Golgi apparatus as the site of "packaging" or modification of secretory products synthesized within the endoplasmic reticulum. Recognition of the lysosomal nature of JGG provides information relative to the mechanism of release of renin. Possibly the proteolytic enzyme renin represents another enzyme of lysosomes in JGC or, conversely, certain lysosomal enzymes in JGC have activities similar to that of renin capable of initiating angiotensin formation and hypertension.

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Lipids of the Living Coelacanth, Latimeria chalumnae

Abstract. *The muscle of* Latimeria chalumnae *contains 30 to 71 percent (dry weight) of lipid deposited extracellularly. Wax esters constituted 90 percent or more of the lipids from muscle and fat storage tissues. These esters, by gas-chromatographic analysis, consisted of* C_{30} *to* C_{40} *homologs with one or two double bonds.*

The living coelacanth fish, *Latimeria chalumnae*, has excited the interest of biologists concerned with vertebrate phylogeny ever since its discovery off eastern South Africa in 1938 (*1*). In the intervening years over 30 specimens have been captured, all but the first in the vicinity of the islands of Anjouan and Great Comoro in the Comoro Archipelago between Madagascar and Mozambique. Several preserved specimens have been carefully dissected, and aspects of their gross and microscopic anatomy and gross chemical composition have been described (*2*). The rarity of the species has combined with the difficulty of access of the Comoros to prevent all but the sketchiest studies of behavior and ecology (*3*). Nothing is known of the physiology and very little of the biochemistry (*4*) of this form.

We recently obtained a newly caught male coelacanth, perfectly preserved by formalin and deep-freezing (*5*). The freshness and excellent condition of this specimen have permitted us to make a detailed study of the pattern of lipid composition of the various tissues. This pattern, while exotic, resembles that found in several species of bathypelagic fishes. We have examined trunk muscle, liver, spleen, the fat-filled presumptive swim bladder, and fat storage tissues in several locations.

The techniques employed (*6*) were: (i) extraction of the lipid from the tissue with chloroform-methanol as solvent; (ii) separation of the various lipid classes by adsorption column chromatography on silicic acid, monitored by thin-layer chromatography (TLC); (iii) saponification of the chromatographically pure wax esters—the principal lipid type present in the muscle and adipose tissues—and separation of the constituent long-chain alcohols and fatty acids on florisil columns; and (iv) gas-liquid partition chromatography (GLC) of the intact wax esters and of the alcohol and acid moieties (as the trifluoroacetate and methyl ester derivatives, respectively) (Tables 1, 2, and 3).

The liver lipid fraction consisted of triglycerides, 78.4 percent; wax esters plus sterol esters, 8.2 percent; and polar lipids (including phospholipids), 9.1 percent. The composition of the triglyceride fatty acids is given in Table 3. The pattern of the spleen lipids was unusual: hydrocarbons, 3.6 percent; wax esters plus sterol esters, 14.7 percent; triglycerides, 6.2 percent; free fatty acids, 16.1 percent; cholesterol, 12.8 percent; and polar lipids, 46.7 percent. The hydrocarbons were identified by GLC as the C_{20} to C_{32} normal alkanes plus squalene (about one-third of the total). No unesterified long-chain alcohols were detected, and the only phospholipid in the polar lipids was lecithin (by TLC; *6*). Lecithin could not have been the principal constituent of this fraction, since 57.8 mg of material on methanolysis gave only 6.0 mg of chromatographically pure methyl esters. In Table 3, therefore, the analysis of the largest homogeneous fraction of spleen lipids is given, namely the free fatty acids. The total fatty acids of the polar lipid fraction were similar, but had lower values for palmitic and palmitoleic acids and correspondingly higher values for stearic acid.

We have not yet compared the fatty acid patterns of wax esters, cholesteryl esters, and triglycerides from a single tissue of the coelacanth since the wax esters so predominate in the lipid from muscle and adipose tissues that isolation of either cholesteryl esters or triglycerides is difficult; indeed, the presence of glycerides in these tissues has not been established. With the organ lipids the problem is the separation of the cholesteryl esters from the wax esters quantitatively.

