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Biosynthesis of Wax Esters in Fish. Metabolism of Dietary Alcohols*

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ABSTRACT: Dietary fatty alcohols are incorporated by the gourami (*Trichogaster cosby*) as alcohols and acids into the roe wax esters. The course of this incorporation was studied by feeding 1-^{3}H- and 1-^{3}H,U-^{14}C-labeled palmityl and oleyl alcohols and by analyzing the lipids 24 hr after ingestion. Levels of incorporation into roe wax esters were 15–60% of ^{14}C but only 0.5–4% of ^{3}H that had been offered. The ratios ^{3}H/^{14}C in the alcohols of those wax esters showed that the dietary alcohols had undergone extensive oxidation to the corresponding fatty acids and were then reduced again for esterification. Some direct esterification of dietary alcohols is indicated by a small amount of tritium in position 1 of the alcohols recovered but it did not exceed 16% of the ^{14}C-labeled chains that were found as alcohols in the wax esters. Direct esterification may occur to a relatively greater extent in body wax esters which are present only in trace amounts. There an enrichment of ^{3}H compared to ^{14}C was observed which remains unexplained. Some of the tritium derived from labeled dietary alcohols is used for reduction of fatty acids since it is found in position 1 of alcohols other than the ones fed. Amounts of tritium similar to those in roe wax esters have entered the glycerol moiety of body triglycerides and phosphatidylcholines, likely by reduction of a triose phosphate. Very little tritium has been used for synthesis of lipid chains.

The roe lipids of the opaline gourami (*Trichogaster cosby*), a tropical freshwater fish, consist mainly of wax esters, whereas the body lipids contain mainly triglycerides (Sand and Schlenk, 1969). We have previously shown that dietary fatty alcohols and acids are efficiently incorporated and interconverted in this fish (Sand *et al.*, 1969). However, those experiments had been carried out with ^{14}C-labeled compounds and they did not indicate the pathways of oxidation and reduction that may be involved when dietary alcohols are incorporated into wax esters. Part of the alcohols was oxidized to acids which were found in wax esters and other lipids but part of them was found as alcohols in the roe wax esters. This latter portion may have been esterified directly or may first have been oxidized to acid and then reduced again to alcohol for esterification. Possibly both of these pathways were concurrently active in the formation of wax esters in opaline gourami.

In order to evaluate these possibilities, palmityl and oleyl alcohols labeled with ^{3}H in position 1 were fed to gouramis either as such or together with U-^{14}C-labeled alcohols. After 24 hr the fish were sacrificed and their lipids analyzed. In roe, the level of ^{3}H in the alcohol bound as wax esters showed that nearly all dietary alcohol had been oxidized and then part of it reduced again to alcohol for esterification. However, some direct esterification of dietary alcohols had taken place since part of the tritium in wax esters was in position 1 of the alcohol that had been fed.

The body lipids of gouramis contain a very small amount of wax esters, and direct esterification may play a greater role in their formation as indicated by the higher specific activity of tritium. Significant amounts of tritium were found in the glycerol moiety of triglycerides and phosphatidylcholines. Very little tritium was in alkyl chains such as 16:0 and 18:1 which the fish can synthesize *de novo*.

Materials and Procedures

Compounds. [1-$^{3}H_2$]Palmityl and oleyl alcohols were prepared from the acid methyl esters (purchased from The Hormel Institute Lipids Preparation Laboratory) by reduction with [^{3}H]$LiAlH_4$ (New England Nuclear Corp., Boston, Mass.). The alcohols were purified by column chromatography on SiO_2 and recrystallized twice from ethanol without

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change of their specific activity. Palmitic acid prepared from [1-$^3H_2$]palmityl alcohol by oxidation with chromic acid (Meakins and Swindells, 1959) had 0.2% of the original molar activity. Stearic acid, prepared from [1-$^3H_2$]oleyl alcohol by oxidation after hydrogenation of the double bond had 0.4% of the original molar activity. Acetylation of alcohols and reconversion of the acetates into alcohols yielded compounds having >95% of the molar activities of the starting material. These tests proved the specific position of the tritium label and showed that the procedures used for analyses are applicable without misleading effects.

