icity is unclear. Free radicals could play an important role (*7*) in that they can inactivate thiol enzymes or coenzymes and damage proteins (*20*). Considerable alterations of permeability of the mitochondrial membrane are correlated with peroxidation of lipoproteins (*21*). Alterations of neuronal membrane permeability might contribute to the effects of oxygen toxicity, especially since active cation transport is now believed to act as a pacemaker for metabolism (*22*).

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Wax Esters in Marine Copepods

Abstract. *Two pelagic copepods,* Calanus helgolandicus *and* Gaussia princeps, *contained wax esters with 28 to 44 carbon atoms as major lipid constituents. In laboratory cultures of the former species, changes in nutrition (amount or species of diatoms fed) affected both the amount of total lipid and the composition of the wax esters. Thus, the wax esters serve as a reserve energy store in this organism.*

The calanoid copepods occupy a key position in the marine food chain because they (i) feed directly on phytoplankton, the primary producers in the oceans, (ii) constitute the largest single fraction of the biomass of the zooplankton in most waters, and (iii) are the principal food at some stage in the life of many fish species of nutritional and economic importance to man, such as the herring, sardine, and anchovy (*1, 2*). We have investigated the lipids of several species of these crustaceans (Table 1). The lipids of the large black *Gaussia princeps* from depths of 650 to 900 m contained a high percentage of wax esters, approaching that found in the muscle of some fishes (*3*). The cosmopolitan *Calanus helgolandicus,* collected some distance offshore in plankton tows to 200 m, contained wax esters in lesser proportions (30 to 37 percent), but still as a major lipid type. Two additional species collected at sea off La Jolla were

Table 1. Composition of the lipids of calanoid copepods. *Calanus helgolandicus* wild type a was collected off La Jolla, California, in April 1969; wild type b was collected off La Jolla, California, in June 1969; laboratory grown c was fed *Skeletonema* at 400 μg of carbon per liter; laboratory grown d was fed *Skeletonema* at 600 to 800 μg of carbon per liter. *Gaussia princeps* wild type was collected over Rodriguez Seamount, off Santa Barbara, California, in April 1967. Values are expressed as weight percent.

Fraction	*Calanus helgolandicus*				*Gaussia princeps* wild type
	Wild type		Laboratory grown		
	a	b	c	d	
Hydrocarbons	Trace	3	3	1	Trace
Wax esters*	37	30	25	41	73
Trigylcerides	5	4	3	12	9
Polar lipids†	14	17	10	16	17
Phospholipids‡	44	45	59	28	
Total lipid (% dry weight)	12.4	15	18.6	28	28.9

* Includes any sterol esters present. † Free acids, cholesterol, mono- and diglycerides, and so forth. ‡ Largely lecithin and phosphatidyl ethanolamine determined by thin layer chromatography.

Table 2. Wax esters of *Calanus helgolandicus* under various dietary regimens. Wild type a was collected off La Jolla, California, in April 1969; 200 adult females plus 200 adult males yielded 2.46 mg of lipid; total phytoplankton available to these animals was estimated at between 30 and 150 μg of carbon per liter. Wild type b (250 adult females) was collected off La Jolla, California, in June 1969; available food similar to wild type a. Wild type c is part of collection b that was kept 7 days in the laboratory without food. The laboratory grown was fed *Skeletonema* at the rates indicated (micrograms of carbon per liter); each column represents data from 20 to 105 adult copepods raised in the laboratory from eggs through the nauplii and copepodite stages over a period of 3 to 4 weeks on the specified diet. Values are expressed as weight percent; N.D., not detected.

Chain length*	Wild type			Laboratory grown			
	a	b	c	800	400	200	100
30	2.8	19.8	65.0	21.0	12.0	80.6	89.7
31		trace	4.1			6.7	5.1
32	8.3	4.3	6.6	32.3	30.6	9.1	4.3
34	16.9	10.4	10.3	11.6	16.5	3.6	N.D.
36	23.2	20.4	11.3	18.2	24.1	N.D.	
38	17.9	16.5	6.0	17.9	13.1		
40	9.5	8.3	N.D.	3.7	2.8		
42	8.9	6.6		N.D.	N.D.		
44	7.1	7.3					
Total lipid (% dry weight)	12.4	15.4	7.7	36.6	18.1	15.0	9.3

* Total carbons, alcohol plus acid.

analyzed by thin layer chromatography (TLC) only—the shallow inshore species *Labidocera trispinosa* (total lipid, 14 percent dry weight), in which the wax esters were estimated to be only 5 percent of the total lipid, and *Eucalanus* sp., a large fragile form from 50 to 100 m, containing about 34 percent lipid but only a trace of wax ester.

