Composition of lipid foams from swim bladders of two deep ocean fish species

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ABSTRACT Lipid-containing deposits within the swim bladders of *Coryphaenoides acrolepis* and *Antimora rostrata* were investigated. Lipid analysis of this material, which was quite uniform from the two species, yielded the following data: neutral lipids, 36.0–41.7%; phospholipids, 53.6–56.7%; and glycolipids, 4.3–8.9%. Cholesterol (mainly in the free form) constituted 60.4%–77.8% of the neutral lipids. Sphingomyelin and phosphatidylcholine were the principal phospholipids, with sphingomyelin highest in the material from *C. acrolepis* and phosphatidylcholine predominant in that from *A. rostrata*. The overall pattern of lipids shows a resemblance to that of plasma membrane, particularly in the relatively high levels of free cholesterol, sphingomyelin, and phosphatidylserine. The lipid-to-protein ratio of the material is approximately 1.5–2 to 1. The lipids of the fine inner lining (tunica interna) of the swim bladder from a shallow water fish, the kelp bass (*Paralabrax clathratus*), had essentially the same composition as the much more abundant swim bladder material from the deep ocean fishes.

SUPPLEMENTARY KEY WORDS cholesterol · phospholipids · plasma membrane · glycolipid · sphingomyelin

PHLEGER AND BENSON (1) have reported that swim bladders of certain deep ocean fishes contain a cholesterol-rich substance. They observed that this material from Pacific rattail fish (*Coryphaenoides acrolepis*) and from another species (*Antimora rostrata*) contained as much as 49% cholesterol (dry weight basis) in approximate 1:1 ratio with phospholipid. That this swim bladder lipid has not been reported in fishes living at or near the ocean surface suggests a relation of the lipid to the stresses of depth on fishes. An opportunity to characterize and investigate this swim bladder lipid more extensively was provided during the Guadalupe Island (Mexico) cruise of the research vessel Alpha Helix (Feb.–Mar. 1970).

Abbreviations: TLC, thin-layer chromatography.

MATERIALS AND METHODS

Materials

The two principal species of fishes employed in this study have the following taxonomic positions:

	Antimora rostrata	*Coryphaenoides acrolepis*
Order	Gadiformes	Gadiformes
Suborder	Gadoidei	Macrouroidei
Family	Moridae	Macrouridae
Common name	Flatnose codling	Pacific rattail

The two species of fishes were caught by a technique in which sunken traps or lines were released to the surface from depths of 1300–2700 m. Under these conditions the swim bladder of *C. acrolepis* ruptures, and the lipid-rich substance is found in the body cavity as well as in the swim bladder; the swim bladder of *A. rostrata* remains intact under these conditions. The material within the bladder of both species has the character of a white foam which completely fills the bladder. It is presumed that the foam structure is an artifact of the rapid pressure change produced by bringing the fish to the surface. A deeper-living species of *Coryphaenoides* (*C. abyssorum*) caught in a slowly rising otter trawl was reported[1] to have the lipid in a surface layer coating the inside of the swim bladder. The foam can be easily lifted from the swim bladder with forceps.

A number of surface teleosts living in the vicinity of Guadalupe Island were caught by net or hook and line. These included sheepshead (*Pimelometopon pulchrum*),

[1] Phleger, C. F. 1970. Personal communication.

whitefish (*Caulolatilus princeps*), and two species of bass (*Paralabrax maculatofasciatus* and *P. clathratus*). While examination showed that none of these fishes contained any lipid material within their swim bladders, the delicate innermost membrane, the so-called tunica interna, was removed from the swim bladder of an individual *P. clathratus* for lipid analysis. Swim bladder material was isolated by dissection of the fish and analyzed either immediately upon bringing the fish to the surface or after overnight cold storage (0–5°C or −30°C) of the fish.

Extraction of the Swim Bladder Material

The wet weights of the fishes and the swim bladder contents were recorded, as were the depths (to bottom) by fathometer at which they were caught. The total swim bladder contents were extracted by the procedure of Folch, Lees, and Sloane Stanley (2), including their technique to wash out nonlipid contaminants from the chloroform phase. Solvent was removed from the lipid extracts on a rotary evaporator under vacuum at 37°C. The solvent-free residues were dissolved and made to known volumes in chloroform (2.0–10.0 ml); aliquots were dried to constant weight.

