LIPIDS AND LIPID BIOSYNTHESIS OF THE EDIBLE SNAIL *OTALA LACTEA*

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1966

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
July, 1968

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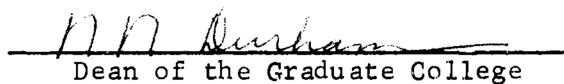
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ACKNOWLEDGEMENTS

I wish to express my appreciation to Dr. Calvin Beames, Jr. for his patience, support, and counsel in the completion of this study and the preparation of this manuscript. I also wish to extend my gratitude to the staff and graduate students of the Department of Physiology and Pharmacology for their helping to create a productive and enjoyable atmosphere.

I express my deepest appreciation to my mother and father for their encouragement and support throughout this and all previous scholastic endeavors.

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CHAPTER I

INTRODUCTION

For land snails the primary adaptation for terrestrial life involves (1) the maintenance of internal water in an aerial environment of variable humidity, (2) respiration of gaseous oxygen, and (3) resistance to or avoidance of a range of temperatures wider than in aquatic environments. Adaptations for terrestrial locomotion, reproduction, excretion, etc. follow. Land snails are generally not as well adapted to a terrestrial environment as are insects and amniote vertebrates, and are limited in distribution (Hunter, 1964). They are cryptozoic in behavior and show nocturnal activity. Terrestrial habitats with extreme ranges of temperature or very low humidity do not support these mollusks (Hunter 1964). In winter months or during unfavorable summer conditions the terrestrial snail withdraws into it's shell and a thin calcareous membrane is formed across the operculum. This calcareous structure protects the animal from the external environment and helps avoid dessication.

During these periods of estivation the animal must rely upon it's stored food reserves for maintenance of life processes. For most animals the principal food reserves are glycogen and fats which are utilized freely during periods of hibernation or estivation. One usually sees a drop in the tissue content of both food reserves during such periods. Thiele (1959) and von Brand (1931) have reported that

the glycogen content of terrestrial pulmonates drops appreciably during estivation, however, there is little change in the lipid content. This raises the question of the physiological role of lipids in snails.

In general, there are four main functions which lipids may serve in an animal. These are: (1) structural and functional components of cell membranes and organelles, (2) a source of potential energy, (3) insulation to reduce energy transfer, and (4) regulation of metabolic processes. Thiele (1959) has shown the lipid content of the terrestrial snail _Helix pomatia_ to be quite low and constant throughout the year. This would suggest that lipid is not a primary source of potential energy or insulation against heat transfer. Therefore, two major functions may still exist, regulation of metabolic processes and structural and functional components of cells. Perhaps the principle function of fatty acid synthesis in the snail is to re-oxidize reduced TPNH formed by the pentose phosphate pathway. Possibly the only mechanism of lipid metabolism in the snail is one that modifies long chain fatty acids. Determining the rate and mechanism(s) of lipid synthesis would help clarify the functional aspects of snail lipids. The objectives of this investigation were to determine the lipid composition and mechanism of fatty acid synthesis in the terrestrial snail _Otala lactea_.

CHAPTER II

LITERATURE REVIEW

Morphology of the Snail

The pulmonate gastropod which is usually used for dissection is _Helix pomatia_, an edible snail, which is morphologically similar to _Otala lactea_. The order Pulmonata to which _Otala lactea_ belongs is the most specialized and has probably evolved more recently than any other group of snails.

Structurally, the animal is divided into three regions, the head, the foot and the visceral hump. The visceral hump is that part which is covered by the shell when the animal is expanded, while the head and foot make up the remainder outside the shell (Borradaile et. al., 1935). The visceral hump contains the hepatopancreas (digestive glands), a two-chambered heart, and a major portion of the alimentary canal and reproductive organs. The foot possesses a flat ventral surface underwhich are longitudinal muscle fibers. The rippling muscles of the foot are coordinated by the action of a ventral nerve network that is common in invertebrates. To facilitate movement over dry surfaces the snail secretes mucus which spreads out as a smooth bed of lubricating fluid over which the snail moves (Borradaile et. al., 1935).

The reproductive organs of _H. pomatia_ are quite complicated. Snails are hermaphrodites, but reproduction occurs mainly by cross-fertilization. Eggs and sperm are produced in the follicle of the

ovotestis. While ripe sperm is found throughout a large part of the year, mature eggs occur only for a very short time. Eggs are fertilized by the sperm and eggs pass through the hermaphrodite duct to the albumen gland. After fertilization the eggs are enveloped in albumen by the albumen gland and then pass to the exterior through the female duct. Sperm pass down the male duct which joins the penis (Borradaile et. al., 1935).

The alimentary canal starts with the buccal mass. The mouth of H. pomatia has a radula which is used for cutting food. Food passes from the buccal cavity down the esophagus to the crop where it mixes with a digestive-mucus mixture that empties into the crop from the hepatopancreas. The hepatopancreas occupies most of the visceral hump (Borradaile et. al., 1935). In H. pomatia the hepatopancreas consists of numerous blind-ending tubules which connect with the stomach by a system of branched ducts. The hepatopancreas functions in the production of digestive enzymes, it is also an organ of absorption, phagocytosis, food storage and excretion (Owen, 1966). Of the thirty or more enzymes associated with the digestive tract of H. pomatia, more than 20 are carbohydrases which are reported to include alpha and beta amylase, cellulases, chitinases and a variety of glycosidases (Owen, 1966). The origin of these cellulases is uncertain, since cellulytic bacteria are usually present in the gut of most gastropods (Owen, 1966).

Most workers (Myers and Northcote, 1958) agree that the digestive juice of H. pomatia shows only slight proteolytic activity. The only recent work on the lipases and esterases of gastropods is that of Ferreri (1958) and Ferreri and Ducato (1959). Using histochemical methods, they were able to demonstrate lipase in the epithelium of the

digestive tracts of H. pomatia.

Carbohydrate Metabolism of Terrestrial Snails

Baldwin (1938) showed an increased oxygen uptake when slices of H. pomatia hepatopancreas were incubated with fructose, galactose, and galactogen. Rees (1953) has shown that H. pomatia hepatopancreas homogenates will increase their oxygen uptake when citrate, cis-aconitate, α-ketoglutarate, succinate, fumarate, malate, and oxalacetate are added to the homogenates. Rees (1953) also demonstrated that the hepatopancreas can synthesize citrate from pyruvate and oxalacetate. He showed the conversion of α-ketoglutarate to succinate and the build-up of α-ketoglutarate when citrate was added to the homogenate. Phosphoglyceric acid appeared to increase oxygen uptake, while other glycolytic intermediates and sugars increased oxygen uptake to a lesser, more questionable extent. Rees (1953) could not demonstrate oxidative phosphorylation in homogenates of the hepatopancreas, indicating either a lack of phosphorylation or destruction of the necessary phosphorylating enzymes.