[U-^{14}C]Palmityl and oleyl alcohols were prepared from the labeled acid methyl esters which we had available from earlier experiments (Gellerman and Schlenk, 1965). The 1-^{3}H- and U-^{14}C-labeled alcohols were mixed for dual-label experiments.

Feeding and Fractionation of Lipids. Mature female gouramis about 8-months old were kept, three fish in one tank, as previously described (Sand *et al.*, 1969). They were offered, together with the food, the following labeled alcohols (dpm): (1) 18.7 mg of [1-$^3H_2$]16:0 (33.3×10^6), (2) 8.8 mg of [1-$^3H_2$]18:1 (46.7×10^6), (3) 8.0 mg of [1-$^3H_2$,U-^{14}C]18:1 (^{3}H, 42.8×10^6; ^{14}C, 1.0×10^6), (4) 18.1 mg of [1-$^3H_2$,U-^{14}C]16:0 (^{3}H, 28.1×10^6; ^{14}C, 10.7×10^6), and (5) 15.4 mg of [1-$^3H_2$,-U-^{14}C]18:1 (^{3}H, 16.6×10^6; ^{14}C, 6.4×10^5).

The fish were sacrificed 24 hr after feeding and their egg and body lipids were extracted separately with $CHCl_3$–CH_3OH (2:1, v/v). The lipids (~500 mg) were fractionated by column chromatography on silicic acid (Sand and Schlenk, 1968). Effluents were monitored by thin-layer chromatography and by counting of aliquots. Appropriate fractions were combined, weighed, and their specific activities determined.

Analysis of Lipids Classes. Wax esters (~400 mg) obtained by column chromatography from egg lipids contained ~1% cholesteryl esters and traces of hydrocarbons. Aliquots (~200 mg) of this material were transesterified in methanol–14% BF_3 (Applied Science Labs, College Park, Pa.) by heating in sealed ampoules to 100° for 1 hr under frequent shaking. The resulting methyl esters, alcohols, and traces of cholesterol and and hydrocarbons were separated by thin-layer chromatography on silica gel G, 30 g on plates of 20 × 20 cm size (Schmid *et al.*, 1967) using hexane–diethyl ether–acetic acid (85:15:1, v/v) as solvent. The bands containing esters and alcohols were extracted with chloroform. Aliquots of the alcohols (~75 mg) were acetylated with acetic anhydride in sealed ampules at 100° for 1 hr. Excess acetic anhydride was removed with a stream of nitrogen at 80° under addition of methanol. The alcohol acetates were purified by thin-layer chromatography before counting and further analyses.

Column chromatography of body lipids yielded wax esters as mixtures with more than 50% cholesteryl esters and traces of hydrocarbons (~7 mg of mixed fraction from 500 mg of lipids). Simple model mixtures in particular of saturated wax esters and cholesteryl esters can be separated satisfactorily on SiO_2 plates or glass fiber paper (Type SA, Gelman Instrument Co., Ann Arbor, Mich.) with tetrachloroethylene–benzene (1:1, v/v) as developing solvent. However, the natural mixtures were not well enough separable for analysis of pure wax esters and cholesteryl esters. Therefore, these mixtures were subjected to methanolysis and subsequent separation by thin-layer chromatography. In addition to thin-layer chromatography, but with little difference in results, chromatography on glass fiber paper was applied to separate the methanolysis products. The chromatograms were cut into 15–18 segments of 1-cm length and these were counted to obtain the relative radioactivity of the three components (Graff *et al.*, 1967).

TABLE I: Major Classes of Lipids in Gourami.[a]

	Roe	Body
Wax ≫ cholesterol esters	86.2	
Wax < cholesterol esters		<1
Triglycerides	0.8	79.3
Intermediate fraction[b]	5.1	6.3
Phosphatidylethanolamine	2.7	7.5
Phosphatidylcholine	5.2	7.8

[a] Weight per cent, by column chromatography. [b] Composition of the intermediate fractions, see text.

