patient's fibroblasts (*3*). Aryl β-glucosidase activity was shown to be 55 percent of controls (*3*); whereas glucosyl ceramide β-glucosidase activity overlapped with controls (Table 1). However, sphingomyelinase activity was found to be only 15 to 17 percent of normal on repeated testing at all stages of cell growth (Table 1). Galactosyl ceramide β-galactosidase activity was about three times higher than normal in this patient (Table 1). In Table 2 is shown the lac-cer β-galactosidase activity assayed according to the methods of Wenger *et al.* (*5*) and Tanaka and Suzuki (*8*), and compared with that reported by Dawson *et al.* (*3*). The lac-cer β-galactosidase activity in this patient was about three times normal when assayed according to the method of Wenger *et al.* (*5*). This is almost the same as the increase in the activity of galactosyl ceramide β-galactosidase found in this patient's fibroblasts (Table 1). When the same culture was assayed according to the method of Tanaka and Suzuki (*8*) the activity of lac-cer β-galactosidase was not different from the normal control. The deficiency in the patient is shown only with the original data of Dawson *et al.* (*3*). In addition to fibroblasts, the liver and brain of the patient were examined for lac-cer–cleaving activity. No deficiency was found with either of the two assay methods.

Therefore the patient with so-called lactosyl ceramidosis was found to have normal or above normal activity for either of the two lac-cer β-galactosidases when assayed by methods recently described (*5, 8*). Dawson *et al.* (*3*) performed their original assays by a method which did not contain either pure or crude sodium taurocholate. They found deficient activity in this patient when they used Triton X-100 as the only detergent. The activity for lac-cer β-galactosidase assayed with Triton X-100 in control fibroblasts was found to be about 1 nmole per milligram of protein per hour, which is about 1 percent of the activity found for controls by the method of Tanaka and Suzuki (*8*) and 5 percent of the activity for controls by the method of Wenger *et al.* (*5*). The present evidence is for two genetically distinct lac-cer β-galactosidase activities. There still is no evidence to support the idea that there may be a third β-galactosidase in humans that degrades lac-cer exclusively. While we cannot conclusively exclude the possibility that the lac-cer–cleaving activity measured in the presence of Triton X-100 might represent the third enzyme, the level of the activity appears too low to be physiologically significant.

Lactosyl ceramide has been reported to be stored to some extent in certain organs in children with specific lysosomal storage diseases such as Tay-Sachs, Niemann-Pick, G_{M1} gangliosidosis, Krabbe's disease, and in the Hurler, Hunter, and Sanfilippo syndromes (*13*). Patients with minimal storage of sphingomyelin, but excess glycolipids including lac-cer, and a partial deficiency of sphingomyelinase activity have been reported (*14*). The reasons for the storage of lac-cer in patients in which the primary lesion has been shown to be the deficiency of a lysosomal enzyme apparently unrelated to lac-cer catabolism is at present unexplained. A partial deficiency (15 to 17 percent of normal) of sphingomyelinase activity found in the fibroblasts may be the primary lesion in this patient. If so, this one patient with so-called lactosyl ceramidosis may be another of the cases of an unusual type of Niemann-Pick disease.

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Visual Membranes: Specificity of Fatty Acid Precursors for the Electrical Response to Illumination

Abstract. *Rat electroretinograms were measured as a function of dietary supplements of purified ethyl esters of linolenic acid, linoleic acid, and oleic acid. Polyunsaturated fatty acids derived from precursors of linolenic and linoleic acids appear to be important functional components of photoreceptor cell membranes, although in equal dietary concentrations, linolenic acid precursors affect electroretinogram amplitudes to a greater extent than linoleic acid precursors. The electrical response of photoreceptor cell membranes appears to be a function of the position of the double bonds as well as a function of the total number of double bonds in fatty acid supplements.*

Vertebrate photoreceptor cells consist of an inner and an outer segment (*1*). The inner segment of the rod cell contains a variety of membrane systems, such as mitochondrial, ribosomal, plasma, and nuclear membranes; the rod outer segments (ROS) are characterized by a stacked array of many hundreds of disk membranes, which are enclosed by a plasma membrane. The visual pigment rhodopsin accounts for 80 to 90 percent of ROS membrane-bound protein (*2*), and phospholipids account for 80 to 85 percent of the total ROS membrane lipid (*3*). Phospholipids are required for both the stability and regenerability of rhodopsin (*4*); and, on the average, each rhodopsin molecule of rat ROS is associated with 60 to 65 phospholipid molecules (*5*).