The aqueous methanol phases of the lipid extractions were treated with an equal volume of acetone. The precipitates in these solutions were collected on filters, washed, and dried to constant weight. This material, which is designated protein, yielded a typical infrared spectrum and amino acid pattern for protein and was not further studied.

Lipid Analysis

The lipids from the swim bladder contents were separated into neutral lipids, glycolipids, and phospholipids by column chromatography. The glass columns (35 × 2 cm) were prepared with 10 g of silicic acid (Clarkson Chemical Co., Williamsport, Pa.) suspended in chloroform. The sample (200 mg) of lipid in 2 ml of chloroform was placed on top of the packing just as it was draining dry. The sample was followed with a few small portions (3–5 ml) of chloroform and then additional chloroform in a total amount of 100 ml. The eluate was collected as the neutral lipid fraction. Acetone (75 ml) was then passed through the column and the glycolipid fraction was collected. This was followed with 200 ml of methanol which eluted the phospholipids. The efficacy of this column operation and the purity of the fractions was established by TLC monitoring. Quantitation of the lipid fractions was accomplished gravimetrically as for total lipids.

TLC on precoated silica gel plates (F-254, Brinkmann Instruments, Westbury, N.Y.) was used to evaluate the purity of the lipid fractions and to identify the components. These plates were developed in petroleum ether–ethyl ether–acetic acid 80:20:1 (v/v/v) to separate neutral lipids. Glyco- and phospholipids were resolved using chloroform–methanol–water–aqueous ammonium hydroxide (28%) 130:70:8:0.5 (v/v/v/v). The Dittmer–Lester spray reagent (3) was used to detect phosphorus-containing lipids; ninhydrin reagent (0.2% in 50% aqueous acetone) was used to reveal -NH_2-containing lipids; and anthrone reagent, 0.2% in H_2O–concd H_2SO_4 2:5 (v/v), was used to detect glycolipids. Further identification of the lipids was accomplished by infrared spectral analysis (KBr pellet) and chromatographic comparisons with authentic reference compounds (Supelco, Inc., Bellefonte, Pa.). The sugars released on acid hydrolysis of the cerebrosides were tentatively identified by the TLC analysis of Gal (4).

The individual phospholipids were quantified by resolving them on two-dimensional TLC plates coated with silica gel HR (Brinkmann Instruments) and analyzing the appropriate areas, detected with iodine vapor, for lipid phosphorus (5, 6). Standard deviations for these analyses did not exceed 12% of the analytical value for components representing >10% of total lipid phosphorus and not more than 31% for minor components (2–10% of total lipid phosphorus). Total cholesterol in the lipid extracts was determined by the method of Abell, Levy, Brodie, and Kendall (7). Free and esterified cholesterol were determined on material scraped from the appropriate areas of silica gel HR plates.

RESULTS

The data of the investigation (Tables 1, 2, and 3) are generally consistent within themselves and with those of Phleger and Benson (1), although they have observed some samples with higher cholesterol content. Findings for *C. acrolepis* no. 1 show the greatest discrepancy. This was the only fish held at 0–5°C for any length of time (12 hr). The others were promptly frozen or analyzed. It is possible that lipase and lecithin:cholesterol acyltransferase action on the swim bladder lipids may account for the discrepant data on this fish. Data for the other two fish of each species agree well and show that the same type of swim bladder material is obtained from both species. The only notable difference in the data is that *C. acrolepis* has more sphingomyelin, and this is compensated for in *A. rostrata* by higher levels of phosphatidylcholine and phosphatidylethanolamine.

The glycolipid in all samples was composed of one main component and two minor ones which had TLC R_F values comparable to a monoglycosyl ceramide. The principal sugar that was found corresponded to

TABLE 1 GROSS COMPOSITION OF SWIM BLADDER MATERIAL FROM TWO SPECIES OF FISHES

	C. acrolepis			*A. rostrata*	
	1	2	3	1	2
Wt of fish (g)	2043	1100	2000	1249	568
Depth (m)	2000	1300	1300	2700	2000
Swim bladder material					
Wet wt (g)	6.5*	10.8	13.8	6.7	1.3
Lipid (g)	0.724	1.176	1.621	0.987	0.183
Protein (g)	0.350	0.729	1.065	0.483	0.092
Lipid composition					
Neutral lipid %	39.2	38.0	41.7	36.2	36.0
Glycolipid %	7.2	6.0	4.3	7.0	8.9
Phospholipid %	53.6	56.0	54.0	56.7	55.1

* Only part of the total bladder contents.

glucose; lesser amounts of a second sugar, with an R_F corresponding to that of galactose, was also detected.