The low percentages of C_{20} and C_{22} polyunsaturated acids in the organ lipids were unexpected. There was no evidence that hydrolysis or autoxidation occurred during the shipping, storage, and extraction of the tissues. The formalin probably would have destroyed any phosphatidyl ethanolamine present (*7*), but it would not be expected to react with the polyunsaturated fatty acids. However, in view of the year which intervened between capture of the fish and completion of the GLC analyses of the methyl esters in the organs, the values in Table 3 for the polyunsaturated acids of the liver

Table 1. Composition of the lipids of *Latimeria chalumnae* tissues.

Total lipid (%)			
Wet weight	Dry weight		Wax ester content
	This work	Millot (*8*)	
	Ventral muscle		
34.1	71.4		94.3
	Dorsal muscle		
7.7	29.7	23.9	92.7
	"Swim bladder"		
61.9	98.9	95	97.2
	Pericardial		
81.0	98.7		92.7
	Pericerebral		
76.2	95.5	92.3	92.9
	Postocular		
60.8	92.7		90.0
	Liver		
24.1	67.7	32.3	8.2*
	Spleen		
3.5	18.6		14.7*

* Includes sterol esters.

Table 2. Wax esters of *Latimeria chalumnae*. The composition was determined by gas-liquid chromatography on a glass column (1.8 m by 4 mm inside diameter) packed with stabilized diethylene glycol succinate polyester, originally 12 percent by weight, on Anakrom ABS (70 to 80 mesh) (*11*). The column was operated at 240°C and an inlet gas pressure of 0.84 kg/cm².

Component*	Muscle tissues		Adipose tissues			
	Ventral	Dorsal	"Swim bladder"	Pericardial	Pericerebral	Postocular
30:0	1.7	1.6	2.7	1.5	2.2	1.9
30:1	0.5	0.4	0.6	0.5	0.6	0.6
32:0	1.8	2.0	2.9	2.4	2.9	2.6
32:1†	9.4	8.7	12.8	9.9	10.8	11.3
32:2	1.9	1.6	2.2	1.9	2.0	2.1
34:0	1.0	trace		trace	0.8	trace
34:1	28.1	27.6	20.6	28.7	26.8	29.6
34:2	11.5	11.5	12.5	12.5	12.0	12.2
36:1	9.9	8.8	11.0	9.7	9.3	9.0
36:2	26.6	30.4	28.8	26.7	25.5	24.8
38:1	1.9	1.2	0.8	1.9	2.0	1.7
38:2	5.3	4.9	4.0	4.0	4.5	3.6
40:2	0.3	1.2	0.9	0.3	0.4	0.5

* The component is designated by the number of carbon atoms in the total compound and the total number of double bonds: hence, 30 : 0 indicates 30 carbon atoms in the ester, and no double bonds. † The retention time for 32 : 1 was 12.8 to 13.9 minutes.

triglycerides, in particular, are best considered minimum figures. Additional work with fresh tissue and other lipid fractions will be required to establish whether or not the coelacanth is an exception to the generalization that the lipids of marine fishes contain large amounts of polyunsaturated C_{20} and C_{22} acids. This uncertainty does not apply to our data on wax esters, since all the naturally occurring examples contain only traces of polyunsaturated acids. The methyl esters from the saponified wax esters of four tissues of the coelacanth were analyzed (the earliest analysis being within 90 days of its capture), and none contained more than 0.3 percent of any individual (0.7 percent total) polyunsaturated C_{20} or C_{22} fatty acid.

Three features are seen in the data: the high content of total lipid in the muscle, the large percentage of wax esters in the lipids of the muscle and adipose tissues, and the great uniformity in the composition of the wax esters from the six tissues examined.

Our data for the lipid content confirms those of Millot (*8*), which are listed in Table 1 for comparison. Observations with the light and electron microscopes indicate that the lipid in the muscle is extracellular, whereas that in liver is enclosed in large intracellular vacuoles. The structures observed for adipose tissues are similar to those in corresponding tissues of other vertebrates (*9*). The high percentage of lipid in the muscle is not exceptional for fishes, but the content of wax esters is slightly higher than that reported in the muscle of the oil fish (*Ruvettus pretiosus*) (*6*) and considerably more than that in the muscle lipid of several lantern fishes (family *Myctophidae*) (unpublished work).

