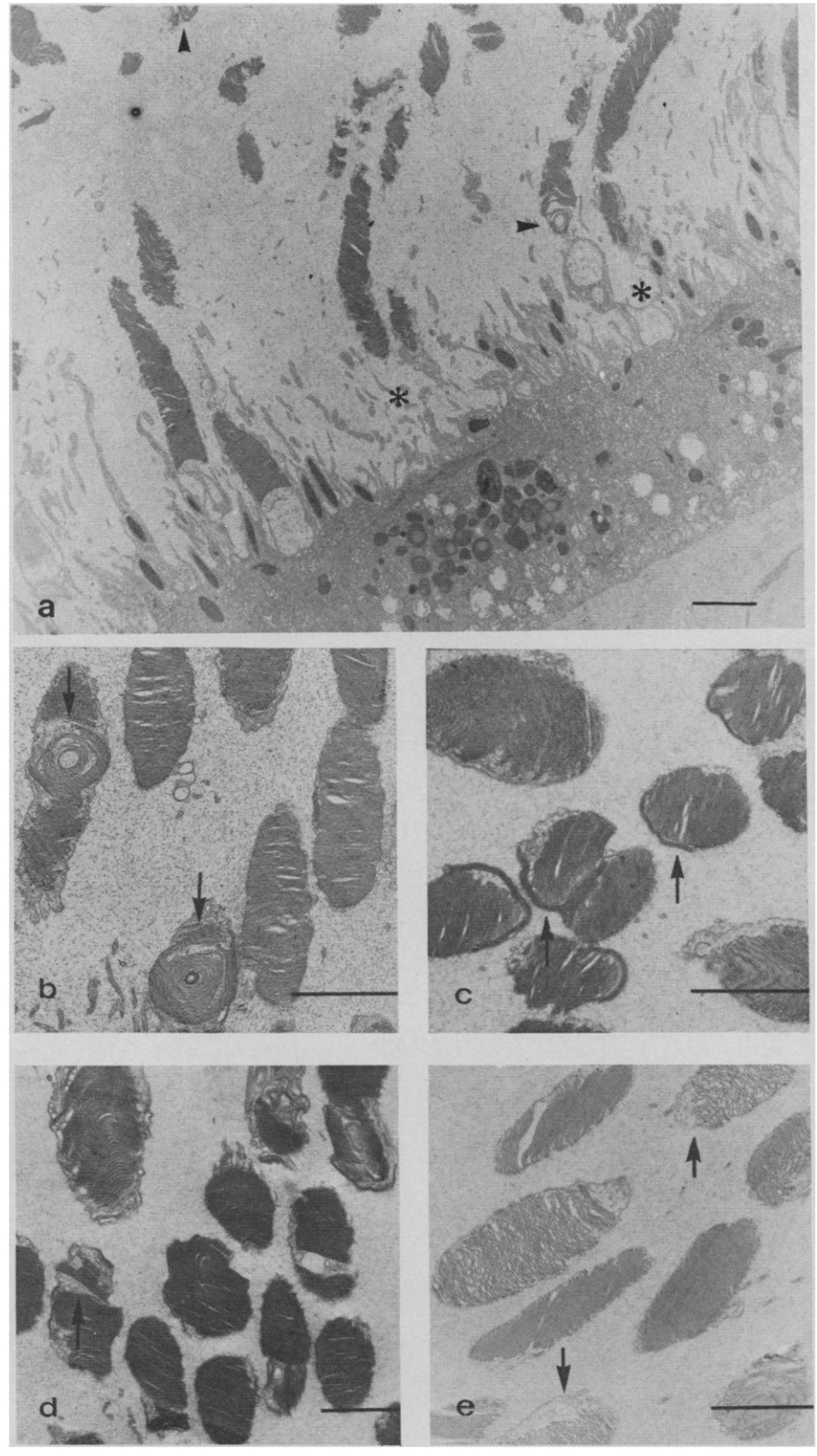

Fig. 1. Electron micrographs of damaged retina. (a) Disarray, separation, and disorganization of outer segments are evident; retraction from pigment epithelium is denoted by the asterisks. Within the disorganized outer segments are disordered lamellae (arrows). Pigment epithelium is relatively unaltered, except for mitochondrial swelling and an increase in lysosomes. (The scale represents 2 μm in all figures.) (b) Two fingerprint-like whorls can be seen here; the disintegration of lamellae into tubules is indicated by the arrows. (c) Lamellar disarray is prominent. The orientation of some lamellae is at right angles to their usual direction (arrows). (d) In addition to altered orientation, lamellae are widely separated (arrow). (e) Outer segments are markedly altered. Lamellae are separated, and there is apparent breakdown of cell membranes (arrows).

two ultrastructural observations are inconsistent with a thermal model of damage: the absence of significant damage to pigment epithelium and its microvilli and the predilection of lamellar disarray for the proximal rather than distal ends of rods and cones. Furthermore, the ultrastructural alterations seen here, though nonspecific in nature, do resemble very closely changes produced with ordinary light at low levels (*5–7*). These changes have been attributed to direct photic effects both on the basis of energy calculations (*7*) and on the basis of studies involving deficiency in vitamin A (*8*). Also, the changes observed do not resemble those produced directly by heat (*6*) or those produced by higher levels of coherent irradiation and, hence, indirectly by heat (*9*).