Docosahexenoic acid, 22 : 6ω3, is the dominant polyunsaturated fatty acid of the phospholipids of vertebrate ROS membranes (*6*). Rats cannot synthesize either ω3 or ω6 fatty acids, and ω3 and ω6 precursors (essential fatty acids) must be obtained from dietary sources (*7*). This dietary requirement, together with the observation that normal rat ROS membranes turn over and are renewed every 9 to 10 days (*8*), suggested that dietary manipulations could be exploited to alter the lipid composition of rat ROS membranes. However, only 8 percent of the polyunsaturated lipids were replaced in ROS mem-

branes when weanlings were maintained on a fat-free diet for 10 weeks (*7*). This unexpectedly low percentage of replacement prompted a subsequent series of experiments which demonstrated that the normal process of membrane renewal was also altered by essential fatty acid deprivation (*9*).

When rats were deprived of essential fatty acids for two generations, (i) more than 50 percent of 22 : 6ω3 was replaced in ROS membranes, (ii) the functional response of the electroretinogram (ERG) was altered, and (iii) rhodopsin concentrations were similar for control and fat-deprived animals (*10*). Although 22 : 6ω3 was the major polyunsaturated fatty acid replaced in the membranes (on a mole percentage basis), alterations were also observed in 22 : 5ω6 and 20 : 3ω9 (*10*). In order to determine the specificity of fatty acid replacements, ERG's have now been measured from adult rats maintained for 40 days (*11*) on fat-free diets supplemented with purified ethyl esters of 18 : 3ω3, 18 : 2ω6, and 18 : 1ω9.

Seventy-two female albino rats (Texas Inbred, Houston) were maintained on a Lab Blox diet (Allied Mills) to age 14 weeks, and then they were randomly divided into six groups of 12 animals each. The groups were placed on the following diets: (i) Lab Blox, (ii) fat-free diet (*12*), (iii) fat-free plus 2 percent by weight of the ethyl ester of 18 : 1ω9 (*12*), (iv) fat-free plus 2 percent 18 : 2ω6, (v) fat-free plus 1 percent 18 : 2ω6 and 1 percent 18 : 3ω3, and (vi) fat-free plus 2 percent 18 : 3ω3. To minimize oxidation, the diets were made up under nitrogen 1 day prior to the onset of the study and stored at 5°C. The fatty acid composition of the diets at days 1 and 20 after mixing are shown in Table 1. The method of storage proved satisfactory, since the sampling variations observed between days 1 and 20 were in the range expected for imperfect mixing of dietary components. The methods for analysis of phospholipids and fatty acids have been described (*7*), and ERG amplitudes were measured as a function of stimulus flux (*10*). The ERG amplitudes were averaged from 14 ± 2 eyes after the animals had been maintained on the respective diets for 40 days (*11*), and ERG data on a given rat were excluded only if the anesthetic (Nembutal, 30 mg/kg) did not completely immobilize the animal or if the animal expired within a week after being tested. Relative ERG amplitudes (*13*) are plotted as a function of fatty acid supplements in Fig. 1, where relative ERG amplitude is defined as the ratio of the average peak ERG amplitude for a specified fatty acid supplement to the average peak ERG amplitude for fat-free diet alone.

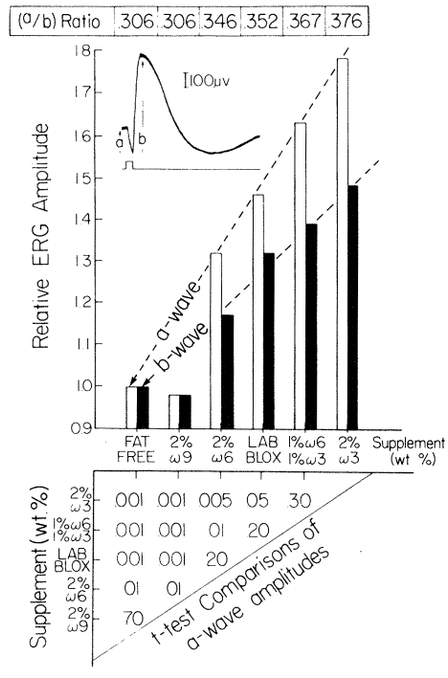

Fig. 1. Relative ERG amplitudes of a-waves (open bars) and b-waves (solid bars) after rats were given the various fatty acid supplements (Table 1) for 40 days. At the top of the figure, ratios of a-wave to b-wave (a/b) are given for each fatty acid supplement. Relative ERG amplitude is the average peak amplitude for a specified fatty acid supplement normalized relative to the average peak amplitude for fat-free diet (averages were derived from 14 ± 2 eyes). On the fat-free diet, average peak amplitudes ± the standard error were 129 ± 7 μv and 419 ± 33 μv for the a-wave and b-wave, respectively. Peak a-wave and b-wave amplitudes for a typical ERG response are shown in the upper trace of the inset, and the 20-msec flash was monitored by the lower trace. Fatty acid supplements are listed in rows and columns in the lower portion of the figure, and the numbers at the intersections of the rows and columns are probability values calculated on two-tailed unpaired *t*-tests. Each intersect specifies the probability that the a-wave amplitudes observed for that row and column were sampled from the same a-wave population.