Recoveries of radioactivity from the chromatograms were >90% when 1 ml of methanol was used per segment for eluting the lipids before adding the scintillation fluid.

Triglycerides of body lipids were transesterified in methanol–5% HCl in sealed ampoules as described before. The reaction mixture was poured into water and the methyl esters extracted with hexane. For determination of radioactivities in the glycerol moiety, methanol and HCl were removed from the transesterification mixture by a stream of nitrogen and eventually under vacuum. Glycerol and fatty esters were then separated by distribution between a small amount of water and hexane. After repeated extraction of the phases, water was evaporated under addition of methanol which was used also for transfers. The crude glycerol was acetylated as described for fatty alcohols. After purification by preparative gas–liquid chromatography ~25 mg of triacetin was obtained from 400 mg of triglycerides.

Phosphatidylethanolamine and -choline from column chromatograms were identified by thin-layer chromatography on silica gel with $CHCl_3$–CH_3OH–NH_3 (15% in H_2O) (65:35:4, v/v) as solvent. Fatty acid methyl esters and triacetin were prepared from phosphatidylcholine as described for triglycerides.

Gas–Liquid Chromatography. Analysis and collection of fatty acid methyl esters and of alcohol acetates were carried out as previously described (Sand and Schlenk, 1969). Preparative purification of triacetin was carried out on a column (0.5 cm i.d. × 122 cm long) of 15% cycloheptaamylose valerate on Chromosorb W, 60–80 mesh (Johns-Manville Product Corp., Chicago, Ill.), at 233° (Schlenk and Sand, 1967). The purity of triacetin was ascertained by chromatography on a column (0.4 cm i.d. × and 185 cm long) of ethylene glycol succinate, 17% on Chromosorb W, 80–100 mesh, at 185°.

Radioactive Counting. All samples were counted in a Tri-Carb 314 EX scintillation counter in toluene containing 0.5% 2,5-diphenyloxazole–0.05% 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene (Packard Instrument Co., Inc., Downers Grove, Ill.). Many of the dual-labeled samples had ratios of $^3H/^{14}C$ outside the range which is acceptable for simultaneous counting (Okita *et al.*, 1957). In such cases, the counts were corrected by reference to a series of known mixtures containing [1-^{3}H]oleyl alcohol and methyl [U-^{14}C]oleate with activities similar to those of the samples to be analyzed.

Separate counting of ^{3}H and ^{14}C, as [^{3}H]H_2O and [^{14}C]CO_2 after combustion, is the method of choice when their ratios vary drastically within one experiment. Some of our samples were analyzed by the combustion method (Packard Tri-Carb sample oxidizer, Model 300) and the results are listed in the tables. Comparison shows that the simultaneous counting

TABLE II: Alcohol and Acid Composition of Lipid Classes.[a]

Chain	Alcohols from Roe Wax	Alcohols from Body Wax	Acids from Roe Wax	Acids from Body Wax + Cholesteryl Esters	Acids from Body Triglycerides	Acids from Roe PE	Acids from Body PE	Acids from Roe PC	Acids from Body PC
14:0	4.0	2.1	0.9	1.4	2.1	0.6	0.2	1.2	0.8
16:0	49.5	47.8	2.4	14.8	25.0	13.4	9.5	26.6	29.6
16:1	4.9	3.3	7.7	6.3	5.3	1.2	1.0	2.4	1.8
18:0	9.0	10.1	0.5	3.4	5.5	17.9	20.6	7.3	5.7
18:1	24.7	22.5	49.8	41.5	37.4	13.0	11.2	15.2	18.4
20:1	2.4	4.3	+	+	+	+	+	+	+
Polyunsaturated ω6	2.1	1.4	22.3	18.5	14.8	13.5	18.2	17.4	16.8
Polyunsaturated ω3	3.0	7.6	16.1	12.7	9.5	39.8	38.7	29.3	26.1

[a] Percentages of gas–liquid chromatographic recorder peak areas using a flame ionization detector. Data from expt 3.

method led essentially to the same results with the inconvenience of the appropriate reference mixtures.

