Pure & Appl. Chem., Vol.53, pp.867-871. 0033-4545/81/040867-05$02.00/0
Pergamon Press Ltd. 1981. Printed in Great Britain.

MARINE WAX ESTERS

J.R. Sargent, R.R. Gatten and R.J. Henderson

Institute of Marine Biochemistry, Aberdeen AB1 3RA, U.K.

ABSTRACT. The composition, biosynthesis and role of wax esters in zooplankton including calanoids and krill is reviewed together with their transformation to triacylglycerols in zooplanktonivorous fish. A special role for 22:1(n-11) alkyl units is indicated.

DISTRIBUTION

Following the discovery (reviewed in refs. 1,2,3) that wax esters are found in large amounts in numerous marine zooplanktonic species it has remained true that these lipids are seldom found in significant amounts outside marine animal phyla. Exceptions are the marine phytoplanktonic cryptomonad, Chroomonas salina, which when grown photoheterotrophically in the presence of glycerol produces large amounts of wax esters (4), and the eye spot of the dinoflagellate, Peridinium foliaceum, which is rich in wax esters (5). Evidence accumulated since the early 1970s continues to support the general hypothesis of Lee and Hirota (6) that wax esters are elaborated by marine animals in environments where short periods of food plenty are followed by long periods of food shortage. Examples of such environments are bathypelagic regions and polar regions. Lee et al (6,7) showed that the wax ester content of calanoid copepod species was correlated with latitudinal distribution, a typical polar species such as Calanus hyperboreus containing more than 70% of its dry weight as wax esters (8). A similar correlation has been established more recently for euphausiids in both hemispheres. The common Antarctic krill Euphausiia superba with a relatively northerly distribution has only traces of wax esters (9,10); its more southerly neighbour E. crystallorophias living close to the ice edge is rich in these lipids (9). In the northern hemisphere the southerly distributed Meganictyphanes norvegica lacks wax esters (11,12) whereas a typically Arctic species such as Thysanoessa inermis contains large amounts of these lipids (12). Thysanoessa sp. in the Antarctic are also rich in wax esters (13).

Both polar and bathypelagic environments have water temperatures seldom greater than 2°C. These low temperatures are intimately associated with a marked temporal variation in primary food density, an association clearly seen in polar regions where cold waters and low phytoplanktonic biomass are inevitable consequences of long periods of low intensity solar radiation during winter. Intense solar radiation during the polar summer generates a large but short seasonal burst of phytoplankton production. It is the polar herbivores, including the calanoid copepods, that are subjected to the most extreme temporal variation in food supply. Larger omnivorous zooplankton including the euphausiids prey heavily on phytoplankton during the summer but can also consume smaller zooplankton and possibly detritus when phytoplankton is scarce (14,15). Therefore the euphausiids are less subject to extreme seasonal variation in food supply and contain lower levels of wax esters than the herbivorous calanoids (8,9,12). It is to be anticipated that extreme variations in polar biomass become more damped at increasingly higher trophic levels, i.e. the content of wax esters is likely to decrease as one progresses from pure herbivores to pure carnivores. Carnivorous polar fish that consume large quantities of zooplankton, e.g. capelin and cod, do not accumulate wax esters in significant amounts.

COMPOSITION AND BIOSYNTHESIS

The wax esters of calanoid copepods contain fatty alcohols that are mainly saturated and monounsaturated, i.e. 16:0, 20:1(n-9) and 22:1(n-11), and fatty acids that are rich in polyunsaturated units (1,2,3). In a recent analysis (16) of wax esters from Calanus finmarchicus from a Norwegian fjord 16:0, 20:1 and 22:1 units accounted for 18%, 27% and 28% respectively of total fatty alcohols; 18:4(n-3) and total polyunsaturated fatty acids accounted for 24% and 44% respectively of total wax ester fatty acids. Calanoid wax esters usually contain only small amounts of 18:1 units, both in the alcohols and acids. Such compositional differences between acids and alcohols of calanoid wax esters have been interpreted as showing that the fatty acids derive preferentially from the animal's dietary lipid whereas the fatty alcohols derive preferentially from de novo biosynthesis of fatty acids from protein and carbohydrate dietary precursors (2,3). Fragments of calanoid copepods in vitro incorporate labelled glucose and amino acids preferentially into the fatty alcohol moieties of their wax esters (17). Wax ester formation may therefore be seen as a mechanism whereby large lipid reserves can be built up through the continuing biosynthesis of lipid (as fatty alcohol) from non-lipid dietary material in the presence of substantial quantities of dietary fatty acids.