Bryant et. al. (1964) and Bryant (1965) followed the incorporation of C^{14} labelled glucose, acetate and succinate into Krebs cycle intermediates by tissue homogenates of several gastropod species. Acetate-1-C^{14} was found in citrate and malate and occasionally in lactate. Succinate-1-C^{14} was found in fumarate, malate and citrate. Whole animal homogenates incorporated C^{14} from glucose into fumarate, malate and citrate. Hepatopancreas homogenates homogenates failed to metabolize glucose or glucose-6-phosphate.

Linton and Campbell (1962) have studied the urea cycle in the

hepatopancreas of Otala lactea. They demonstrated the enzymes associated with the urea cycle except for carbamyl phosphate synthetase. It was possible for them to show that carbamyl phosphate was utilized by the system. Campbell and Bishop (1963) demonstrated that O. lactea hepatopancreas tissue incorporated $C^{14}O_2$ into the ureido carbon of citrulline, again suggesting the presence of the urea cycle.

When comparing these various results found with the hepatopancreas, indications are that the sliced hepatopancreas retains most of its normal metabolic function. When the hepatopancreas is homogenized, however, metabolic activity is frequently difficult to demonstrate. This is especially true when ATP is required.

Snail Lipids

Thiele has done a large majority of the investigations of terrestrial snail lipids. Thiele (1959) found that in the snail H. pomatia the lipid per cent of dry weight varied from 7 per cent in March to 9 per cent in November. The dry weight is about 20 per cent of the wet weight in all seasons. Thus the total lipid of H. pomatia dropped only insignificantly in the winter. However, the iodine number varied from 65 in November to 95 in April. The phosphorus content of total lipid was quite high, peaking in the early months of the year and decreasing in the summer and fall months. The data suggested that 45 per cent of the total lipid was phospholipid. Thiele (1959) also found the nitrogen content to be quite high. Plasmalogen was about 10 per cent of the phospholipid and reached its peak content in March, toward the end of estivation, and decreased sharply during the mating season in June. The glycogen content varied from 13 per cent of dry weight in November

to 5 per cent of the dry weight in May.

In a later study, Thiele (1960) found cholesterol to be twice as high in winter snails as in summer snails; the glyceride level was correspondingly lower in winter. In summer snails, the level of trienoic acids was about two times as great as tetraenoic acids; the amounts were reversed in winter snails. The main unsaturated fatty acids were those with 18 carbons. Of the saturated fatty acids present, stearic acid was the main constituent. The unsaponifiable fraction consisted mainly of cholesterol.

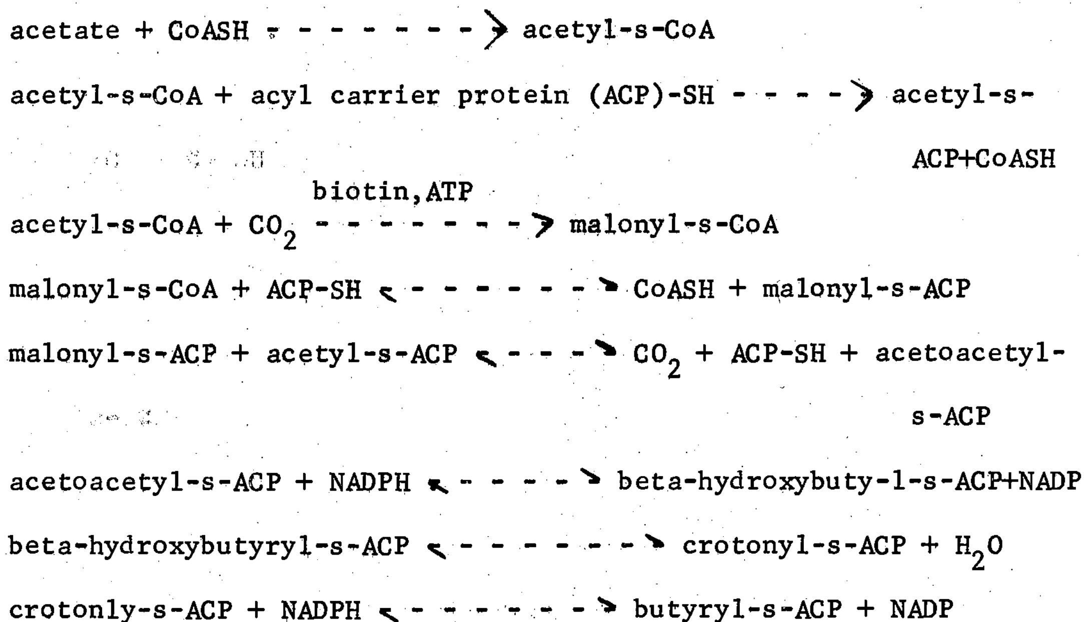
Thiele and Krober (1963) determined the fatty acid composition in free fatty acids, diglycerides, triglycerides, and cholesterol esters of lipid extracts of *H. pomatia*. For the free fatty acids they found a large per cent of 20:2, 20:4 and 18:2 fatty acids. The major constituent of cholesterol ester fatty acids was not identified, but its relative retention time suggested the acid was between 16:0 and 18:0 carbons in chain length. Palmitic acid was the second most prevalent acid esterified to cholesterol. In diglycerides and triglycerides, the main components were 16:0, 18:0 and 18:1 fatty acids.

Large amounts of work have been done on carbohydrate and nitrogen metabolism in land snails while an almost insignificant amount of work has been done on lipid synthesis. Vonk et. al. (1956) studied the metabolism of *H. pomatia* with deuterio-acetate. Addink and Ververgaert (1963) also reported on acetate incorporation into fatty acids, but their data is presented in such a way that it was impossible to accurately interpret the results. Apparently, acetate-1-C^{14} is incorporated into fatty acids, with more radioactivity in the unsaturated fatty acids. Radioactivity from acetate-1-C^{14} was also found in waxlike substances

and cholesterol. When Addink and Ververgaert (1963) analyzed the fatty acids of *H. pomatia*, the main components were reported to be 11 per cent palmitic, 19 per cent stearic, 13 per cent linoleic, 20 per cent oleic and 12 per cent eicosadienoic. These values for the molar ratio of fatty acids in *H. pomatia* differ appreciably from that reported by Thiele and Krober (1963).