TLC analysis of the neutral lipids for all samples revealed only three components: free and esterified cholesterol and free fatty acids. The values for free fatty acids in Table 2 were therefore determined by difference.

The unknown phospholipid X reported in Table 3 was composed of two slightly detectable spots on TLC plates which moved in a manner similar to cardiolipin in the two-dimensional solvent system of Patton, Hood, and Patton (8), i.e., somewhat beyond phosphatidylethanolamine in the ammonia-containing solvent and about the same as the phosphatidylethanolamine in the acetic acid-containing solvent.

Phospholipid composition (% of total phospholipids) of the tunica interna membrane of kelp bass, *P. clathratus*, was: sphingomyelin, 22.3; phosphatidylcholine, 45.2; phosphatidylinositol, 0.38; phosphatidylserine, 12.5; and phosphatidylethanolamine, 19.5. TLC analysis of the neutral lipids from this membrane revealed cholesterol, cholesteryl esters, and free fatty acids in approximately the same proportions as for the swim bladder material from the two bathypelagic fishes. The ratios of weight of cholesterol to weight of phospholipid for the two sources of lipid were approximately the same (0.5).

TABLE 2 COMPOSITION OF NEUTRAL LIPIDS IN SWIM BLADDER MATERIAL FROM TWO SPECIES OF FISHES

No.*	*C. acrolepis*			*A. rostrata*	
	1	2	3	1	2
	% of neutral lipids				
Total cholesterol†	60.4	76.0	72.9	76.5	77.8
Free cholesterol	44.0	67.2	67.6	71.7	68.6
Ester cholesterol	28.7	15.4	9.3	8.4	16.1
Free fatty acids	27.3	17.4	22.8	20.8	15.3

* Corresponds to fish numbers in Tables 1 and 3.

† Cholesterol results are averages of duplicates. Cholesteryl esters are calculated assuming a mol wt of 676.

TABLE 3 COMPOSITION OF THE PHOSPHOLIPIDS IN SWIM BLADDER MATERIAL FROM TWO SPECIES OF FISHES

No.*	*C. acrolepis*			*A. rostrata*	
	1	2	3	1	2
	% of total phospholipids				
Phospholipid†					
LPC	20.5	2.30	2.99	1.57	0.69
SP	67.2	42.2	52.3	24.5	21.3
PC	12.0	33.2	24.4	48.6	47.9
PI	nd	0.56	0.71	1.19	0.43
PS	nd	8.10	7.90	8.15	11.1
PE	0.25	13.3	11.5	16.4	18.3
X	nd	0.17	nd	0.08	0.22

* Corresponds to fish numbers in Tables 1 and 2.

† Mean values from four TLC separations for the *C. acrolepis* lipids and from three separations for the *A. rostrata* lipids. Abbreviations: LPC, lysolecithin; SP, sphingomyelin; PC, phosphatidylcholine; PI, phosphatidylinositol; PS, phosphatidylserine; PE, phosphatidylethanolamine; X, unknown; nd, not detected.

DISCUSSION

The principal purpose of the gas-filled swim bladder in fishes is to maintain a satisfactory state of buoyancy. A few fishes have swim bladders filled with lipid. For example, Nevenzel, Rodegker, Mead, and Gordon (9) reported that the swim bladder of the coelacanth *Latimeria chalumnae* is 61.9% lipid wet wt, 98.9% lipid dry wt; this lipid was 97.2% wax esters. However, no one has previously found swim bladders containing the cholesterol-rich material discovered by Phleger and Benson (1). Our research confirms that the swim bladders of *C. acrolepis* and *A. rostrata* contain such material. We find further that the material is also rich in phospholipid and protein and that it exists as a foam in fishes brought from the deep. The state of this material in the living fish awaits development of procedures to deal with depth and pressure problems of the deep ocean.[2] The fact that specimens of *A. rostrata*

[2] For example, efforts to bring fishes slowly from the deep on open lines in order to minimize pressure changes on structure can be frustrated by predators such as sharks. None of the deep ocean fishes in this study reached the surface alive.