Since 20:1 and 22:1 fatty alcohols are prominent in the wax esters of some Arctic amphipods and decapods (18) they are not necessarily associated with purely herbivorous animals such as the calanoids. The fatty alcohols of the euphausiid wax esters do not contain 20:1 and 22:1 fatty alcohols; 14:0 followed by 16:0 are the major alcohols in wax esters from Euphausiia crystallorophias whereas 16:0 followed by 14:0 are major alcohols in wax esters from Thysanoessa inermis (9,12). The fatty acids of both these wax esters are dominated by 18:1 and contain very much lower amounts of polyunsaturated fatty acids than the calanoid wax esters (9,12). Nevertheless preliminary experiments have shown that labelled glucose is still preferentially incorporated into the alcohol moieties of euphausiid wax esters in in vitro systems (19). Curiously, phytol accounts for some 10% of the total alcohols in the wax esters of T. inermis; wax esters whose sole alcohol is phytol are also present in significant amounts in T. raschii which is not a wax ester-rich species (12,20). Clearly the phytol in such esters stems from phytoplanktonic material. The only other known occurrence of phytol in marine wax esters is in the eye spot of the dinoflagellate Peridinium foliaceum where the esters are composed solely of phytol esterified to a range of highly polyunsaturated fatty acids (5). It may be noted that the phytol consumed in large quantities by the calanoid copepods is ultimately metabolised to pristane (21).

The wax esters of bathypelagic invertebrates contain mainly 16:0 and lesser amounts of 18:1 fatty alcohols; 18:1 tends to be a dominant fatty acid and polyunsaturated fatty acids are present in amounts similar to those in euphausiid wax esters (2). A similar situation exists for the wax esters of bathypelagic fishes except that 18:1 alcohol now assumes as much importance as 16:0 alcohol (2).

ROLE OF WAX ESTERS IN ZOOPLANKTON

Although roles for wax esters in buoyancy regulation and the provision of metabolic water have been proposed (1) it is certain that the major function of any large deposit of neutral lipid lies in the provision of metabolic energy. Wax ester accumulation in the calanoid copepods occurs maximally at stages IV and especially V, i.e. immediately before development to the sexually mature males and females (stage VI). Since calanoid copepods commonly overwinter prior to reaching full sexual maturation in the following spring, it is possible that wax esters are consumed in the provision of metabolic energy for maintenance during the potentially long winter when phytoplankton food is scarce. It is equally possible that the animals overwinter in a relatively inactive metabolic state, akin to hibernation, without consuming significant amounts of wax esters. The lipids could then be used during reproduction in the following spring immediately before the appearance of phytoplankton food in the water. Current information suggests that the latter of these two possibilities is by far the more important.

When wax ester-rich Euchaeta are starved their wax esters are depleted but only at a slow rate (22). We have maintained wax ester-rich E. norvegica in the laboratory at ambient temperatures for up to two months without significant loss of lipid. It follows that the maintenance requirement of these animals is very low. Euchaeta produces eggs rich in wax esters so that direct transfer of the lipids from the adult female to eggs occurs (22,23). Calanus produces eggs rich in triacylglycerols possibly indicating transformation of wax esters in the adult to triacylglycerols prior to transfer into the eggs (23,24). A recent study has established that some 30% of the lipid in prespawning female Euchaeta is transferred to the eggs with another 30% being recovered in the spent female (25). It is probable that the remaining lipid is combusted to produce metabolic energy for the elaboration of the female reproductive apparatus since it has been established (24) that some 50% of the lipid, largely wax esters, is lost when stage V Calanus helgolandicus develop to sexually immature females, i.e. females that have well developed and recognisable ovaries but which have not yet produced eggs. That is, the metabolic energy cost of producing the female reproductive apparatus (ovaries and ancillary organs) of a calanoid is about one half of the total lipid level in the animal, and a sizeable proportion of the remaining lipid is ultimately transferred to eggs. It has also been estimated that approximately one half of the triacylglycerols in E. superba (deficient in wax esters) is transferred to eggs (10). While quantitative data are not yet available for the wax ester-rich T. inermis, a seasonal survey of lipid levels in this species has shown a marked depletion of wax esters during ovarian development and egg production (15). Overall, therefore, the present evidence strongly suggests that wax esters, and indeed neutral lipids generally, are elaborated in large amounts by zooplankton not so much for survival during winters in the absence of food but much more for reproduction during the following spring.