Results

Roe and body of the gouramis contained about 0.3 and 0.4 g of lipids, respectively, per fish and the wax esters of the roe represented one-third of the total lipids. Typical proportions of lipid classes are given in Table I. Wax esters, cholesteryl esters, and traces of hydrocarbons are found in the least polar fraction of column chromatograms. The hydrocarbons of some of these fractions were isolated by thin-layer chromatography and were tentatively identified by gas–liquid chromatography as *n*-nonacosane with minor amounts of homologs. They did not show any significant radioactivity.

Intermediate fractions (Table I) were analyzed by preparative thin-layer chromatography. From roe lipids they contained fatty acids, 0.8%; alcohols, 0.5%; cholesterol, 1%; triglycerides, <1%; and other components migrating similar to diol lipids, 2% relative to the total lipids. The composition of intermediate fractions from body lipids was similar, except for higher values of cholesterol and triglycerides. All triglycerides of roe lipids were collected in this mixture whereas the large amount of triglycerides from body lipids was obtained pure with little trailing into the intermediate fraction.

Analyses of roe and body PC and PE,[1] and of body wax esters from gourami (Table II) have not been previously reported. The composition of alcohols in body and in roe wax esters is similar, with 16:0 and 18:1 being predominant. They contain some 20:1 alcohol but the corresponding acid occurs only in traces. Comparison of acids from the two sources is limited by the presence of relatively large amounts of cholesteryl esters in the fraction from body lipids. This fraction contains about 15% palmitic acid which may be bound in wax esters and/or cholesteryl esters, whereas roe wax esters contain an unusually small amount of this acid. Palmitic acid occurs in other lipid classes at levels from 10 to 30%.

Radioactivities of roe and body lipids are listed in Table III. Levels of incorporation are drastically different for ^{3}H and ^{14}C, *e.g.*, 6.2% *vs.* 68.2% in expt 3. Tritium occurs not only in lipids with alcohols as constituents but also in other lipid classes. Most of the remaining tritium is in roe wax esters and in body triglycerides but the specific activities of these major portions are lower than those of the phospholipids and in particular of body wax esters. Preparative thin-layer chromatography as well as glass paper chromatography showed that tritium in the intermediate fractions was associated with free alcohols and triglycerides.

Interesterification of the wax esters and subsequent procedures revealed that tritium is mainly in their alcohol moieties (Table IV). Distribution of ^{14}C between alcohols and acids is similar to that previously reported for 16:0 and 18:1 chains (Sand *et al.*, 1969). The location of tritium in roe wax alcohols was determined in samples from expt 2 and 3 (Table V). By gas–liquid chromatography and counting of the alcohol acetates it was ofund that tritium was at highest level in C_{18} alcohols. Chemical oxidation of the alcohols and analysis of the resulting acids as methyl esters showed that most tritium of C_{18} and some of the other alcohols was lost and, therefore, had been in position 1.

The location of tritium in body triglycerides (see Table III) was determined by preparing triacetin and the fatty acid methyl esters (Table VI). Virtually all tritium was found in the glycerol, whereas hardly any ^{14}C had entered that part of the triglycerides. The same difference in distribution of ^{3}H and ^{14}C was qualitatively demonstrated for PC and it also appears likely for PE.

Discussion

Palmityl and oleyl alcohols are the major alcohol components of roe wax esters and, therefore, were chosen for this study. When the alcohols tritiated in position 1 were fed, less than 0.5 and 2.5%, respectively, of the radioactivity offered to the fish was found in these waxes (Table III, expt 1 and 2). However, U-^{14}C-labeled palmityl and oleyl alcohols had been incorporated previously at levels 10–20 times higher (Sand *et al.*, 1969). These differences in incorporation of ^{3}H and ^{14}C are verified by use of dual-labeled alcohols (expt 3–5). The loss of ^{3}H is much greater than that of ^{14}C and it proves that

[1] The abbreviated form of the Geneva nomenclature is used for description of alcohol and acid chains; *e.g.*, 16:0 for palmityl alcohol or palmitic acid, 18:1 for oleyl alcohol or oleic acid. Abbreviations used are: PE, phosphatidylethanolamines; PC, phosphatidylcholines.

most of the alcohol in roe wax esters had been oxidized and then reduced again for esterification.

