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Effect of Temperature Acclimatization on the Fatty Acid Composition of Goldfish Intestinal Lipids

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1. The fatty acid composition of whole goldfish, whole-intestinal mucosa, intestinal mucosal membranes and individual phospholipids extracted from mucosal membranes were measured, fish adapted to different temperatures being used. 2. Alterations of the adaptation temperature did not noticeably affect the fatty acid composition of the whole-fish lipids, but there were marked changes in the fatty acids of lipids extracted from homogenates of goldfish intestinal mucosa. These changes were more pronounced in a membrane fraction prepared from these homogenates. Raising the adaptation temperature by 20°C halved the percentage of $C_{20:1}$, $C_{20:4}$ and $C_{22:6}$ fatty acids and nearly doubled the percentage of $C_{18:0}$ and $C_{20:3}$ fatty acids recovered. 3. Choline phosphoglycerides constituted about one-half and ethanolamine phosphoglycerides about one-quarter of the total membrane phospholipids. 4. The fatty acids of choline and ethanolamine phosphoglycerides were more susceptible to temperature-dependent changes than were the phosphoglycerides of inositol or serine. 5. The increase in $C_{18:0}$ fatty acid that occurred in membranes of warm-adapted fish was greatest for ethanolamine phosphoglycerides, but increases also occurred in other phospholipid fractions and in membrane neutral lipids.

It is now well known that the fatty acid composition of both plant and animal tissues can be modified by a change in environmental temperature, the proportion of unsaturated to saturated fatty acids increasing when the environmental temperature falls (see Hilditch & Williams, 1964). This proportional increase in unsaturated fatty acids will lower the mesomeric transition temperature, and it has been suggested that such an alteration is essential if basal metabolic processes are to function normally at lower environmental temperatures (Chapman, 1969). More detailed experimental information as to how such a mechanism might operate is not available.

The present experiments were carried out to establish whether temperature-dependent changes in fatty acid composition, already described for goldfish brain and muscle (Johnston & Roots, 1964; Knipprath & Mead, 1968), also occur in goldfish intestine. Later work involved the analysis of membranes isolated from homogenates of goldfish intestinal mucosa by a process of differential centrifugation.

Membrane adenosine triphosphatase prepared from homogenates of goldfish intestinal mucosa changes its ability to be activated by $Na^+ + K^+$ in response to a change in environmental temperature, the Mg^{2+}-stimulated adenosine triphosphatase activity increasing and the $Na^+ + K^+$-stimulated triphosphatase activity falling as the environmental temperature is raised (Smith, 1967). Some evidence that phospholipids are involved in this change was provided when phospholipase C was incubated with intestinal membranes prepared from fish adapted to different temperatures (Smith & Kemp, 1969). It was therefore decided to test whether the fatty acid composition of individual phospholipids isolated from these membranes might also change with the environmental temperature of the fish.

EXPERIMENTAL

Fish. Goldfish weighing 40–80g were obtained from Perry's, Enfield, Middx., U.K. They were kept for the first week in aerated water at room temperature and then transferred to smaller acclimatization tanks where the water was maintained at different temperatures. The total period of acclimatization varied between 3 and 5 weeks. The appetite of the goldfish depended on the temperature at which they were kept, warm-adapted fish eating more than cold-adapted fish. Duffields anglers' groundbait (Buxton Distributors Ltd., Buxton, Norwich, U.K.) was given once or, for warm-adapted fish, twice daily in slight excess of their requirements. Goldfish

acclimatized to 14°C or less were kept in a cold-room where the period of artificial light was controlled automatically to coincide with the normal period of daylight. Experiments were carried out in the summer and late autumn and slight differences in the fatty acid composition in the later series of experiments are mentioned in the text where relevant.

Reagents. Reagents were of A.R. grade where available and all solvents were checked for trace impurities that might mimic fatty acid methyl esters on g.l.c. Some solvents were redistilled and reagent-blank samples run in parallel with test samples throughout the procedures outlined below, to check that all but minor contributions to the final g.l.c. analysis were absent. Butylated hydroxytoluene was added to maintain a concentration of approx. 5mg/100ml in all solvent solutions.

Extraction of lipids. (1) Diet. A 1g sample from a finely ground batch of fish food was extracted three times by shaking with 100ml of chloroform–methanol (2:1, v/v). These extracts were pooled after filtration through glass-fibre paper (Whatman GF81) and washed once with 0.25vol. of 0.9% NaCl solution (Folch, Lees & Sloane-Stanley, 1957). The lower phase was dried on a rotary evaporator at 35°C. The lipid was extracted with chloroform and finally stored in benzene solution under N_2 at −16°C.