Table 3. Component fatty acids and alcohols of *Latimeria chalumnae* lipids (results are percentages by weight).

Component*	Fatty acids				Alcohols from wax esters	
	Spleen free fatty acids	Liver triglycerides	Wax esters			
			Dorsal muscle	"Swim bladder"	Dorsal muscle	"Swim bladder"
14:0	1.5	2.4	3.2	3.8	0.05	0.09
15:0	0.45	0.23	0.25	0.24	0.25	0.27
16:0	31.6	20.9	3.1	2.6	45.0	40.1
16:1	6.0	7.3	13.9	18.8	0.04	trace
17:1	0.75	0.74	1.2	1.7	.08	0.50
17:1†	1.1	0.56	1.1	1.5		
18:0	12.8	1.4	0.68	0.35	11.2	8.8
18:1	31.9	60.2	70.6	64.2	40.4	46.7
18:2	0.64	0.10	0.54	0.92		
19:1	0.42	.25	0.27	0.25	0.1	0.39
20:1	2.9	3.5	4.1	3.5	2.7	2.9
20:4	4.6	0.22				
22:1		.59	0.64	0.68		
22:3	3.0	.10				

* The notation 14:0, for example, indicates an acid or alcohol with a total of 14 carbon atoms and no double bond. † Branched chain.

The lipid of the fat storage tissues (postocular, pericranial, pericardial, and the "swim bladder") consists almost entirely of wax esters. This fact is support for the idea that *Latimeria* deposits pure wax esters as reserve lipid.

The precision of the GLC analyses of the wax esters is estimated to be better than 9 percent of the observed values for major constitutents (>12 percent of total mixture) and better than 20 percent for minor constituents. Therefore the differences in the compositions given in Table 2 for the wax esters from six different tissues are negligible, with the possible exception of the values for monoenoic C_{34} compound in the "swim bladder." This suggests a common site for the synthesis of wax esters in *Latimeria*, but there is no evidence of where this site might be.

Our study of *Latimeria* lipids, together with the data on *Ruvettus pretiosus* (*6*), unpublished work with other gempylid and with myctophid species, and data reported by others, is consistent with the hypothesis that many fishes deposit massive amounts of wax esters in order to approach neutral buoyancy. The other species are all bathypelagic; *Latimeria* may also have this characteristic.

It is not known why wax esters are deposited rather than the more usual triglycerides. Nor is it obvious which chemical or physical properties of the two lipid types may be relevant to the problem. If bouyancy is the objective, then the densities would be important; the meager data available indicate that the wax esters have slightly lower densities (perhaps by 5 percent) than do the triglycerides of a comparable degree of unsaturation (*10*). Study of the site and pathway of biosynthesis of the wax esters, and the rate of turnover of this lipid type in vivo may provide an answer.

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10. The density of oleyl oleate is 0.8600 at 30°C; that of triolein is 0.8988 at 40°C [F. Richter, Ed., *Beilsteins Handbuch der Organischen Chemie*, 3rd suppl. (Springer Verlag, Berlin, ed. 4, 1961), vol. II-2, pp. 1415, 1423].
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Survival of Mammals Breathing Organic Liquids Equilibrated with Oxygen at Atmospheric Pressure

Abstract. *Because oxygen and carbon dioxide are very soluble in certain silicone oils and fluorocarbon liquids, these liquids will support respiration of mammals. Mice and cats respiring silicone oil die shortly after return to air breathing, while those breathing fluorocarbon survive for weeks. The respiration of mice is optimally supported by these organic liquids at about 20°C. In cats, arterial oxygenation is excellent, but there is some impairment of carbon dioxide elimination. All animals have suffered some pulmonary damage from breathing fluorocarbon liquids. Continued investigation of organic fluid respiration may lead to development of a safe method to support the respiration of man by liquids equilibrated with gases at atmospheric pressure.*

Oxygen is at least ten times as soluble in silicone and fluorochemical liquids as in plasma or saline. A given volume of oxygen-saturated silicone oil contains half-again as much oxygen as the same volume of air or whole blood, while a given volume of oxygen-saturated fluorochemical liquid contains three times as much (*1*, *2*). It therefore seems reasonable to expect that they might be capable of supporting the respiration of intact animals. These organic liquids are available in viscosities near that of water, they are poor solvents, and are generally regarded as biologically inert (*3*). The silicone oils (polymethylsiloxanes) are immiscible with the fluorocarbon liquids (perfluorobutyltetrahydrofurans) and with water. Both have significant vapor pressures.