The significance of these observations is not fully established. If this morphologic phenomenon proves to be extensively reproducible, its functional effects and persistence or resolution remain undetermined. At present, we conclude that very low levels of coherent radiation are capable of producing ultrastructural alterations in sensory retina.

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Inhibition of Chemical Carcinogenesis by Viral Vaccines

Abstract. *The incidence of 3-methylcholanthrene–induced subcutaneous tumors was significantly reduced by a single injection of inactivated type C RNA viral vaccine. Rauscher leukemia virus vaccine reduced the incidence of sarcomas from 78 to 50 percent in the BALB/cCr mouse. Radiation leukemia virus vaccine and a vaccine from a wild murine leukemia virus derived from a 3-methylcholanthrene tumor reduced the incidence of sarcoma from 86 percent to 33 and 37 percents, respectively, in the C57BL/6 mouse. These reductions in tumor incidence by virus vaccines help support the concept that type C RNA viruses serve as determinants of chemically induced cancer; additional studies of vaccines made with more purified virus preparations are necessary.*

Transplantation tests with chemically induced tumors have demonstrated tumor-specific antigens (TSA) which do not protect against other chemically induced transplantable tumors (*1*). These antigenic specificities differ from those of antigens in virus-induced tumors where cross-reactivity is a common feature (*2*). Huebner and his associates demonstrated that cytoplasmic group specific antigens of the type C RNA virus were present in chemically induced tumors (*3–7*). Because of this it seemed feasible to undertake studies to determine whether type C RNA virus vaccines would inhibit induction of sarcoma by 3-methylcholanthrene (3MC) in BALB/c mice and C57BL/6 mice.

BALB/cCr mice were obtained from Microbiological Associates, Inc., Bethesda, Maryland, and C57BL/6Cum mice from Cumberland View Farms, Clinton, Tennessee. For virus preparations and viral titrations, newborn mice 24 to 72 hours old were used. Female mice, weanling (4 weeks old), were used for vaccination studies. Mice were housed, fed, and observed as described (*5, 6*).

Rauscher leukemia virus (RLV) (*8*) was partially purified and concentrated by the Moloney procedure (*9*) as modified by Huebner *et al.* (*10*) from infected BALB/c spleen tissue and resuspended in phosphate-buffered saline. The concentrated virus was titrated by the spleen antigen test in which BALB/c newborn mice were inoculated intraperitoneally with 0.05 ml per $\log_{10}$ dilution of virus; two litters were used for each dilution, and the spleens were harvested individually for half the mice at 21 days and for the remaining mice at 42 days; induction of infection was determined by testing the spleens in the complement-fixation test for the group specific (gs) antigen of type C RNA virus (*11*). Tests for both 21- and 42-day titrations indicated that the infectious dose (ID_{50}) was $10^{5.5}$ per milliliter. The virus was kept for 2 weeks at 4°C in formalin (final concentration of 0.1 percent), and inactivation was established by inoculating (intraperitoneally) undiluted viral

Table 1. Effect of inactivated type C RNA virus vaccine on 3-methylcholanthrene tumor induction in BALB/c and C57BL/6 mice. CI, Carcinogenic index; PBS, phosphate-buffered saline.

Treatment		Tumor			
		Incidence			
Vaccination	3MC* (μg)	Tu/T†	Percent	Average latency (weeks)	CI
		BALB/c mice			
None	None	0/24	0		
RLV + adjuvant	None	0/24	0		
None	150	21/27	78	14.8	75
PBS + adjuvant	150	22/29	76	14.0	77
RLV + adjuvant	150	12/24	50	15.6	46
		C57BL/6 mice			
None	None	0/25	0		
RadLV + adjuvant	None	0/27	0		
PBS + adjuvant	150	19/22	86	17.3	71
RadLV + adjuvant	150	9/27	33	17.8	27
Wild MuLV + adjuvant	150	10/27	37	16.3	33

* All mice received trioctanoin, the vehicle, whether or not they were given 3MC. † Tu/T, Number of tumors/total numbers of mice at risk for 5 months.