Fatty Acid Synthesis

Animal tissues contain at least three metabolic pathways for the synthesis and interconversion of the various fatty acids. They are: (1) *de novo* synthesis of saturated acids (mainly palmitic acid), (2) systems which bring about the elongation of fatty acids and (3) a system for desaturating fatty acids. Palmitic acid occupies a central position in synthesis since it is the main end product of *de novo* synthesis and the smallest fatty acid to be elongated by the enzymes in mitochondria (Wakil, 1964):



the butyryl-s-ACP then condenses with another malonyl-s-ACP which forms

a beta-keto acid and the cycle repeats itself. The fatty acid is usually re-cycled until the chain is 16 carbons long. Then the acid is released from the acyl carrier protein. Palmitic acid comprises 60 to 80 per cent of the products of de novo synthesis while 10 to 20 per cent is stearic and 5 to 10 per cent is myristic acid (Wakil, 1964). Palmitic and stearic acids can be desaturated to palmitoleic or oleic acid by microsomes in the presence of O_2 and $NADPH_2$ (Wakil, 1964).

The mitochondria may elongate saturated 16, 18, 20 and 22 carbon fatty acids by the addition of acetyl-CoA (Wakil, 1964). The palmitoleic or oleic acids can be elongated by the addition of one or more acetyl-CoA units to form longer chain mono-unsaturated acids. Oleic acid can be desaturated further to the 18:2 acid which can be elongated to longer chain acids. The microsomes also have the ability to elongate fatty acids by the addition of malonyl-CoA (Nugteren, 1965).

Mitochondrial phospholipids contain a large amount of unsaturated acids (McFarlane, 1960). Phospholipids have been demonstrated to be involved in membrane transport (Hopkin, 1963). It is believed that the mitochondria may serve the purpose of providing fatty acids for structural or transport functions. As was pointed out earlier, analysis of the snail tissues for the above pathways should provide clues to the physiological role of lipids in the Mollusca.

CHAPTER III

MATERIAL AND METHODS

The Animal

Otala lactea were obtained from S. Scozzaro and Sons Inc., Brooklyn, New York, and maintained in estivation at 55°F. At least seven days before individual snails were used in experiments they were placed in moist aquaria at room temperature (75°F) and allowed to feed *ad libitum* on lettuce and cabbage leaves.

The snail shell was cut off with scissors and the desired tissue was removed for experimentation. Both the left and right hepatopancreas (digestive glands) were used. Care was taken to remove the intestine from the hepatopancreas. Albumen glands were easily removed and handled separately. Reproductive tissue included the dart sac, flagellum, mucous glands, oviduct, penis and sperm duct but not the ovotestis. The foot tissue was taken only from the bottom region of the foot and was mainly muscle with the associated mucous glands, epithelium, etc. Foot tissue of the other areas of the extended foot and head was discarded. In some experiments, the soft body minus the above dissected parts was also used.

Chemicals

Adenosine triphosphate (ATP), coenzyne A (CoA), and nicotinamide adenine dinucleotide, reduced form (NADH) were obtained from Pabst

Laboratories (Milwaukee, Wisconsin). Sodium acetate-1-C^{14} was obtained from Nuclear-Chicago Corporation (Des Plaines, Illinois). Acetyl-1-C^{14} CoA was obtained from Nuclear New England Corporation (Boston, Massachusetts). All other chemicals used were commercial products of reagent grade.

In Vivo Incubations

Sodium acetate-1-C^{14} (7.5μmoles, 4.6 x 10^6 dpm) was mixed with an equal volume of .05M potassium phosphate buffer (pH7.5). The snail was teased with a sharp instrument so that it would withdraw into its shell and thus void excess body fluids. A one-eighth inch hole was carefully drilled in the shell with an electric drill. A total of fifty μl of buffered sodium acetate-1-C^{14} was placed in the hole with a micropipette and the opening was immediately sealed with wax. Twenty-four hours after the acetate was injected the animals were dissected. Similar tissues from individual snails were pooled and the lipids were extracted as described by Folch et. al. (1951). The organic solvent was removed under vacuum at 45°C and the lipid residue was adjusted to known volume with chloroform. Aliquots were taken for analyses.

Mince Tissue Incubations

O. lactea body organs were carefully dissected and placed in ice cold containers. The tissue was minced with the aid of two scalpels. Appropriate amounts of the mince were suspended in 5.9 mls of phosphate buffer (.05M, pH7.5) in a 50 ml erlenmeyer flask. The mixture was allowed to equilibrate for 5.0 minutes and 0.1 ml of sodium acetate-1-C^{14} (30μmoles, 1.87 x 10^7 dpm) was added to the system. The flasks

were flushed with 99.9 per cent nitrogen for 40 seconds, stoppered, and shaken in a metabolic shaker at 37° or $26^{o}C$ for two hours. At the end of this period the reaction was stopped by adjusting the reaction mixture to pH 1 with 10N sulfuric acid. The suspension was transferred to 50 ml centrifuge tubes and centrifuged at 2000 rpm for 1 minute. The resulting pellet (tissue mince) was extracted as described below.

Tissue Extracts

O. lactea was removed and placed on ice cold containers. When sufficient tissue had been collected it was homogenized (1:3, w/v) in cold 0.25M sucrose containing 1.2×10^{-4} M $CaCl_2$. A Potter-Elvejhem homogenizer with a teflon pestle was used for all preparations.

Cell debris was removed by centrifuging at 500 x g for 15 minutes at $2^{o}C$ in a Servall model RC-2B refrigerated centrifuge. The supernatant was decanted and centrifuged at 9500 x g for 20 minutes at $2^{o}C$ to remove the mitochondria. The supernatant (S_1) was saved and the mitochondria re-suspended in cold sucrose. The re-suspended mitochondria were again centrifuged at $2^{o}C$ for 20 minutes at 9500 x g. The mitochondria were saved and the supernatant from this centrifugation discarded. Microsomes were isolated from the (S_1) supernatant by centrifuging at $2^{o}C$ for one hour at 100,000 x g in a Beckman ultracentrifuge (Spinco Model L2). Protein was routinely determined by the Lowry Method (Lowry et. al., 1951).

Incubations

Tissue extract was added to screw cap tubes containing acetate-1-C^{14} and appropriate cofactors to give a final volume of 3 mls. Each

tube was flushed with nitrogen for 30 seconds, capped, and incubated in the metabolic shaker for two hours at 37° or 26°C. Incubations were stopped by the addition of 3 mls of 10 per cent methanolic KOH to each tube. Fatty acids were extracted from the individual tubes following the procedure described by Beames et. al. (1967).