Zooplanktonic wax esters are sufficiently rich in polyunsaturated fatty acids to be excellent precursors of biomembrane lipid (phospholipid). Equally the esters are very rich in 14:0, 20:1 and 22:1 alkyl moieties that are not major constituents of polar lipids. The fate of these fatty acids, therefore, is solely to be combusted to produce metabolic energy (ATP).

WAX ESTER SLICKS

Interest has recently focussed on apparently natural phenomena that generate the formation of extensive surface slicks of wax esters. A major example of this is the occurrence of large deposits of waxy material washed on to the shores of Bute Inlet, B.C. Canada (1). This has been established as consisting mainly of wax esters composed of 20:1 and 22:1 fatty alcohols together with 14:0 and 16:0 fatty acids (1,26). These constituents are very prominent in the wax esters of Calanus plumchrus, the dominant calanoid copepod in the inlet. That the Bute Inlet wax derives from the indigenous calanoid in the inlet is supported by its containing small but significant amounts of pristane (26). It has been considered that the wax found on the shores stems ultimately from large numbers of calanoids being killed due to a decrease in the surface salinity of the Inlet, e.g. due to an influx of fresh water stemming from a sudden thaw in late winter (26). Mass mortalities of copepods have frequently been witnessed in Norwegian fjords following the entrainment of the animals in fresh water gyres generated through discharges of hydroelectric generating stations into fjords (27). The oil released from the dead calanoids is considered to be aged resulting in destruction of the relatively labile polyunsaturated fatty acids leaving only saturated and monounsaturated wax esters (26). An extensive slick of wax esters occurred in the North Sea in 1975 with a composition almost identical to that of the Bute Inlet wax (16). A similar slick occurred in the North Sea in 1980 (28). It was established (16) that the earlier North Sea slick consisted of two wax esters 34:1 and 36:1, the former composed almost entirely of the alcohol acid combination 20:1-14:0 and the latter of 22:1-14:0 (78%) + 20:1-16:0 (19%). Wax esters of virtually the same composition predominated in fresh Calanus oil and indeed oil of identical composition to the slick oil could be simply generated by argentation chromatography of total Calanus oil to eliminate the polyunsaturated units (16). Decreased salinity cannot readily be invoked to explain massive mortalities of zooplankton in open sea waters such as the North Sea (16) or the N.W. Pacific (29). Both North Sea slicks were associated with periods of unusually high temperatures. The possibility remains that special conditions were created leading to overgrowth and collapse of plankton populations and consequent death of large numbers of calanoids. In none of these instances was there any reason to suspect death from pollution. The incidence of such large scale phenomena and the ultimate fate of the aged wax esters is not known.

METABOLISM BY FISHES

Wax esters are inevitably consumed in large quantities by zooplanktonivorous fish which include most clupeoids and many salmonids at least at some stage in their life history. The great majority of teleost fishes including those consuming zooplankton contain triacylglycerols as their major neutral lipid with at most traces of wax esters. It is known from direct feeding experiments that even a freshwater fish such as the rainbow trout that does not experience wax esters in its natural diet can assimilate more than 90% of dietary wax esters fed in the form of calanoid copepods (30). Wax esters in the gut lumen appear to be hydrolysed by a non-specific lipase, similar to the cholesteryl esterase of mammalian pancreas that also hydrolyses triacylglycerols to glycerols and free fatty acids (31). Nonetheless the rate of hydrolysis of wax esters is less than that of triacylglycerols when both lipids are present (31). The non-specific lipase is widely distributed in fish (32). Fatty alcohols and fatty acids generated in the fishes' intestinal lumen are absorbed by the intestinal epithelial cells and there converted to triacylglycerols by a process where the NAD-dependent oxidation of fatty alcohol to fatty alcohol is coupled to the NADH-dependent formation of glycerophosphate from glucose or amino acids (33). Triacylglycerols finally formed in the endoplasmic reticulum are processed into very low density lipoprotein (VLDL) particles and ultimately to chylomicron particles, and discharged serosally from the intestinal cells (33,34). The overall stoicheoimetry of the conversion appears to be 3 wax ester molecules converted to 2 triacylglycerol molecules, a stricture that necessitates the locating of fatty acids such as 20:1 and 22:1 (stemming from wax ester alcohols) on both position -1 and position-2 of the triacylglycerol-glycerol backbone (33).