TABLE 4 LIPID COMPOSITION OF SWIM BLADDER MATERIAL FROM *A. rostrata* AND OF PLASMA MEMBRANE FROM RAT LIVER

Liquid Component	Swim Bladder*	Plasma Membrane*
	% of total lipid	
Neutral lipid	36.1	39.0
Glycerides	nil	7.3
Cholesterol (free)	25.3	18.1
Cholesterol (ester)	4.4	2.0
Free fatty acid	6.5	7.9
Phospholipid†	55.9	55.4
LPC	0.6	1.8
SP	12.7	9.8
PC	27.0	19.3
PI	0.5	4.1
PS	5.4	5.0
PE	9.7	10.3
Glycolipid	8.0	5.6

* Values for swim bladder are an average of two fish; values for plasma membrane are from Ray et al. (13).

† See footnote (†) to Table 3 for abbreviations.

could be brought to the surface with swim bladders intact and containing the foam suggests that a substantial volume of the living swim bladder in these fish contains gas.

The physiology of swim bladders has been reviewed (10, 11). Gas is supplied to the swim bladder by a structure known as the gas gland. This gland is a complex of capillaries and is normally located in the anterior wall of the swim bladder. Through a salting out and counter-current multiplication effect the gas content of these capillaries is greatly increased above the normal level carried by the blood. The gas in swim bladders of deep ocean fishes is mainly oxygen, which indicates that the gas-concentrating effect in these fishes is primarily a hemoglobin unloading phenomenon (12). Scholander (12) has observed that the swim bladder of the living codfish, *Gadus callarias*, secretes gas bubbles. Copeland (11) has studied the mechanism of gas transport into the swim bladder at the ultrastructural level. He concluded that final release is in the form of microbubbles from a secretory epithelium. It is a point of interest here that in some of his photomicrographs myelin figures and lipid-like substances are evident at the secretory cell–gas phase (membrane) boundary.

In this connection we call attention to similarities in the lipid composition of material from the swim bladder of *A. rostrata* and the plasma membrane from rat liver. The comparison (Table 4) utilizes, as an example, the data of Ray, Skipski, Barclay, Essner, and Archibald (13).[3] The idea that a secretory process may accumulate membrane material at the point of secretion, the plasma membrane, is consistent with the current understanding of Golgi vesicles as vehicles in secretory processes. The membranes of such vesicles appear to interact with the plasma membrane at secretion (14). However, while such a process would be in harmony with the ultrastructural observations of Copeland (11) and may account for the manner in which the material in question accumulates in the swim bladder, it does not explain the marked differences in the amount of this material in surface-living fishes compared with the deep ocean species. Our data for the kelp bass suggest that epipelagic fishes have traces (a few milligrams) of the material in their swim bladder lining in contrast to abundant deposits (grams) in the swim bladders of *A. rostrata* and *C. acrolepis*. One variable which may be of importance under these conditions is gas density. As pointed out by Alexander (10), the density of oxygen at an ocean depth of 6000 m is 0.6 g/ml. The ultrastructural process of secreting a gas under these conditions may have some of the characteristics of secreting a liquid. For example, in the case of milk fat droplet secretion, plasma membrane is removed from the cell as an envelope around the droplet (15).

It is possible to suggest several functions for the swim bladder material, the validity of which can be established only by further research: (*a*) it protects surrounding tissue from the effects of a relatively dense and enriched oxygen atmosphere; (*b*) in the manner of a surfactant it facilitates secretion of gas; and (*c*) it buffers against rapid changes in the gas content and composition within the swim bladder. To the extent that the swim bladder material resembles plasma membrane (d 1.15–1.17), a role in positive buoyancy is excluded in light of the density of sea water, 1.026.

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[3] Ray et al. (13) summarized results from a number of other investigations in comparison with their own on the lipid composition of rat liver plasma membrane. The total summarized data are not inconsistent with the point of similarity we are suggesting, but their data are more complete.

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