These findings clarify reports (*4*) that long-chain alcohols were the principal components of the nonsaponifiable fraction of the lipids of *Calanus finmarchicus*; together with the high percentages of this nonsaponifiable fraction consistently reported for copepod lipids (*5*), they suggest that the wax esters are at least minor constituents of many of the species in the suborder Calanoidei. The wax esters of the marine copepods are very probably also the main source of the long-chain alcohols observed in marine sediments (*6*) and of some ocean surface lipids such as Bute Inlet wax (*7*).

We investigated the effect of different food species and concentrations on the wax esters of *C. helgolandicus*. The copepods were raised at 15°C in agitated seawater (salinity, 34 per mille) from eggs to adulthood. With a new culture method (*8*), it was possible to keep the diatom food suspended uniformly at up to 800 μg of carbon per liter, and the copepods generally evenly distributed at a concentration of 6 to 12 animals per liter. The diatoms belong to the most abundant algal species in the Pacific Ocean off La Jolla. The lipids were investigated (*9*) by fractionation into component lipid types by chromatography on silicic acid columns and analysis by gas liquid chromatography (GLC) of the native wax esters, the long-chain alcohols (as trifluoroacetates), and the fatty acids (as methyl esters). The amount of total lipid available from most experimental groups was less than 1 mg, and in these cases only analyses of wax esters were attempted. For this purpose we injected portions of the crude total lipid directly onto a column (1.8 m by 3.2 mm, outside diameter) of 3 percent OV-1 on 60/80 mesh Gas-Chrom P (Loenco model 70 Hi-Flex apparatus fitted with a flame ionization detector and operated isothermally at 300°C and with nitrogen carrier gas pressure of 2.1 kg/cm^2). The observed retention time for authentic dodecyl palmitate (28 : 0 wax ester) was 2.4 minutes. Only partial resolution of components that differed in degree of unsaturation was obtained, and so only summation values for each chain length are given (Table 2).

Because of limitations of our GLC techniques we were unable to determine directly the degree of unsaturation of the wax esters of the various chain lengths. However, since both the alcohol and acid moieties of the group fed 400 μg of carbon per liter (*Skeletonema*) were largely unsaturated—19 and 20 percent monoenes (*10*) and 65 and 58 percent polyenes, compared to only 16 and 19 percent saturated components—it is apparent that a minimum of 65 percent of these wax esters must contain two or more double bonds per molecule. The bimodal distribution of wax ester homologs seen in animals fed 400 to 800 μg of carbon per liter in the laboratory is consistent with an observed minimum at C_{18} for both the alcohols and fatty acids. Although it is not widely recognized, this minimum at C_{18} is very common for fatty acids of phytoplankton (*11*), and is pronounced with *Skeletonema costatum*.

The effect of nutrition on the total amount of fat, the rapid depletion of this total fat during starvation (involving at least a proportionate loss of wax esters), and the extensive changes in the composition of the wax esters during starvation establish that, in *Calanus helgolandicus*, the wax esters are primarily a reserve energy store. We rationalize these findings as follows. When *C. helgolandicus* feeds on organisms rich in lipids, such as *Skeletonema*, a second liquid phase of lipid forms around the intestine (*2*, p. 105; *12*). This oil droplet is particularly rich in wax esters (*13*) that must be synthesized by the copepod during the digestion and absorption of the dietary lipid, because the diatom lipids contain no wax esters. In *C. helgolandicus* food passes through the gut in as little as 20 minutes; digestion and absorption must be on a comparable time scale, so that very rapid wax ester biosynthesis occurs. In turn, this requires a rapid synthesis of long-chain alcohols, which apparently is accomplished mainly by reducing part of the dietary fatty acids, rather than by *de novo* synthesis. As the amount of nutrient offered (carbon in *Skeletonema*) increases, both the alcohol and fatty acid moieties tend to approach the homolog distribution of the dietary fatty acids. The very large proportion of polyunsaturated alcohols found in well-fed laboratory animals — even higher than for wax esters in fish roe (*14*)—and the minimum observed at C_{18} for both the alcohol and acid moieties in these animals support this hypothesis. Between meals the oil droplet may disappear, as the animal metabolizes the lipids, partly for energy and partly for synthesis of lipids characteristic of the species. In *C. helgolandicus* these characteristic lipids include wax esters in which C_{30} homologs predominate. In starved animals the reserve wax esters have been thoroughly modified, the C_{20} and C_{22} polyunsaturated alcohols and acids (and therefore longer chain wax esters) largely metabolized, and only the characteristic, shorter homologs survive; the latter may well be synthesized *de novo* from acetate.

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