(2) Carcass. The weighed whole bodies, less the gut, of groups of three specimens of fish were homogenized in 10vol. (v/w) of methanol. A 50ml sample of the homogenate was taken and 100ml of chloroform added. The mixture was filtered and extracted twice more with 100ml of chloroform–methanol (2:1, v/v). The pooled filtered extracts were then treated as described under 'Diet.'

(3) Homogenates and membranes. A 1ml portion of homogenized mucosal scrapings or mucosal membrane fractions (5–10mg of protein in 1 or 2ml of 0.9% NaCl) was extracted three times with 60ml of chloroform–methanol (2:1, v/v). The extracts were filtered and pooled and then treated as described under 'Diet'.

Separation of neutral and phospholipid fractions. The total membrane lipids were applied in the minimum of chloroform to a 3g column of silicic acid (Mallinckrodt, 100–200 mesh) previously washed with 50ml of methanol, 50ml of chloroform–methanol (1:1, v/v) and 50ml of chloroform. Neutral lipids were eluted with 15ml of chloroform, and the phospholipids and any other polar lipids with 15ml of chloroform–methanol (1:1, v/v) followed by 15ml of methanol.

Thin-layer chromatography. The total phospholipid fractions were applied as bands across a plate coated with silica gel H (E. Merck A. G., Darmstadt, Germany) and developed in chloroform–methanol–water (19:7:1, by vol.) in an S-chamber (Parker & Peterson, 1965). Authentic markers of choline, ethanolamine and inositol phosphoglycerides were run at the edges of the plates. The marker lanes and the edges of the test sample were sprayed with ninhydrin to detect amino groups and then with the phospholipid spray of Vaskovsky & Kostetsky (1968). Five bands were scraped off, placed in small glass columns plugged with glass wool and eluted with chloroform–methanol–acetic acid (20:20:1, by vol.). Band 1, the fastest running, probably contained phosphatidic acid, but this was not characterized. Band 2 consisted of ethanolamine phosphoglyceride and band 3 of choline phosphoglyceride. Band 4 was a mixture of serine and inositol phosphoglycerides and band 5 probably contained sphingomyelin and lyso compounds. Since bands 3 and 4 were not always well separated, because of the streaking of inositol and serine phosphoglycerides, they were rechromatographed in chloroform–methanol–water–aq. ammonia (sp.gr. 0.88) (110:50:5:3, by vol.). The eluates were dried in a stream of N_2 and stored in benzene at −16°C.

Preparation of fatty acid methyl esters. Samples freed from solvents were hydrolysed by refluxing with 5ml of 6% (w/v) KOH in 95% (v/v) ethanol. After cooling and the addition of water, unsaponifiable material was extracted with hexane. The solution was acidified with HCl, and free fatty acids were extracted with hexane. The hexane was removed in a stream of N_2, and the fatty acids were methylated with fresh diazomethane.

Gas–liquid chromatography. Fatty acid methyl esters were separated on a column (1.5m×4mm) of 10% poly(ethylene glycol adipate) on Diatomite C (Pye Unicam Ltd., Cambridge, U.K.) with a Pye 104 gas–liquid chromatograph fitted with dual flame ionization detectors. The column was run at 197°C with N_2 as carrier gas. Some 20 major peaks were identified by reference to Burchfield & Storrs (1962) and to known standards.

The areas under the peaks were calculated by the method of Carroll (1961) and expressed as a percentage of the total peak area. Myristic acid was not included since it was obscured by the peak of butylated hydroxytoluene. Runs on Apiezon L indicated that only small amounts of myristic acid were present. No significant peaks were eluted after the $C_{22:6}$ fatty acid.

Preparation of intestinal membranes. Each fish was decapitated, the anterior intestine dissected in a cold-room (6°C), and the mucosa scraped out, weighed and homogenized in 10 vol. (v/w) of ice-cold 0.25M-sucrose solution containing 5mM-EDTA, 60mM-histidine and 0.1% sodium deoxycholate buffered to pH7.1 with HCl. The homogenate was centrifuged in the cold in a Spinco model L preparative ultracentrifuge (rotor AH50) for 15min at 10000*g*. The pellet was rejected and the supernatant centrifuged for a further 60min at 20000*g*. This pellet, washed once in 0.25M-sucrose solution containing 10mM-histidine–HCl, pH7.1, was used for lipid analysis. Previous examination of similar fractions showed them to consist of membrane sheets of different sizes (Smith, Colombo & Munn, 1968). On occasion protein determinations were carried out on these membrane suspensions by the method of Lowry, Rosebrough, Farr & Randall (1951), with crystalline bovine serum albumin as standard.