Extraction of Lipids From Mince Tissue

After the reaction was stopped with 10 N sulfuric acid, the aqueous solution and minced tissue was separated by centrifugation. The tissue was extracted overnight with chloroform:methanol (2:1) and filtered. The filtrate was saved and the tissue was discarded. The aqueous incubation fraction was placed in a separatory flask and extracted with 20 ml of ether. The solvent was removed under a slight vacuum at room temperature. The lipid residue was dissolved in the chloroform:methanol extract and the mixture was washed following the method of Folch et. al. (1951).

Saponification of Total Lipids

Aliquots of the lipid extract were evaporated and the residue was suspended in a 10 per cent KOH-50 per cent methanol solution (1 ml per.1 g. lipid) and refluxed for 4-6 hours. The resulting soap solution was evaporated at 50°C under a stream of nitrogen to approximately one-half volume. An equal volume of water was added and the solution was extracted 3 times with 30 ml of ethyl ether to remove the non-saponifiable lipid. The remaining soap solution was acidified to pH 1 with HCL and the fatty acids were removed by extracting the solution 3 times with 30 ml of ethyl ether. The ether extract was dried for two hours

with anhydrous sodium sulfate, filtered and evaporated at room temperature under a slight vacuum. The fatty acid residue was adjusted to volume with chloroform and aliquots were taken for analysis.

Separation of Neutral and Phospholipids

Neutral lipids were separated from phospholipids by silicic acid column chromatography as described by Beames (1964). The solvents from each fraction were removed *in vacuo* at 40°C and lipid residues were adjusted to volume with chloroform. The method was tested for completeness of phosphorus separation by the method of Bartlett (1959) as modified by Bottcher et. al. (1961). Aliquots of the neutral lipids and phospholipids were weighed and the per cent of each in the total lipid extract was calculated. Fatty acid composition of each fraction was determined by gas liquid chromatography.

Thin Layer Chromatographic Analysis

Analytical thin layer chromatography (TLC) was carried out on 20 x 20 cm plates coated with Silica Gel G. The methods of developing the plates, detecting the spots and identifying the various lipids were those described by Beames et. al. (1967). To determine the distribution of acetate-1-C^{14} in the various lipids, the spots were scrapped from the plates and placed in scintillation vials. Scintillation fluid was added, and the radioactivity was measured with a Packard Tri-Carb Spectrometer. Quenching was corrected by the channel ratio method described by Herberg (1965).

Gas Chromatographic Analysis

Methyl esters of non-volatile fatty acids were separated and identified by gas chromatography. The solvent was removed from aliquots of extracts containing non-volatile fatty acids and methylation was accomplished with diazomethane solution. A Barber-Colman Model 5000 gas chromatograph equipped with an ionization detector was used with a 6-ft. glass column (4mm i.d.) packed with 14.5 per cent ethylene glycol succinate of Gas-Chrom CLP, 100-120 mesh (Applied Science, State College, Pennsylvania). The conditions for analysis were: column temperature 175°C, detector temperature 215°C, and injector port temperature 250°C. The gas-flow rate was 100 mls per minute. Fatty acid esters were identified by comparing their retention time of known standards. When standards were not available, the fatty acid esters were tentatively identified by plotting the $\log_{10}$ of the retention time versus the number of carbon atoms. The radioactive effluent was trapped in glass capsules containing anthracene (Karmen, 1965). The vials were capped, placed in specially prepared holders and counted directly in a Packard Tri-Carb spectrometer. Collection of the effluent was facilitated by using a Packard gas chromatography fraction collector. With this method 60-70 per cent of the radioactivity placed of the column was recovered.

Decarboxylation of Fatty Acids

The method of Brady et. al. (1961) for decarboxylating long chain fatty acids was used with the following modifications. Approximately 2,000 counts per minute of experimental fatty acids or palmitic-1-C^{14} acid (used as a standard) and non-labelled carrier fatty acid was added

to Thunberg tubes (8.5 cm long). The tubes were kept ice cold while adding chemicals. Hyamine solution was added to the side arm. Greased (grease M.P. 100°C) tops were placed on the tubes and secured with rubber bands. The tubes were evacuated for 10 seconds while still on ice. Once the tops had been turned and the system made a closed vacuum, the tubes were placed in a 80°C water bath for one and one-half hours with intermittant shaking. After removal from the water bath the tubes were placed in a metabolic shaker for approximately 20 minutes. The hyamine solution in the sidearm was quantitatively transferred to a scintillation vial with 10 ml of scintillation fluid. The $C^{14}O_2$ was counted in a Packard Tri-Carb Spectrometer. Control experiments with palmitic-1-C^{14} acid showed 90 per cent recovery of $C^{14}O_2$.

CHAPTER IV

RESULTS

Total Lipid Analysis of Snail Tissues

To obtain an idea of the total lipid in *Otala lactea*, the various tissues were extracted, separated into neutral and phospholipids and analyzed by gravimetric methods. The results are shown in Table I. The hepatopancreas with 2.2 per cent, had the most lipid per gram wet weight. Foot tissue had 1.78 per cent; albumen gland, 1.60 per cent; and reproductive tissue, 1.43 per cent. Thiele (1959) found the per cent lipid of the soft parts of *H. pomatia* to be approximately 1.7 per cent of the wet weight throughout the year. Thus, the results with *O. lactea* are in relative agreement with those reported for *H. pomatia*.

Although the total lipid content of the various tissues does not show great variation, the neutral to phospholipid ratios are quite different between tissues. It is not surprising that the albumen gland contained rather high concentrations of neutral lipid. If this gland contributes significantly to the food stored in the eggs, this suggests that lipids are an important source of energy for the developing embryo. The per cent neutral lipid in the total lipid of each of the various tissues was as follows: hepatopancreas, 48 per cent; reproductive tissue, 32 per cent; and foot tissue, 27 per cent. A high phospholipid content is in keeping with the structural role of lipids in tissue such as the foot where there is extensive membrane networks in the cells.

TABLE I

TOTAL LIPID AND PER CENT NEUTRAL AND PHOSPHOLIPID

OF SELECTED SNAIL TISSUES

	Foot Tissue (8.0 g. wet wt.)			Hepatopancreas (19.9 g. wet wt.)			Albumen Gland (9.0 g. wet wt.)			Reproductive Tissue (7.5 g. wet wt.)		
	wt (gm)	%wet wt	%total lipid	wt (gm)	%wet wt	%total lipid	wt (gm)	%wet wt	%total lipid	wt (gm)	%wet wt	%total lipid
Total Lipid	0.14	1.78	100.0	0.44	2.21	100.0	0.14	1.60	100.0	0.11	1.43	100.0
Neutral Lipid	0.04	0.48	27.0	0.21	1.06	48.0	0.09	0.96	60.0	0.04	0.46	32.0
Phospholipid	0.10	1.30	73.0	0.23	1.15	52.0	0.05	0.64	50.0	0.07	0.97	68.0

Thiele (1959) calculated the phospholipid of all soft parts of H. pomatia to be approximately 45 per cent. The present results agree very well with that value.