Since rats should be able to synthesize ω9 fatty acids de novo, 18 : 1ω9 is not an essential fatty acid. However the 18 : 1ω9 group provides a control for nutritional supplement of an 18-carbon fatty acid. The 18 : 1ω9 group behaved as expected (see Fig. 1), and the a-wave amplitudes for animals maintained on a fat-free diet supplemented with 2 percent 18 : 1ω9 were equivalent to amplitudes observed with fat-free diet alone at a *t*-test probability of 0.7 ($P = .7$). The a-wave amplitudes for 2 percent 18 : 2ω6 were greater than the amplitudes observed with fat-free or 18 : 1ω9 ($P = .01$), while amplitudes for the three groups supplemented with substantial amounts of 18 : 3ω3 (Lab Blox, 1 percent ω6 + 1 percent ω3, and 2 percent ω3) were all greater than the amplitudes of groups supplemented with 18 : 1ω9 or fat-free alone ($P < .001$).

Except for the Lab Blox diet, the number of double bonds in fatty acid supplements increases from left to right in Fig. 1, and ERG amplitudes correlate with the number of double bonds in the purified fatty acid supplements. Although the total number of double bonds per gram of diet was greatest for the Lab Blox diet, ERG amplitudes were greater for the groups with greater weight percentages of ω3 fatty acids, and the amplitudes increased in the order of increasing ω3 concentrations.

Except for Lab Blox and the fat-free ration, the diets in Fig. 1 were isocaloric. Yet a-wave amplitudes observed for supplements of 2 percent ω3 and 1 percent ω6 + 1 percent ω3 were greater than a-wave amplitudes observed for the group supplemented only with 2 percent ω6 ($P = .005$ and .01, respectively). While a general nutritional requirement for ω6 fatty acids is well documented, our data also indicate a selective functional role in the visual system for ω3 fatty acids.

Table 1. Fatty acid analyses of diets at day *t* after mixing (percentage by weight).

Diet	*t* (days)	Unsaturated fatty acids			Miscellaneous fatty acids*	Total fatty acids
		18 : 1ω9	18 : 2ω6	18 : 3ω3		
Fat-free (FF)	1	0.03	0.01	0.01†	0.07	0.10
	20	0.04	0.01	0.01	0.08	0.12
FF + 2% 18 : 1ω9	1	2.02	0.08	0.03	0.06	2.30
	20	1.79	0.08	0.02	0.07	1.96
FF + 2% 18 : 2ω6	1	0.02	1.82	Tr	0.01	1.88
	20	0.02	1.96	0.01	0.01	2.02
FF + 1% 18 : 2ω6 + 1% 18 : 3ω3	1	0.02	1.00	1.16	0.02	2.18
	20	0.02	1.02	1.22	0.02	2.13
FF + 2% 18 : 3ω3	1	0.02	0.09	1.82	0.05	2.08
	20	0.05	0.10	1.77	0.05	1.90
Lab Blox	U‡	1.10	2.00	0.20	0.75§	4.24

*Predominantly 14 : 0, 16 : 0, and 18 : 0, with no detectable polyunsaturated acids except for those in Lab Blox. §Lab Blox contained 0.06 percent of 22 : 5ω6 and 0.13 percent of 22 : 6ω3. ‡Unknown. †Tr.

Relative mole percentages of the phospholipid classes were not detectably different between groups. However, there was a trend toward positive correlation between the fatty acid precursors of the dietary supplements and the fatty acid composition of the phospholipid classes of the ROS membranes. With only 20 eyes for assay for each dietary group, we estimate an uncertainty of 5 percent for major fatty acid components of rat ROS, and hence it appears that the ERG achieved a steady state when no more than 5 percent of the polyunsaturated compounds were replaced in ROS membranes [also see (*14*)]. Inasmuch as the photocurrents are probably controlled by plasma membranes (*15*), it is interesting that ROS plasma membranes contribute no more than 5 percent to the total of rat ROS membranes. In addition, ROS plasma membranes apparently are renewed in a relatively brief time interval in which only a small percentage of total ROS membranes are renewed (*16*). These considerations suggest that perhaps the observed electrical alterations are associated with fatty acid substitutions in the plasma membrane, but our ERG measures are powerless to discriminate between electrical contributions from specific membrane systems such as plasma membranes, disc membranes, and mitochondrial membranes.