RESULTS

Fatty acid composition of goldfish diet and of lipids extracted from goldfish carcass and intestine. Warm-adapted goldfish eat more than do cold-adapted fish and the fatty acid composition of food given was therefore determined. The diet contained large amounts of $C_{18:1}$ and $C_{18:2}$ fatty acids and the proportions of both were slightly higher in the carcass of the warm-adapted fish (Table 1). The proportion of $C_{20:4}$ fatty acid was marginally higher

Table 1. *Fatty acid composition of lipids extracted from the carcasses and intestines of goldfish adapted to different temperatures compared with that of lipids extracted from fish food*

Only those fatty acids present in amounts greater than 0.5% of the total amount recovered are shown. Values for whole carcasses are based on samples taken from three fish; those for whole intestines are mean values from two determinations, each determination being made on a pooled sample from three fish. All fish had been adapted to 8 or 30°C for 3–4 weeks.

Material	Fatty acid composition (%)												
	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$	$C_{20:1}$	$C_{20:2}$	$C_{20:3}$	$C_{20:4}$	$C_{22:5}$	$C_{22:6}$
Diet	16.6	0	2.2	22.5	50.5	3.3	3.9	0	0	0	0	0	0
8°C-adapted-fish carcass	21.2	10.9	5.4	30.0	12.3	5.5	0.6	2.3	2.3	0.7	3.1	1.8	1.9
30°C-adapted-fish carcass	17.4	6.2	3.3	37.3	15.8	5.2	0	3.1	1.9	0.7	2.9	0.8	1.9
8°C-adapted-fish whole intestine	20.9	7.5	7.8	21.4	15.4	3.4	1.7	2.7	2.3	2.8	5.7	2.8	4.4
30°C-adapted-fish whole intestine	20.1	5.6	7.0	31.3	15.4	2.9	0.5	2.5	2.3	2.3	3.9	2.5	2.5

Table 2. *Fatty acid composition of membrane fractions prepared from the intestinal mucosa of goldfish adapted to different temperatures*

Only those fatty acids present in amounts greater than 2% of the total recovered are shown, except where lower values complete the series for different adaptation temperatures. Each set of values is based on one analysis of lipids extracted from the intestines of five fish. The amount of myristic acid could not be determined since its retention time coincided with that for butylated hydroxytoluene.

Acclimatization temperature (°C)	Fatty acid composition (%)										
	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{20:1}$	$C_{20:2}$	$C_{20:3}$	$C_{20:4}$	$C_{22:5}$	$C_{22:6}$
3	15.6	2.2	12.4	7.7	14.3	2.2	1.8	3.9	13.7	3.0	18.2
14	19.6	2.2	15.3	7.1	12.9	1.8	4.1	4.5	13.0	4.3	11.7
23	17.8	1.2	19.1	7.9	15.9	1.5	5.1	5.4	8.5	3.6	10.5
32	17.3	0.9	19.5	11.9	21.1	1.2	4.2	6.4	6.0	2.9	5.5

in the cold-adapted fish. An analysis of goldfish muscle by Knipprath & Mead (1968) showed the predominant fatty acid to be $C_{18:1}$ (cold-adapted, 45.3%; warm-adapted, 37.4%) with $C_{18:2}$ 7.5% (cold-adapted) and 9.6% (warm-adapted) of the total recovered, the amount of $C_{20:4}$ fatty acid being actually higher in the warm-adapted fish (1.2 and 2.5% respectively). In our experiments the fatty acid composition of the fish as a whole was not changed markedly either by alterations in environmental temperature or by differences in the amount of food eaten by the two groups of fish. The proportion of $C_{18:1}$ fatty acid from intestines of 30°C-adapted fish was higher than that from intestines of 8°C-adapted fish (Table 1). This might reflect the different eating habits of the fish, but the proportions of $C_{18:2}$ fatty acid did not change (15% for intestines of both cold- and warm-adapted fish), though this fatty acid made up half of the total dietary fatty acids. The intestines contained larger proportions of long-chain unsaturated fatty acids than did the carcasses and there was a clear temperature-dependent difference, the intestines from 8°C-adapted fish containing nearly twice as much $C_{20:4}$ and $C_{22:6}$ fatty acids.