The Non-volatile Fatty Acids

Neutral and phospholipids were saponified and the fatty acids were extracted for separation and tentative identification by gas-liquid column chromatography. The results, presented in Table II, show that in most tissues a majority of the fatty acids contained 16 or 18 carbons. Tabulation shows that 39.9 per cent of the fatty acids of all the tissues are saturated.

For the hepatopancreas, both neutral and phospholipids contained a significant amount of palmitic acid and larger amounts of saturated and unsaturated 18 carbon fatty acids. Fatty acids with 20 carbons account for over 35 per cent of the acids in the phospholipids from the hepatopancreas. Neutral lipid from this tissue had lesser amounts of 20 carbon fatty acids, and more fatty acids with 12 carbons.

Fatty acids from the albumen gland show the most atypical pattern since 40 per cent of the fatty acids from the neutral lipids was tentatively identified as 13 carbon fatty acid. This 13 carbon fatty acid may correspond to fatty acids that Thiele (1963) could not identify. He found two non-identifiable fatty acids in the range between 13 and 15 carbons in the fatty acid extracts of the cholesterol ester fraction of H. pomatia. Fatty acids from the albumen gland phospholipids were predominantly palmitic acid and unsaturated 18 and 20 carbon fatty acids.

Fatty acids from the neutral and phospholipids of foot tissue were mainly palmitic acid and 18 carbon unsaturated fatty acids. Longer

TABLE II

DISTRIBUTION OF FATTY ACIDS IN NEUTRAL AND PHOSPHOLIPIDS OF SELECTED SNAIL TISSUES

Mole Per cent of Total Fatty Acids

Fatty Acid	Hepatopancreas		Albumen Gland		Foot Tissue		Reproductive Tissue	
Methyl Ester	Neutral	Phospholipid	Neutral	Phospholipid	Neutral	Phospholipid	Neutral	Phospholipid
10:0				.42				
11:0			11.39					
12:0	11.17							.14
13:0?			40.20					
14:0			.50	1.05	6.07	3.04		1.08
14:1		.98						
14:2?		1.34		3.94				3.25
15:0					3.78			
16:0	18.61	12.41	11.16	14.60	29.37	18.76	7.32	8.44
16:1				.86	6.79			.22
16:2		2.28			1.02	.73		
16:3					2.43?	12.18		

TABLE II (Continued)

Fatty Acid Methyl Ester	Hepatopancreas Neutral	Hepatopancreas Phospholipid	Albumen Gland Neutral	Albumen Gland Phospholipid	Foot Tissue Neutral	Foot Tissue Phospholipid	Reproductive Tissue Neutral	Reproductive Tissue Phospholipid
18:iso?			4.00					
18:0	7.12	9.54	9.60	9.76	6.41		12.21	23.30
18:1	22.72	11.32		12.03	13.10	11.58	13.15	26.01
18:2	12.44	23.77	8.37	15.78	23.35	25.39	21.00	11.84
18:3	9.00				7.76			
20:iso			1.51					
20:0		5.17		.52		3.55	17.79?	1.08
20:1		1.96	2.90	1.61			6.77	8.36
20:2	18.91	11.36	8.00	5.80		8.12	21.74	16.26?
20:3		19.74		14.25				
22:0			2.50			11.17		
22:1						3.65		

chain 20 and 22 carbon fatty acids from the phospholipids were also present in appreciable amounts.

Some palmitic acid was present in the lipids from the reproductive tissue but the largest mole percentage of fatty acids contained 18 or 20 carbons. There is slightly more unsaturated fatty acids in the tissue. There were more 20 carbon fatty acids in the reproductive tissue than in any other tissue studied. Thiele (1963) found that terrestrial snail fatty acids are predominantly saturated 16 carbon and saturated and unsaturated 18 and 20 carbon fatty acids. Our results support this observation.

In Vivo Incorporation of Acetate-1-C^{14}

In vivo incubations were performed to determine the ability of the whole snail and individual tissues to incorporate acetate into lipids. Eleven snails were injected with sodium acetate-1-C^{14}. After 24 hours they were dissected and the total lipid was extracted from the hepatopancreas, albumen gland, other reproductive tissue, foot tissue, and all remaining soft tissue. Distribution of the various lipids is presented in Table III. The values for the whole soft body were computed from determinations of the various parts. When the whole snail is considered, some 65 mμmoles of acetate was incorporated into fatty acid per gram of wet weight. The hepatopancreas incorporated 88 mμmoles of acetate per gram wet weight, - the highest amount of all the tissues studied. The foot tissue incorporated 27 mμmoles of acetate per gram wet weight. The albumen gland and other reproductive tissue had a much lower amount of incorporation. The albumen gland incorporated 9 mμmoles of acetate and the other reproductive tissue incorporated 15.6

TABLE III

IN VIVO INCORPORATION OF ACETATE-1-C^{14} INTO TOTAL

LIPIDS OF SELECTED SNAIL TISSUES

	Whole Soft Body (33.04 g. wet wt.)	Foot Tissue (1.89 g. wet wt.)	Albumen Gland (0.94 g. wet wt.)	Hepato-pancreas (5.42 g. wet wt.)	Reproductive Tissue (1.97 g. wet wt.)	All Other Soft Tissue* (22.80 g. wet wt.)
Total dpm incorp.	1,333,677.0	43,554.0	5,291.0	296,102.0	19,099.0	969,802.0
$\frac{\text{dpm incorp.}}{\text{dpm injected}}$ X (100)	2.63	0.08	0.01	0.58	0.04	1.95
dpm incorp. per g. wet wt.	40,374.0	22,992.0	5,610.0	54,608.0	9,685.0	42,524.0
mμmoles acetate incorp. per g. wet weight	65.11	37.1	9.0	87.9	15.6	68.6

Eleven snails were injected with 50 μl of buffered sodium acetate-1-C^{14} (7.5 μmoles, 4.6 x 10^6 dpm). The various tissues were dissected, bulked, and extracted 24 hours after injection.

*This includes the gut, mouth parts, mantle, nervous tissue, salivary glands, and other organs and tissues in the "head."

mμmoles of acetate per gram of wet weight.

To determine the distribution of radioactivity in the various lipid components, aliquots of the lipid extracts were separated by thin layer chromatography using a neutral lipid solvent. The results are summarized in Table IV. The very small amount of radioactivity in the albumen gland prevented its analysis by this method.