The ERG is an external field parameter which results from the currents generated by cells of the retina in response to illumination. As a consequence ERG alterations may arise from either or both of the following: (i) from alterations in the currents generated by retinal cells; (ii) from alterations in the passive impedance characteristics of the external field, where the external field includes extraretinal tissues of the eye such as the lens and aqueous and vitreous humors. However, the latter possibility seems extremely unlikely here because of the following considerations. If there were differential impedance changes in the extraretinal tissues of fat-free and supplemented animals, the changes were only resistive changes in the regions of the frequency domain occupied by the ERG response (*17*). Yet as a purely resistive attenuator the extraretinal tissue would modify all components of the ERG by the same percentage; that is, the ratio of a-wave to b-wave (a/b) would remain constant if the fatty acid substitutions only produced passive resistive changes in extraretinal tissue. This result was not observed. On a percentage basis, the a-wave was altered more than the b-wave, and the (a/b) ratios were different for different dietary groups (see Fig. 1). Equally compelling are the observations that (i) the a-wave amplitude continued to change as a function of time on a fat-deprived diet when the b-wave had achieved a steady-state value, and (ii) the rate constants for the a-wave and b-wave differed by several days (*11*). In summary, the b-wave provides an internal control against the possibility that these a-wave alterations were simply the result of passive impedance changes in extraretinal tissues of the eye.

The a-wave of the ERG seems to be primarily a response function of the photoreceptor cells of the retina, while the b-wave appears to arise from the response of other cells in the retina (*18*). With the b-wave as an internal control, we conclude from the a-wave data presented here that the electrical response of photoreceptor cell membranes is a selective function of $\omega 3$ and $\omega 6$ fatty acids.

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11. T. G. Wheeler, thesis, Graduate School of Biomedical Sciences, University of Texas Health Science Center at Houston (1974). In adult rats the amplitude of the a-wave of the ERG declined to a steady-state value after 40 days of fatty acid deprivation, and this steady-state amplitude was the same as that observed after two generations of fatty acid deprivation. The b-wave achieved the steady-state value after only 25 days of deprivation. The exponential time constant was 10 days for the a-wave and 7 days for the b-wave.
12. Fat-free diet was supplied by Nutritional Biochemicals. This diet contained traces of fatty acids associated with supplements of fat-soluble vitamins A, E, and K (see Table 1). All purified ethyl esters of fatty acids were supplied by NuCheck Prep (Elysian, Minn. 56028).
13. Although Fig. 1 shows only relative response amplitudes for 20-msec flashes at one value of stimulus flux (700 microwatts), relative ERG amplitudes were similar to those of Fig. 1 over the entire flux range from threshold to saturation in intervals of 0.5 log unit.
14. If data between 14-week-old adults are comparable to data on 3-week-old weanlings, approximately a 4 percent reduction in 22 : $6\omega 3$ would be expected after 40 days of fatty acid deprivation. This estimate is based on a linear extrapolation of an 8 percent change in 22 : $6\omega 3$, which was observed when 3-week-old weanlings were placed on a fat-free diet for 70 days (*7*).
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Parasite Reproductive Strategy and Evolution of Castration of Hosts by Parasites

Abstract. *A modification of the Euler equation is used to describe a simple parasite life history in which survival decreases with age at a rate determined by mortality of the parasitized host. The advantages of castration of hosts by parasites are discussed using the modified equation in which castration is equivalent to reducing parasite virulence in the host to near zero or zero. It is suggested that castrating parasites can infest a wider range of hosts with higher mortality rates and that within parasite groups having castrating and noncastrating species, the former should infest hosts with higher mortality rates and relatively larger gonads.*

Parasites of many taxa, from protozoans to vertebrates, castrate their hosts. In some groups, such as digenetic trematodes infesting mollusks, castration of the host is the rule (*1*). Ecological life history models have not been used to study this phenomenon. The following general approach is proposed to investigate reproductive strategies of parasites with respect to the advantages of host castration.

Ecological descriptions of life histories use life table data. The life table is an actuarial list of estimations of probabilities of survival to age x, and fecundity in the age