Not only is the natural diet of zooplanktonivorous fish rich in wax esters but it is also rich in 22:1 alkyl chains. The (n-9) isomer of 22:1, erucic acid, is known to cause transient cardiac liposis when fed to rats in large amounts as rapeseed oil (35). A similar effect is shown by partially hydrogenated commercial marine oils (35) which are rich in the (n-11) isomer of 22:1, cetoleic acid, which originates in the 22:1 (n-11) fatty alcohol of zooplanktonic wax esters and is passed up the food chain to zooplanktonivorous fish, gadoids and the marine mammals (36,37). Liver perfusion studies have shown an enhanced chain shortening of erucic acid shortly after feeding rats diets rich in either rapeseed oil or partially hydrogenated marine oil (38). Such enhanced chain shortening occurs via a cyanide-insensitive pathway in liver (39). It has been proposed that the catabolism of high dietary levels of 22:1 isomers in liver occurs via the cyanide-insensitive beta oxidation pathway in perixosomes, rather than the conventional cyanide-sensitive pathway of mitochondria (39). An enhanced oxidation of fatty acyl CoA by liver perixosomes after feeding rats diets rich in partially hydrogenated marine oil has been observed (40) and a mechanism for the preferential oxidation of a dietary excess of long chain monoenoic acids by liver peroxisomes has been proposed (41). At the same time it has been claimed that perixosomal oxidation of fatty acids in liver is quantitatively much less important than mitochondrial oxidation (42).

While rainbow trout fed diets rich in wax ester-containing zooplankton have an active peroxisomal fatty acid oxidation pathway in their livers, the pathway is no more active than in the livers of fish fed commercial diets lacking 22:1 fatty acid, i.e. feeding large quantities of 22:1 units to fish in the form of wax esters does not induce peroxisomal fatty acid oxidation in the liver (43). At the same time fish fed the wax ester-rich diet showed a markedly selective catabolism of fatty acids in the order 22:1>20:1>14:0, while the more usual 16:0 and 18:1 were selectively retained (43). Selective catabolism of 22:1 fatty acid by the peroxisomal pathway in organs other than liver, e.g. intestine, or by other mechanisms remains to be studied.

The utilisation of wax esters during the formation of reproductive tissue in female calanoids mentioned earlier involves *a priori* an extensive catabolism of 22:1(n-11). A similar extensive catabolism of 22:1(n-11) appears to occur in capelin prior to spawning (44). These fish approach their spawning grounds with oil contents in the region of 10-15% of their weights, such oil commonly containing around 20% of its total fatty acids as 22:1(n-11). Prior to spawning the oil content of the fish has decreased to 2-3% of their body weight; the roe produced ultimately by the fish has lipid which contains only 4% of its total fatty acids as 22:1(n-11). It can be concluded confidently that a massive and probably selective catabolism of 22:1(n-11) has occurred prior to spawning. Thus the large stores of lipid in zooplankton and zooplanktonivorous fish, both rich in 22:1(n-11), appear to be catabolised to a surprisingly large extent in the provision of metabolic energy during the elaboration of female reproductive tissue. The endocrinological controls that undoubtedly underlie this process remain to be investigated.