In foot tissue, reproductive tissue, and the remainder of the soft body, most of the radioactivity from acetate was found in the phospholipids. None of the other lipids in these three extracts contained an appreciable amount of radioactivity except perhaps for the triglycerides, sterols and hydrocarbons from the reproductive tissue. Monoglyceride spots were not detected in these three extracts.

Unlike other tissues, hepatopancreas acetate incorporation was primarily into neutral lipids. The phospholipids contained 25 per cent of the radioactivity. Monoglycerides, free fatty acids, triglycerides, and sterol esters and hydrocarbons each contained 14 to 18 per cent of the radioactivity. Diglyceride contained 4 per cent and cholesterol 6.8 per cent of the radioactivity.

Tissue Mince Incubations

Finding radioactivity in a tissue after _in vivo_ incubation does not necessarily mean that tissue has the ability to synthesize lipids from acetate-1-C^{14}. Lipid could be formed in one tissue and transported to another. A series of experiments were designed to determine the ability of isolated tissues to incorporate acetate into lipids.

The results of such experiments with tissues of _O. lactea_ are presented in Table V. The results show that increasing amounts of

TABLE IV

DISTRIBUTION OF RADIOACTIVITY IN LIPIDS OF SNAIL PARTS AFTER

IN VIVO INCORPORATION OF ACETATE-1-C^{14}

Lipid	Per cent of Total dpm Incorporated			
	Foot Tissue	Reproductive Tissue	Hepatopancreas	Soft Body Minus Dissected Tissue
Phospholipids	83.3	65.9	18.4	85.4
Monoglycerides	———	———	14.1	———
Diglycerides	2.0	1.7	3.9	1.3
Triglycerides	5.7	15.6	18.4	4.5
Fatty Acids	2.0	5.1	15.7	1.9
Cholesterol	4.0	2.6	6.8	2.5
Sterol Esters and Hydrocarbons	3.0	9.0	15.7	4.4

Lipids were separated on Silica Gel G coated thin layer chromatographic plates. The plates were developed twice with hexane: ether: acetic acid (60:40:1). Albumen gland lipid separation was omitted due to its low incorporation.

TABLE V

INCORPORATION OF ACETATE-1-C^{14} INTO LIPIDS

BY ISOLATED TISSUE MINCES

	g. wet wt.	total incorp.-dpm	mμMoles acetate incorp.
Hepatopancreas	.20	1,286.	2.07
	.40	3,470.	5.58
	.80	4,976.	8.01
Foot Muscle	.22	5,986.	9.63
	.48	13,821.	22.25
	.75	15,951.	25.68
Reproductive Tissue	.20	8,546.	13.76
	.44	10,046.	16.17
	.79	16,121.	25.95
Albumen Gland	.29	4,896.	7.88
	.38	7,101.	11.43
	.66	7,796.	12.55

Tissue minces were incubated in 5.9 mls. potassium phosphate buffer (.05M., pH 7.5) and .1 mls Na acetate-1-C^{14} (30 μmoles, 1.87×10^7 dpm) for two hours with N_2 at 35°C on a metabolic shaker. Boiled control values were subtracted to give the above experimental values.

isolated tissue mince incorporate increasing amounts of acetate into lipid. The values in Table V represent the amount of radioactivity above that of controls run at the same time. The highest rates of lipid synthesis from acetate were observed with foot and reproductive tissue. The albumen gland mince had considerably less incorporation. Contradicting previous suggestions, the hepatopancreas mince incorporated less acetate-1-C^{14} into lipid than did the foot or reproductive tissue. It is important to realize the physical nature of the dissected organs. The albumen gland and hepatopancreas are very soft, jelly-like tissues while the foot and reproductive tissue are very tough and fibrous. It is very easy to destroy the integrity of the hepatopancreas during dissection since the intestine is imbedded in it. The albumen gland, even though it is soft, is easy to remove intact. It may be that the small amount of incorporation into the hepatopancreas is due to the disruption of the tissue during removal and mincing with the resulting release of digestive enzymes. Also, the conditions of these mince incubations may not have been optimal. However, from the results of Table V it is definite that all tissues studied have the ability to incorporate acetate-1-C^{14} into lipids. The hepatopancreas proved to be quite heat stable. Mince of this organ had to be boiled at least 10 minutes to prevent lipid synthesis.

Hepatopancreas lipids of _in vivo_ incubated snails contained the largest amount of acetate incorporation per gram of wet weight while the isolated mince incorporated the least amount of acetate. These results suggest that either the mince loses its ability to incorporate acetate into lipids or the experimental conditions were not optimal. To demonstrate the effect of experimental conditions upon acetate incorporation

in the original mince, the hepatopancreas mince was incubated at varying pH's. As shown in Figure I, the optimum pH for hepatopancreas mince incubations appeared to be 9.0 and not 7.5, the pH that had been used in the previous mince experiments to demonstrate isolated tissue incorporation. The incorporation of acetate-1-C^{14} at pH 9.0 was approximate twice as much as at pH 7.5. It may be that the pH conditions of the other tissue mince incubations were also not optimal. However, the determination of the optimum conditions was not the purpose of these incubations. The main purpose was to determine if the isolated tissue could incorporate acetate into lipids.

Subcellular Incubations

Hepatopancreas was used for further studies because it was plentiful enough to obtain in sufficient quantities. Homogenizing and fractionating the foot tissue proved unfruitful due to the mucous and fibrous nature of the tissue. Other tissues could not be dissected in adequate quantities.

Determination of the subcellular site of acetate incorporation could give some indication of the pathway(s) of fatty acid synthesis in the tissues of *Otala lactea*. To obtain an idea as to where in the cell the acetate-1-C^{14} was incorporated into fatty acids, subcellular incubations of the hepatopancreas were performed. Table VI shows the amount of incorporation obtained with the hepatopancreas subcellular fractions. Only the homogenate minus the cell debris (500 x g supernatant) and the mitochondria showed any definite incorporation. Therefore, it appears that the mitochondria was the most active fraction for incorporation of acetate into lipids. Addition of cofactors made no difference in incorporation except perhaps in the soluble cytoplasm.