Fatty acid composition of goldfish intestinal membranes. The results for fish adapted to 3, 14, 23 or 32°C are given in Table 2. The proportion of an unknown fatty acid (probably a $C_{22:6}$ isomer) that immediately preceded the $C_{22:6}$ peak on elution changed in a similar way to the $C_{22:6}$ fatty acid when the environmental temperature of the fish was altered. The proportion of the unknown was always about one-tenth that of the $C_{22:6}$ fatty acid, and both have been added together to give the values for $C_{22:6}$ fatty acid. The proportion of $C_{20:4}$ plus $C_{22:6}$ fatty acids in membranes prepared from cold-adapted fish was three times that from membranes of warm-adapted fish, and these fatty acids together constituted 32% of the total recovered from membranes of cold-adapted fish. In addition, the proportion of $C_{20:1}$ fatty acid appeared high in membrane fractions prepared from cold-adapted fish. The rise in $C_{20:4}$ and $C_{22:6}$ fatty acids was compensated for by a fall in the amounts of $C_{18:0}$ and $C_{20:3}$ fatty acids recovered. Although the amounts of $C_{18:1}$ and $C_{18:2}$ fatty acids increased by 50% when the the temperature was changed from 3 to 32°C, the probability that this was due to chance, considering all four temperatures (3, 14, 23 and

Table 3. *Fatty acid composition of neutral lipids and phospholipids extracted from membranes prepared from the intestinal mucosa of goldfish adapted to different temperatures*

Each set of values is based on one analysis of lipids prepared from membranes pooled from five fish. Abbreviations: PL, phospholipids; NL, neutral lipids.

Acclimatization temperature (°C)	Type of lipid	Fatty acid composition (%)										
		$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{20.1}$	$C_{20:2}$	$C_{20:3}$	$C_{20:4}$	$C_{22:5}$	$C_{22:6}$
3	PL	18.9	2.3	13.0	7.6	13.6	2.6	1.3	3.9	14.0	2.3	15.8
	NL	22.8	6.0	14.4	16.4	17.0	1.6	1.3	2.4	4.3	5.3	3.9
14	PL	21.5	2.1	17.4	7.2	7.0	2.0	4.8	5.1	14.5	4.4	10.9
	NL	10.3	1.5	18.0	18.4	13.5	2.3	3.7	3.9	9.8	4.0	9.3
23	PL	19.8	1.1	19.2	7.0	16.2	1.5	5.2	5.6	8.9	3.0	9.2
	NL	19.6	3.4	17.3	18.0	18.0	1.6	3.3	3.7	4.7	2.7	4.7
32	PL	20.1	0.8	17.8	9.7	24.2	1.0	4.6	7.5	4.9	2.2	4.3
	NL	16.3	2.3	23.3	17.6	14.4	1.3	3.4	4.0	6.3	2.7	5.7

Table 4. *Significance of temperature-dependent changes seen in the fatty acid composition of lipids extracted from goldfish intestinal membranes*

Values of r (correlation coefficient between acclimatization temperature and % of fatty acid recovered) and P (probability that this correlation is due to chance) were calculated from the experimental values for neutral lipids and phospholipids (Table 3) and for total lipids (Table 2).

Fatty acid	Total lipid		Phospholipid		Neutral lipid	
	r	P	r	P	r	P
$C_{18:0}$	+0.97	<0.05	+0.81	>0.20	+0.90	<0.10
$C_{20:1}$	−0.99	<0.01	−1.00	<0.01	−0.40	>0.30
$C_{20:3}$	+0.98	<0.02	+0.97	<0.05	+0.82	>0.10
$C_{20:4}$	−0.96	<0.05	−0.91	<0.10	+0.08	>0.30
$C_{22:6}$	−0.98	<0.05	−0.98	<0.02	+0.15	>0.30

32°C), was still greater than 0.05 and these fatty acids have not been considered further.