Biological oxidation of dietary alcohol prior to its incorporation as alcohol explains some results from previous experiments where [U-^{14}C]16:0 and 18:1 had been fed as alcohols and as acid methyl esters. The respective chains were found in wax esters at proportions alcohol–acid, 10/1 for 16:0 and 3/2 for 18:1 regardless whether given as alcohol or as methyl ester. The present experiments demonstrate efficient oxidation and reduction. Thus the form administered has little influence on the final proportion of alcohol and acid.

Incorporation of ^{14}C. It had been observed previously with the gourami that chain conversions, in particular of 18:1, do not occur to a significant extent. The same inertia to conversions was indicated here when 93 and 98% of ^{14}C in roe wax esters was found in C_{18} chains (Table V, expt 3). There is no doubt that this radioactivity is in 18:1 rather than 18:0. Palmitic acid may undergo conversions to a slightly greater extent than oleic. However, 24 hr after feeding [U-^{14}C]16:0 alcohol or acid not more than 7% ^{14}C had been found in other chains (Sand *et al.*, 1969). The present experiments were carried out over the same time period and one can accept that ^{14}C essentially represents the 16:0 chain in which it had been administered.

The chains of palmityl and oleyl alcohols are incorporated more in roe wax esters than in body triglycerides (Table III) although these lipids are about equal in amount. In roe wax esters, the ratios alcohol/acid are 6.5/1 for 16:0 and 0.5/1 for 18:1 whereas the ratios are about equal, 2/1, for both structures in the body wax fraction. The difference may be due to the presence of acids from cholesteryl esters in the latter but may also be the consequence of a different route in the synthesis of body wax esters.

Incorporation of ^{3}H. Tritium had been specifically in position 1 of the dietary alcohols and most of it was lost without degradation of the chains (Table III). From the distribution of the tritium retained it is evident that indirect pathways play a relatively greater role for incorporation of ^{3}H than of ^{14}C. Tritium may enter the lipids by direct esterification of the labeled alcohol; or it may have been abstracted from the alcohol group and reused for hydrogenations or possibly hydrations. An aldehyde-type intermediate as highest oxidation step before incorporation as alcohol would combine both preservation *in loco* and transfer to other compounds. However, there was no indication for aldehydes, free or bound, with particularly high specific activity. Also in view of the equal results from dietary alcohols and acids having the same chain structure there is no reason to postulate an aldehydic compound as more than a transitory entity which remains bound during the oxidation and reduction of alcohol and acid.

Direct esterification of dietary alcohol to wax esters preserves tritium in position 1. Such tritium is removed when the alcohol is chemically oxidized to acid and it can be indirectly quantified this way. From these results (Table V) and from the composition of the alcohols (Table II), one can specify the distribution of tritium in the wax esters (Table VII). The ratio of 1-^{3}H/U-^{14}C in C_{18} alcohols is 8150/1200 dpm per mg (derived from data of Table V), *i.e.*, 7.1/1 whereas it had been 42.8/1 (expt 3) in the oleyl alcohol fed. Therefore, only 16.6% of the ^{14}C-labeled 18:1 alcohol in roe wax has been esterified directly. The true value must be slightly lower since some tritium has been reused for reduction of acids including 18:0 and 18:1. The results with palmityl alcohol are in principle the same (Tables III and IV). Less tritium has been retained from palmityl than from oleyl alcohol although both

TABLE III: Incorporation of Radioactivities.[a,b]