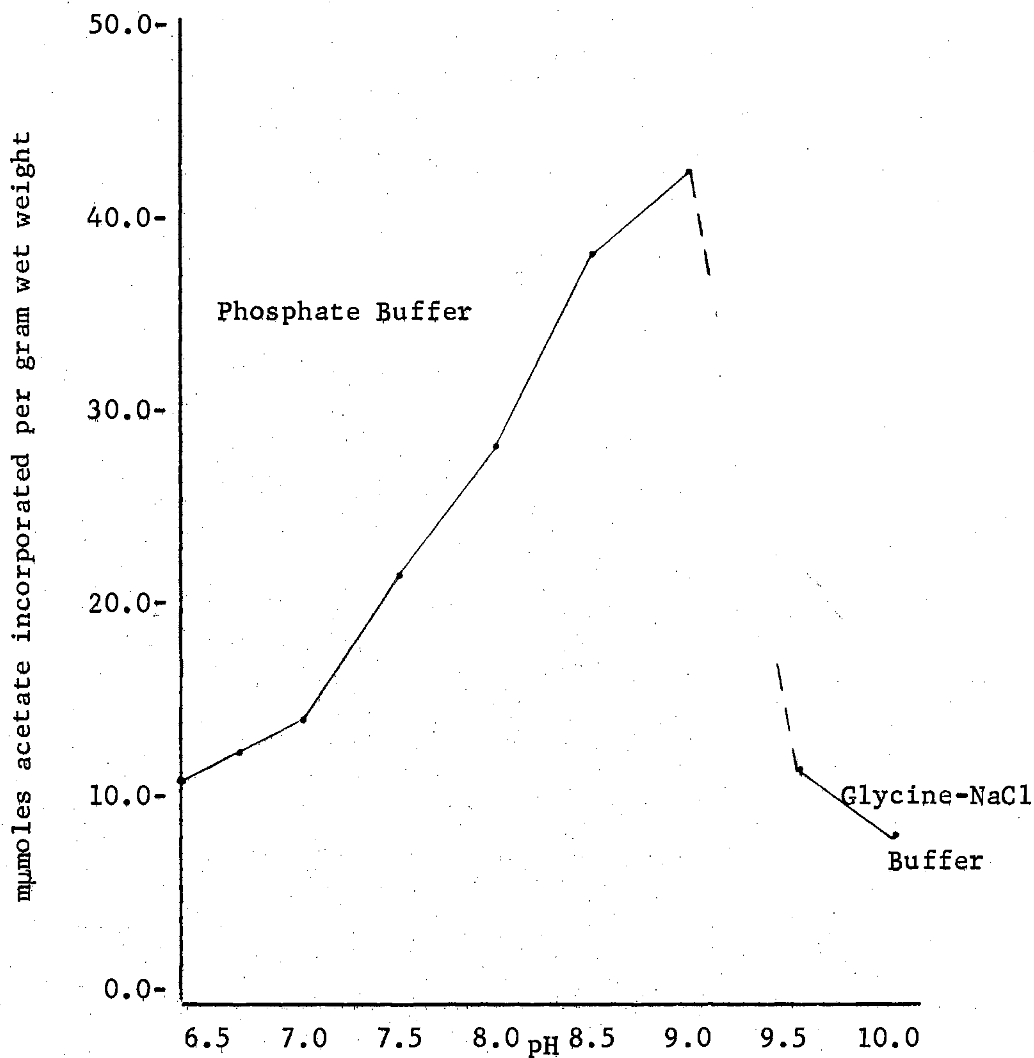


Figure 1. The Effect of pH on the Incorporation of Acetate Into Lipids of Hepatopancreas Mince.

Hepatopancreas mince (.3-.4g) was incubated in buffer (pH 6.0-9.0, potassium phosphate buffer; pH 9.5-10.0, glycine-NaCl buffer) with sodium acetate-1-C^{14} (7.5 μmoles, 4.6 x 10^6 dpm). Total volume was 6 mls. The mixture was flushed with N_2 and put on a metabolic shaker at 26°C for two hours.

TABLE VI

INCORPORATION OF ACETATE-1-C^{14} INTO FATTY ACIDS BY SUBCELLULAR FRACTIONS OF THE HEPATOPANCREAS

	Incorporation in dpm and mμmoles acetate per mg. protein per two hours			
	Additions to Cell Fractions			
	None		Cofactors	
Cell fraction	dpm	mμmoles	dpm	mμmoles
500 x g Supernatant	1756.	.102	1723.	.101
Mitochondria	2376.	.194	1674.	.135
Microsomes	203.	.052	179.	.046
Soluble Cytoplasm	124.	.006	420.	.024

Each value represents the average of six tubes. Total incubation volume was 3 mls. All incubations contained 30 μmoles Na acetate-1-C^{14} (1.86×10^7 dpm) and 50 μmoles potassium phosphate (pH 7.5). The cofactor incubations also contained: 4 μmoles ATP; .4 μmoles CoA; 1μmole NADPH; 3 μmoles $MnCl_2$; 60 μmoles Na malonate; 20 μmoles $KHCO_3$; and 10 μg. biotin. The tubes were incubated in a metabolic shaker at 26°C for two hours. Incubations were under N_2.

The same experiment as in Table VI was tried several times with the same results. Potassium fluoride, which is known to inhibit ATPase, was added in one whole homogenate experiment, with no difference in results. The whole homogenate was also incubated at pH 9.0 with no difference in results. The action of an ATPase is known to sometimes change the pH of a system as the reaction occurs. When the pH of the incubation system was followed, there was no change in the pH as time passed; however, the buffer could be adequate to prevent pH change. To allow by-passing of the requirement for ATP in fatty acid synthesis, the 500 x g supernatant was incubated with acetyl-1-C^{14} CoA. Again, there was no incorporation of acetate into fatty acids. In an effort to isolate the enzymes for lipid synthesis from their inhibitors, the soluble cytoplasm was fractionated by ammonium sulfate precipitation. Incubation of the various fractions with acetyl-1-C^{14}CoA and known cofactors produced no fatty acid synthesis. As one progressively breaks down and disrupts the hepatopancreas, there is increasingly less or no incorporation of acetate into fatty acids.

Gas-liquid Chromatographic Separation of Radioactive Fatty Acid Methyl Esters

It is known that mitochondrial elongation of fatty acids usually takes place by elongating palmitic acid. Palmitic acid and lower carbon fatty acids are either synthesized *de novo* or acquired in the diet. Detection of significant quantities of radioactivity in fatty acids with 16 or less carbons would suggest that there was *de novo* synthesis. Fatty acids isolated from the *in vivo* experiments with the hepatopancreas

were methylated and analyzed by gas-liquid chromatography. The individual methyl esters were collected on anthracene as they were eluted from the column. Results of the distribution of radioactivity in the various esters is shown in Table VII. While 7.5 per cent of the recovered radioactivity was in 14 carbon fatty acids, 27 per cent was in the 16 carbon fatty acids. This would suggest that there is a *de novo* synthesis in the snail. The 18 carbon fatty acids contained 43 per cent of the recovered radioactivity; 20 carbons, 9.5 per cent; and 22 carbons, 4.4 per cent. The activity in the 18, 20 and 22 carbon fatty acids suggests that there is an elongation system(s) for producing fatty acids with more than 16 carbons.