Table 3 shows that most of the changes seen in total lipid extracts could be accounted for by changes in the phospholipid fraction. Correlation coefficients between adaptation temperature and percentage of fatty acid recovered were estimated for each fatty acid, and those showing a statistically significant dependence on environmental temperature ($P<0.05$ for total lipids) are compared with others calculated for the same acids in the neutral-lipid and phospholipid fractions in Table 4. The changes in the longer-chain fatty acids ($C_{20:1}$, $C_{20:3}$, $C_{20:4}$ and $C_{22:6}$) were confined to the phospholipids, whereas that in the $C_{18:0}$ took place in the neutral-lipid fraction. This was contrary to other results shown below where individual phospholipids were analysed. In these cases the proportion of phospholipid $C_{18:0}$ fatty acid also increased at a higher adaptation temperature.

The percentages of phospholipid $C_{20:3}$, $C_{20:4}$ and $C_{22:6}$ fatty acids and the neutral-lipid $C_{18:0}$ fatty acid are shown at four different adaptation temperatures in Fig. 1. The $C_{20:4}$ and $C_{22:6}$ fatty acids were present in roughly equal proportions in mucosal membrane phospholipids, and the concentration of each was halved by a 20°C increase in acclimatization temperature. These changes were qualitatively reproducible, but the absolute change for $C_{20:4}$ and $C_{22:6}$ fatty acids appeared to depend on the season of the year (see below). The proportion of $C_{20:3}$ fatty acid was generally low and not so dependent on the environmental temperature of the fish (a 30°C increase in acclimatization temperature approximately doubling the proportion present). It could nevertheless represent a critical point in the regulation of longer-chain fatty acids, an increase in the amount present possibly indicating a diminished ability of the mucosa to put an extra double bond into the C_{20} chain. In the neutral-lipid fraction the proportion of $C_{18:0}$ fatty acid was the only one to vary significantly with the acclimatization temperature of the fish. The statistical significance of this change was low ($P<0.1$), and the

change in proportion of phospholipid $C_{18:0}$ fatty acid (13 to 19% prepared from fish adapted to 3 and 32°C respectively) was not significant.

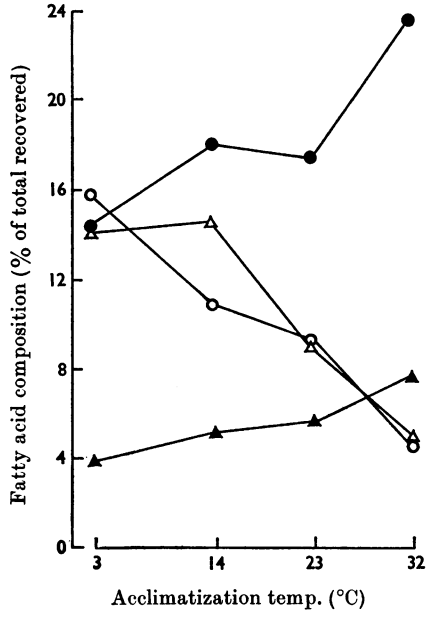

Fig. 1. Percentage recovery of some fatty acids extracted from the intestinal mucosal membranes of goldfish adapted to different temperatures. ●, $C_{18:0}$ fatty acid; △, $C_{20:4}$ fatty acid; ○, $C_{22:6}$ fatty acid; ▲, $C_{20:3}$ fatty acid. The $C_{20:3}$, $C_{20:4}$ and $C_{22:6}$ fatty acids were from a phospholipid fraction and the $C_{18:0}$ fatty acid from a neutral-lipid extract of total lipids. Each point gives the value from one determination with five fish.

The absolute amounts of fatty acids recovered from neutral-lipid and phospholipid extracts were not measured, but g.l.c. of equal amounts of the two fractions showed that the fatty acids were about equally distributed between the two. Fig. 1 shows that a very large percentage of total fatty acids (about 20% of the total) changed with the environmental temperature of the fish and indicates that changes in fatty acid metabolism may be of importance to the function of these epithelial membranes.