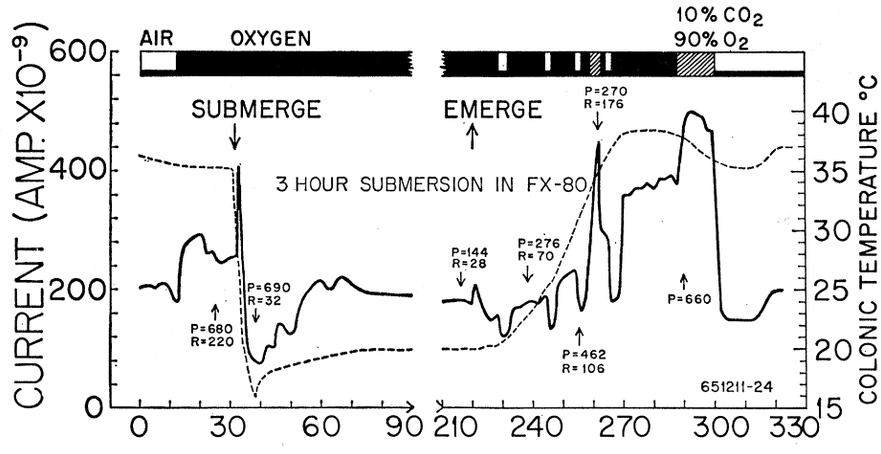

Fig. 1. Brain oxygen cathode current during liquid fluorocarbon breathing in the mouse. The polarographic brain oxygen cathode current is indicated as the solid line and colonic temperature as the dotted line. Time in minutes is shown on the horizontal axis. Pulse and respiration rates are indicated by *P* and *R*. The gas being breathed or being equilibrated with the fluorocarbon is indicated on the bar at the top of the graph. Cathode: platinum, 40 gauge, 4 mm; reference: AgCl-Ag; respiration fluid: fluorocarbon FX-80, continuously bubbled with the gas indicated.

Preliminary experiments (*4*) indicated that mice and rats could survive complete immersion in oxygen-saturated silicone oils for prolonged periods and that the length of time they were able to continue breathing the oils was related to its viscosity and temperature. Thus, in groups of five mice, survival averaged 4, 5, 16, and 35 minutes in oxygen-saturated oil at 24°C, having viscosities of 10, 5, 2, and 1 centistoke, respectively. Silicone oils having a viscosity of 0.65 centistoke proved to be too toxic to use. The optimum temperature for survival was about 18°C; several animals breathed oil at this temperature for over 6 hours, while respiration usually ceased after 5 minutes at 35°C. The mean brain oxygen cathode current (*5*) at 20°C was approximately half that obtained during air breathing at the same temperature. Several cats survived respiration with 1-centistoke silicone oil for 1 hour, but the arterial pO_2 fell, the pCO_2 increased, and the *p*H decreased. All of the mammals succumbed between 10 minutes and 5 hours after removal from the silicone fluid. Goldfish survived under silicone oil for several weeks.

Mice breathing the liquid fluorocarbon for 1 hour, in contrast to those breathing the silicone oils, survived for several weeks after removal from the fluid. Immersion survival times averaged about 4 hours at 18°C, 40 minutes at 25°C, and 15 minutes at 30°C. One animal continued to breath the liquid for 20 hours at 18°. Schlieren could be seen in the expired liquid even after the temperatures of the mice were the same as that of the oil, indicating a change in the refractive index or density of the liquid, depending on its gas content. The addition of Fluothane (1 cm^3 per liter of FX-80) arrests the swimming motions of the mice when submerged and the animals survive.

Brain oxygen cathode currents were recorded in a number of mice before, during, and after immersion in the fluorocarbon and a typical result is shown in Fig. 1. It can be seen that the cerebral oxygen tension during fluid breathing is roughly equivalent to that