REFERENCES

1. A.A. Benson, R.F. Lee and J.C. Nevenzel, *Biochem. Soc. Symp.* 35, 175-187 (1972).
2. J.R. Sargent, R.F. Lee and J.C. Nevenzel, in The Chemistry and Biochemistry of Natural Waxes (ed. P. Kolattukudy), pp. 50-91, Elsevier (1976).
3. J.R. Sargent, *Sci. Progr.* Oxford 65, 437-458 (1978).
4. N.J. Antia, R.F. Lee, J.C. Nevenzel and J.Y. Cheng, *J. Protozool*, 21, 768-771 (1974).
5. N.W. Withers and J.C. Nevenzel, *Lipids*, 12, 989-993 (1977).
6. R.F. Lee and J. Hirota, *Limnol. Oceanogr.* 18, 227-239 (1973).
7. R.F. Lee, J.C. Nevenzel, G.A. Paffenhofer, *Naturwissenschaften* 59, 406-411 (1972).
8. R.F. Lee, *Mar. Biol.* 26, 313-318 (1974).
9. N.R. Bottino, *Comp. Biochem. Physiol.* 50B, 479-484 (1975).
10. A. Clarke, *J. Exp. Mar. Biol. Ecol.* 43, 221-236 (1980).
11. R.G. Ackman, C.A. Eaton, J.C. Sipos, S.N. Hooper and J.D. Castell, *J. Fish. Res. Bd.* Canada 27, 513-533 (1970).
12. J.R. Sargent and S. Falk-Petersen, *Mar. Biol.* MS submitted (1980).
13. A. Clarke, personal communication.
14. J. Mauchline and L.R. Fisher, *Adv. Mar. Biol.* 7, 1-454 (1969).
15. S. Falk-Petersen, R.R. Gatten, J.R. Sargent and C.C.E. Hopkins, *J. Exp. Mar. Biol. Ecol.* MS submitted (1980).
16. J.K. Volkman, R.R. Gatten and J.R. Sargent, *J. Mar. Biol. Assn. U.K.* 60, 623-632 (1980).
17. R.J. Henderson and J.R. Sargent, *Mar. Biol.* 56, 1-6 (1980).
18. R.F. Lee, *Comp. Biochem. Physiol.* 51B, 263-266 (1975).
19. R.J. Henderson and J.R. Sargent, unpublished data (1980).
20. J.R. Sargent and R.F. Lee, *Mar. Biol.* 31, 15-23 (1975).
21. J. Avigan and M. Blumer, *J. Lipid Res.* 9, 350-352 (1968).
22. R.F. Lee, J.C. Nevenzel and A.G. Lewis, *Lipids*, 9, 891-898 (1974).
23. R.F. Lee, J. Hirota, J.C. Nevenzel, R. Sauerheber and A.A. Benson, *Calif. Mar. Res. Comm. Cal Cofi Rept.* 16, 95-102 (1972).
24. R.R. Gatten, J.R. Sargent, T.E.V. Forsberg, S.C.M. O'Hara and E.D.S. Corner, *J. Mar. Biol. Assn. U.K.*, 60, 391-399 (1980).
25. U. Båmstedt, *Mar. Biol.* 54, 135-142 (1979).
26. R.F. Lee and J.C. Nevenzel, *J. Fish Res. Bd. Canada* 36, 1519-1523 (1979).
27. K. Wiborg, personal communication.
28. R.R. Gatten and J.R. Sargent, unpublished observation.
29. R.F. Lee and P.M. Williams, *Naturwissenschaften* 61, 505-506 (1974).
30. J.R. Sargent, R. McIntosh, A. Bauermeister and J.H.S. Blaxter, *Mar. Biol.* 51, 203-207 (1979).
31. J.S. Patton, J.C. Nevenzel and A.A. Benson, *Lipids* 10, 575-583 (1975).
32. J.S. Patton and A.A. Benson, *Comp. Biochem. Physiol.* 52B, 111-116 (1975).
33. A.E.M. Bauermeister and J.R. Sargent, *Biochim. Biophys. Acta* 575, 358-364 (1979).
34. A.E.M. Bauermeister, B.J.S. Pirie and J.R. Sargent, *Cell Tissue Res.* 200, 475-486 (1979).
35. J.L. Beare-Rogers, *Prog. Chem. Fats Other Lipids* 15, 29- (1977).
36. J.C. Pascal and R.G. Ackman, *Chem. Phys. Lipids* 16, 219-223 (1976).
37. R.G. Ackman and R. Hardy, *Proc. Int. Conf. Fish Sci. Technol.* July 1979, Aberdeen, *in press*.
38. E.N. Christiansen, M.S. Thomassen, R.Z. Christiansen, H. Osmundsen and K.R. Norum, *Lipids* 14, 829-835 (1979).
39. R.Z. Christiansen, E.H. Christiansen and J. Bremer, *Biochim. Biophys. Acta* 573, 417-429 (1979).
40. C.E. Neat, M.S. Thomassen and H. Osmundsen, *Biochem. J.* 186, 369-371 (1980).
41. H. Osmundsen and C.E. Neat, *FEBS Letters*, 107, 81-85 (1979).

42. G.P. Mannaerts, L.J. Debeer, J. Thomas and P.J. DeSchepper, J. Biol. Chem. 254, 4585-4595 (1979).
43. R.J. Henderson and J.R. Sargent, unpublished data.
44. P.M. Jangaard. Bulletin of Fish. Res. Bd. Canada No. 186 The Capelin (1974).