Separation and analysis of individual phospholipids from goldfish intestinal membranes. As the changes in fatty acid composition in mucosal membranes were confined largely to the phospholipids, these were extracted from fish acclimatized to 8 or 30°C and were separated by t.l.c. to test whether the changes were common to all phosphoglycerides. The approximate proportions of individual phospholipids/mg of protein, calculated by measuring the total peak areas given by their fatty acids during g.l.c., were found not to vary with the environmental temperature of the fish (Table 5). The percentage of fatty acids that changed with temperature in the different phospholipids is also given. The change in $C_{18:0}$ fatty acid, which had been shown previously to occur in neutral-lipid extracts, was also seen in all the samples of phospholipids analysed. The greatest percentage change in $C_{18:0}$ fatty acid occurred in the ethanolamine phosphoglyceride fraction (nearly a threefold increase for a 22°C rise in adaptation temperature). These phosphoglycerides represent 27% of all phospholipids extracted from the membranes, so that the bulk of the total increase in this fatty acid must also occur in this phospholipid. The same

Table 5. *Analysis of different phospholipids extracted from membrane fractions prepared from goldfish adapted to different temperatures*

Analyses were performed on membranes prepared from four fish (30°C-adapted) or eight fish (8°C-adapted). Abbreviations: PS, PI, PC and PE, phosphoglycerides of serine, inositol, choline and ethanolamine respectively; PX and PY, mixtures of unidentified phospholipids appearing on t.l.c. in regions corresponding to lysolecithin and phosphatidic acid respectively; $T_{accl.}$, temperature of acclimatization.

Phospholipid	$T_{accl.}$ (°C)	% of total phospholipid	Fatty acid composition (%)				
			$C_{18:0}$	$C_{20:1}$	$C_{20:3}$	$C_{20:4}$	$C_{22:6}$
PY	8	8.0	15.2		5.0	3.5	8.7
(band 1)	30	7.8	16.9		6.0	3.1	4.6
PE	8	26.7	10.9	4.2	3.7	13.9	23.3
(band 2)	30	27.3	27.0	1.1	5.2	12.2	12.8
PC	8	54.3	9.4	1.8	7.6	9.7	10.1
(band 3)	30	50.6	11.7	0.8	7.6	7.6	5.2
PS+PI	8	6.3	19.2		8.0	6.5	10.5
(band 4)	30	9.2	32.5		7.6	6.3	11.5
PX	8	4.7	16.7		8.4	3.7	4.1
(band 5)	30	5.0	17.7		7.5	1.4	2.8

can be said for the $C_{20:3}$ fatty acid (a 50% increase for a 22°C rise in adaptation temperature). Ethanolamine phosphoglycerides showed a corresponding fall in the percentage of $C_{22:6}$ fatty acid recovered, the proportion falling from 23 to 13% for a 22°C rise in adaptation temperature. Choline phosphoglycerides showed a similar fall (10 to 5%) in the recovery of this fatty acid. Ethanolamine and choline phosphoglycerides were present in these membranes in the proportion 1:2, so that the total change in unsaturation brought about by the changes in $C_{22:6}$ fatty acid was similar for both phospholipids. The $C_{20:4}$ fatty acid recovered from choline and ethanolamine phosphoglycerides increased in the membranes of cold-adapted fish, but the difference was not as great as seen above for total phospholipids. The large percentage change in this fatty acid, which was found in the PX band, could not explain this difference, since these phospholipids represented only one-twentieth of the total phospholipid content of the membranes. Since the amounts of phospholipids used were always very small we were not able to check that the recoveries after t.l.c. were complete. The $C_{20:1}$ fatty acid was associated with the choline and ethanolamine phosphoglycerides. The percentage was low, but there was in each case a marked change with the environmental temperature of the fish. The percentage at both adaptation temperatures was lower in the choline phosphoglycerides, but the total change in this fatty acid was similar for both phospholipids.

Seasonal variation in the fatty acid composition of goldfish intestinal lipids. The main group of experiments reported in this paper was performed in July 1968. Further experiments in the late autumn, when membrane lipids were again analysed for their constituent fatty acids, showed a lower proportion of $C_{20:4}$ fatty acid in 8°C-adapted fish compared with that for fish adapted to 3 or 14°C in the summer (9% compared with 13%). The proportion of $C_{22:6}$ fatty acid remained high in the fish acclimatized in the autumn (14% in membranes of 8°C-adapted fish compared with 15% for membranes of 3 and 14°C-adapted fish in the summer), and fish acclimatized to 30°C in the late autumn still contained less $C_{20:4}$ and $C_{22:6}$ and more $C_{20:3}$ fatty acids than did 8°C-adapted autumn fish. Seasonal factors or individual variation may be responsible for these changes in the fatty acid pattern of membrane lipids, or a mixture of both effects might have occurred.