Alcohol Fed[c]	[^{3}H]16:0 (Expt 1)		[^{3}H]18:1 (Expt 2)		[^{14}C-^{3}H]18:1 (Expt 3)				[^{14}C,^{3}H]16:0 (Expt 4)				[^{14}C,^{3}H]18:1 (Expt 5)			
					Roe		Body		Roe		Body		Roe		Body	
Radioactivity Recovered in	Roe ^{3}H	Body ^{3}H	Roe ^{3}H	Body ^{3}H	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C
Total Lipids	0.7	3.3	3.0	1.5	4.7	61.8	1.5	6.4	0.8	15.4	2.6	14.1	2.6	27.9	5.0	23.2
Wax ≫ cholesterol esters	0.46		2.40		3.92	58.8			0.49	13.6			1.85	24.22		
													2.46	*24.0*		
Wax < cholesterol esters		0.57		0.17			0.15	0.29			0.42	0.42			1.56	1.76
Triglycerides		1.75		0.62			0.48	3.40			1.51	10.7			2.11	17.55
							0.52	*2.98*							*3.99*	*17.8*
Intermediate	0.12	0.59	0.17	0.04	0.25	1.79	0.10	0.44	0.13	0.96	0.26	1.16	0.40	2.79	0.67	1.62
Phosphatidylethanolamines	0.03	0.09	0.11	0.11	0.15	0.31	0.12	0.37	0.05	0.18	0.08	0.35	0.13	0.25	0.14	0.46
Phosphatidylcholines	0.09	0.30	0.30	0.57	0.38	0.99	0.65	1.88	0.13	0.63	0.33	1.44	0.22	0.64	0.52	1.81

[a] Per cent of radioactivity offered to the fish. [b] Data in italics are by separate counting of isotopes after combustion. [c] Labeled 1-^{3}H or 1-^{3}H,U-^{14}C.

TABLE IV: Radioactivity in Alcohols and Acids of Wax Esters.[a,b]

Wax Origin	Component[c]	Fed 18:1 (Expt 3)		Fed 16:0 (Expt 4)		Fed 18:1 (Expt 5)	
		^3H	^{14}C	^3H	^{14}C	^3H	^{14}C
Roe	Alcohols	92.4	30.0	99.0	85.6	93.4	33.8
		90.0	*31.1*			*84.1*	*35.4*
	Acids	7.6	70.0	0.7	13.4	6.6	66.2
		10.0	*68.9*			*15.9*	*64.3*
Body	Alcohols			89.8	67.4	94.5	59.5
	Acids[d]			8.5	30.6	4.0	38.7

[a] Per cent of radioactivities in roe or body wax fractions. [b] Data in italics are by separate counting of isotopes after combustion. [c] Radioactivities in the front line of chromatograms and in cholesterol were <2%. [d] Includes acids from cholesterol esters.

TABLE V: Radioactivities in Chains of Roe Wax Esters.[a]

Chain Length	Alcohol	Oxidized Alcohol[b]	1-^3H in Alcohol	Wax Acid
	Fed [1-^3H]18:1 (Expt 2)			
C_{14}	670	200	470	830
C_{16}	670	490	180	620
C_{18}	6170	210	5960	240
C_{20},C_{22}	930	200	730	240

	Alcohol		Oxidized Alcohol[b]		1-^3H in Alcohol	Wax Acid	
	^3H	^{14}C	^3H	^{14}C		^3H	^{14}C
	Fed [1-^3H,U-^{14}C]18:1 (Expt 3)						
C_{14}	1050	30	400	80	650	920	170
C_{16}	1080	20	850	20	230	780	110
C_{18}	8470	1200	320	1100	8150	1100	1400
C_{20},C_{22}	1270	320	400	200	870	300	170

[a] Disintegrations per minute per milligram. [b] Measured as methyl esters after hydrogenation and separation by gas–liquid chromatography.

TABLE VI: Radioactivities in Body Triglycerides.[a,b]

	Fed [^3H]16:0 (Expt 1)	Fed [^3H]18:1 (Expt 2)
	^3H	^3H
Triglycerides	1790	270
Acids	33	9
Glycerol[c]	1690[d]	252

	Fed [^3H,^{14}C]18:1 (Expt 3)		Fed [^3H,^{14}C]16:0 (Expt 4)		Fed[^3H,^{14}C]18:1 (Expt 5)	
	^3H	^{14}C	^3H	^{14}C	^3H	^{14}C
Triglycerides	190	36.5	820	2530	410	1540
	192	*29.7*			*720*	*1460*
Acids	6.3	8.8		820		510
	6.3	*7.7*	*53*	*754*	*70*	*463*
Glycerol[c]	174.7	0.3	920	19	440	11

[a] Disintegrations per minute per micromole; the average molecular weight of the fatty acids is 284. [b] Data in italics are by separate counting of isotopes after combustion. [c] Weighed and counted as triacetin, except where specified differently. [d] Calculated by subtraction.