DISCUSSION

The goldfish has been used on several occasions to demonstrate changes in fatty acid composition associated with changes in environmental temperature. Fatty acids from the total lipids of whole fish (the present investigation) or muscle (Knipprath & Mead, 1968) show only very small changes in composition with temperature as opposed to total lipid extracts of brain (Johnston & Roots, 1964) and intestinal mucosa (the present work). Although the proportion of phospholipids to neutral lipids varied in the different samples analysed, this could not account for these differences. Phospholipids constitute about 20% of the total lipids in goldfish muscle (Knipprath & Mead, 1968) and about 50% in intestinal mucosal membrane preparations. Only the 'kephalin' of muscle phospholipids changed its fatty acid composition with temperature, and this was not sufficient to change the fatty acid pattern of the total phospholipids. It seems then that the phospholipids of some membranes (e.g. those from the intestinal mucosa) are more labile than others. The reason for this might be connected with the importance of this tissue in allowing the fish to function efficiently at different temperatures.

Fractionation of the phospholipids showed that the most marked changes occurred in the ethanolamine and choline phosphoglycerides (Roots, 1968; the present work). In the intestinal mucosa we found that the phospholipid/protein ratio did not change to any appreciable extent with change in adaptation temperature, nor did the relative amounts of the individual phospholipids. Similar results were reported by Roots (1968). Thus the changes seen here are mediated either by exchange of phospholipid molecules or by partial deacylation and reacylation of the resulting lyso compounds with new fatty acids (Eibl, Hill & Lands, 1969).

It seems at least possible that different tissues, because of the specialized function of their component membranes, might adapt differently to temperature changes. In brain at low temperatures the percentage of $C_{16:1}$ fatty acid increased and that of $C_{18:0}$ acid fell in ethanolamine and choline phosphoglycerides (Roots, 1968). There was no obvious increase in the unsaturation of the C_{20} and C_{22} fatty acids at these low temperatures. In mucosal phospholipids, however, the major changes were found in the long-chain unsaturated acids ($C_{22:6}$, $C_{20:3}$, $C_{20:1}$ and to a variable extent $C_{20:4}$). These tissue-specific differences were very obvious, but it must be remembered that seasonal variation or variation between batches of fish might have been responsible for part of the difference. We did obtain evidence that some changes in fatty acid composition might be due to factors not connected with temperature. It is also surprising that changes in the amounts of long-chain unsaturated fatty acids extracted from brains of cold-adapted fish (Johnston & Roots, 1964) should not be associated with the phospholipids (Roots, 1968). It is difficult to imagine that these fatty acids were found only in the neutral lipids. We have found that these

long-chain fatty acids are very easily oxidized, even when stored in benzene with butylated hydroxytoluene at −16°C under nitrogen. It is conceivable that autoxidation could have altered some of the brain phospholipid fatty acids examined by Roots (1968). Lastly, the mucosal fractions analysed contained mainly microsomal and ruptured microvillar membranes with little contamination from mitochondria, whereas Roots (1968) analysed whole-brain phospholipids. The metabolism of fatty acids and the fatty acid composition of mitochondrial membranes also change with environmental temperature of the fish (Anderson, 1968; Caldwell, 1967) and the nature of these changes might be different in detail from those taking place in the plasma membranes.

It would not be surprising, however, if changes in the fatty acid composition of intestinal plasma membranes did occur more quickly and were more dramatic than in other tissues. The rate of cellular renewal in this tissue is high, particularly in warm-adapted fish (Hyodo, 1965), and the rate of protein synthesis within the mucosa is five times that in liver and 15 times that in brain (M. W. Smith & D. Morris, unpublished work). Warm-adapted fish eat more than cold-adapted fish, and this might independently influence the fatty acid composition of such a metabolically active tissue, but in fact there was no evidence of this. Linolenic acid was the most unsaturated acid found in the diet and only minute traces of fatty acids eluted after $C_{20:0}$ on g.l.c. could be detected, yet it was the proportions of these longer-chain unsaturated fatty acids that were changing with temperature. Apart from maintaining a high rate of metabolism, the intestinal mucosa has to ensure that the rest of the fish continues to receive an adequate supply of nutrients at the different environmental temperatures. Part of this regulation probably involves a reorganization of membrane protein, but there is evidence that the membrane adenosine triphosphatase system behaves differently in membranes containing different fatty acids (Smith & Kemp, 1969) and that the rate at which amino acids pass across the intestine might in part be governed by their lipid solubility in these different fatty acids (M. W. Smith, unpublished work). These facts taken together suggest that membrane lipids might exert a modifying influence on transport processes.

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