TABLE VII: Distribution of Tritium in Roe Wax Esters.[a]

Fed	[1-^{3}H]18:1 (Expt 2)	[1-^{3}H,U-^{14}C]-18:1 (Expt 3)
^{3}H in roe wax esters	2.40	3.92
1-^{3}H in C_{18} alcohols	1.85	2.95
1-^{3}H in other alcohols	0.12	0.19
^{3}H in alcohol chains except position 1	0.31	0.64
^{3}H in acid chains	0.12	0.15

[a] Per cent of ^{3}H offered to the fish.

chains have been incorporated as alcohol in about equal amounts. The distribution and position of tritium has not been determined in experiments with palmityl alcohol but all other data indicate that the extent of direct esterification is less with palmityl than with oleyl alcohol. As in other conversions of lipids, the enzymatic reactions involved here seem to have a selectivity depending on the structure of the chains.

Tritium of body wax esters is mainly in the alcohols (Table IV) as it had been observed for the roe wax esters. One may assume that also in body wax alcohols the greater portion of the tritium is in the original alcohol. According to the data presently available (Tables III and IV, expt 4 and 5), tritium has been enriched in alcohols by about 30% in reference to ^{14}C. This appears unlikely but a similar enrichment of tritium has been reported when dual-labeled lipids were used as precursors in the formation of glyceryl ether lipids and several possibilities have been considered to explain the unexpected phenomenon (Thompson, 1966; Friedberg and Greene, 1967). For the present case one can merely conclude that the data indicate a greater role of direct esterification for body than for roe wax.

A relatively large percentage of lipid-bound tritium is found in the glycerol moieties of triglycerides and PC (Tables III and V). Most likely, tritium has entered the glycerol by reduction of a derivatized triose. Reduction of dihydroxyacetone phosphate with NADH (Kornberg and Price, 1953) and of acyldihydroxyacetone phosphate with NADPH (Hajra and Agranoff, 1968; Agranoff and Hajra, 1971) are well-established routes to phosphatidates and triglycerides. Also, glyceraldehyde has been suggested as possible precursor (Rao *et al.*, 1968; Antony *et al.*, 1969). Any of these versions would explain the tritium label in the glycerol moieties.

The oxidation of fatty alcohols to acids and the reverse reaction have been shown in mammals decades ago by Schoenheimer and his coworkers (Schoenheimer and Hilgetag, 1934; Stetten and Schoenheimer, 1940) and these reactions became of interest again in connection with studies on alkoxyglycerol lipids (Snyder, 1969) and wax esters (Nevenzel, 1970).

For aquatic animals the interconversion of acid and alcohol was demonstrated with dogfish (*Squalus acanthias*) (Malins, 1966), lantern fish (*Latimeria chalumneae*) (Nevenzel and Kayama, 1968), and in particularly high yields with gouramis (Sand *et al.*, 1969). Incubation of [U-^{14}C]palmitic acid with gourami eggs (unpublished data from this laboratory) yielded 10% incorporation into wax esters. The ratios of 16:0 alcohol/acid were 0.2/1 but they changed to 4/1 which is rather close to that encountered *in vivo*, when glucose, glucose-1-P, or similar compounds were added. They may provide with their metabolism NADH and ATP which have been found to play a role for reduction of fatty acids in cell-free systems from Euglena (Kolattakudy, 1970), Clostridium (Day *et al.*, 1970), and mouse preputial gland tumor (Snyder and Malone, 1970). One may surmise that the reduction of acids in gourami eggs proceeds similar as in the foregoing purified systems whereas oxidation of alcohols may take place mainly in the course of intestinal absorption with concurrent formation of triglycerides.

A small portion of the dietary alcohols escapes oxidation and, by mechanisms not yet investigated for the gourami, is esterified directly to appear foremost in body wax esters.

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