Decarboxylation of Radioactive Fatty Acids

To further determine the mechanisms of fatty acid synthesis in the snail, hepatopancreas fatty acids from *in vivo* incubations were decarboxylated (Table VIII). If the incorporation of acetate-1-C^{14} was by only a mitochondrial elongation, removal of the carboxyl group would remove almost 100 per cent of the radioactivity of the fatty acid. If the incorporation of acetate-1-C^{14} into fatty acids was by *de novo* synthesis, every other carbon would be radioactive. Thus, removal of the terminal carboxyl group would remove a much smaller per cent of the total radioactivity from the fatty acids.

Decarboxylation removed 19.2 per cent of the radioactivity from the fatty acids. The removal of only one-fifth of the radioactivity from the experimental fatty acid indicates that there is both *de novo* and mitochondrial incorporation of acetate-1-C^{14} into fatty acids in the snail *Otala lactea*.

TABLE VII

DISTRIBUTION OF HEPATOPANCREAS FATTY ACIDS AND RADIOACTIVITY IN FATTY ACIDS EXTRACTED FROM *IN VIVO* INCUBATIONS WITH ACETATE-1-C^{14}

Fatty Acid Methyl Ester	Mole Per cent of Total Fatty Acids	Per cent of Radioactivity
10:0	.01	.12
12:0	.55	.22
12:1?	2.17	.33
14:0	1.02	7.48
15:0	.23	1.45
15:1	.39	.46
16:0	5.04	26.03
16:1	.24	1.80
16:2	.09	1.48
16:3	7.03	2.39
18:0	11.82	21.63
18:1	15.30	10.68
18:2	12.10	8.99
18:3	6.89	1.68
20:0?	.20	0.0
20:1	3.83	2.74
20:2	11.05	6.81
22:iso?	1.10	.75
22:0	13.60	2.56
22:1	4.02	1.13
23:0?	1.34	.89
23:1?	1.34	.36

TABLE VIII

DECARBOXYLATION OF HEPATOPANCREAS FATTY ACIDS FROM

IN VIVO INCUBATIONS WITH ACETATE-1-C^{14}

	DPM			
	Fatty Acid	$C^{14}O_2$	$C^{14}O_2$/Fatty Acid	Fatty Acid/$C^{14}O_2$
Experimental	2477.	412.	.192	6.0
Control	3201.	2913.	.910	1.1

Each Thunberg tube (8.5 cm. long) contained 2,000-4,000 dpm of hepatopancreas fatty acids or palmitic-1-C^{14} acid and palmitic acid, 50 mg. sodium azide, .5 mls. fuming H_2SO_4: conc. H_2SO_4 (1:3), and the side-arm contained .3mls. hydroxide of hyamine. Decarboxylation was in an 80°C water bath for 1.5 hours and at 37°C for 20 minutes in a metabolic shaker.

CHAPTER V

DISCUSSION

The total lipid content of selected tissues of *Otala lactea* appeared to be rather low when compared to mammals. The per cent of wet weight varied from 1.4 per cent in reproductive tissue to 2.2 per cent in the hepatopancreas, as compared to 2 to 3 per cent for humans (Guyton, 1967). Previous investigation by Thiele (1959) showed the lipid content of *H. pomatia* soft tissue to be approximately 1.7 per cent of wet weight.

The lipids of hepatopancreas and albumen gland were composed of approximately equal amounts of neutral and phospholipid, while the foot and reproductive tissue was approximately 70 per cent phospholipid. The fatty acids were predominantly fatty acids with 18 carbons, however, significant amounts of palmitic acid and some fatty acid with 20 carbons was also present. The distribution of fatty acid in *O. lactea* appears to be quite similar to that found by Thiele (1963) in *H. pomatia*. The albumen gland contained 40 per cent of a fatty acid which is tentatively identified as a fatty acid with 13 carbons. Thiele (1963) was unable to identify a fatty acid of this approximate molecular weight which he isolated from the cholesterol esters of *H. pomatia*. Forty mole percent of all the fatty acids from the four tissues extracted in January were saturated fatty acids. In *H. pomatia* whose lipids were extracted in the month of May, Thiele (1963) found 56 per cent of the fatty acids were

saturated. It is hard to make correlations between investigations since the amount of unsaturation of total lipids is known to be higher in the late winter and increasingly lower through the summer months (Thiele, 1959). Of course, the snails are not of the same species.

With the _in vivo_ experiments the distribution of radioactivity (on a per gram wet weight basis) in the total lipids was greater in the hepatopancreas than in any of the other individual tissues. The incorporation of acetate-1-C^{14} was extremely low in the albumen gland and other reproductive tissue; thus, and not surprising, the two tissues with the lowest lipid content contained the least amount of radioactive lipids. The foot tissue had the second highest amount of radioactivity per gram wet weight. All parts contained radioactivity in relative proportion to their lipid per cent of wet weight.

Acetate was incorporated into phospholipids in the majority of the snail tissues. With the _in vivo_ experiments, 83 per cent of the radioactivity was in phospholipids in all tissues studies except for the hepatopancreas. In hepatopancreas, 75 per cent of the radioactivity was in the neutral lipids. The higher per cent of radioactivity in neutral lipids of hepatopancreas suggests that the hepatopancreas may be: (1) synthesizing the neutral lipids for distribution to other areas, (2) act as a storage area for newly synthesized neutral lipids or (3) serving as a site for the re-oxidization of reduced TPNH.

All isolated tissue minces incorporated acetate-1-C^{14} into lipid; however, the distribution of radioactivity on a per gram wet weight basis was not exactly the same as the _in vivo_ experiments. With the _in vivo_ incorporation, the foot tissue incorporated 37 mμmoles of acetate per gram of wet weight while the hepatopancreas incorporated

88 mμmoles. At pH 7.5, the foot mince incorporated 40 mμmoles of acetate per gram wet weight and the hepatopancreas mince incorporated 11 mμmoles of acetate per gram of wet weight. At optimum pH, 9.0, the hepatopancreas mince incorporated 43 mμmoles of acetate per gram of wet weight, which is still below the *in vivo* experiment. These results suggest that *in vitro* either the hepatopancreas loses some of its ability to incorporate acetate-1-C^{14} into lipids or the incubation conditions were not optimal.

Incubation of hepatopancreas homogenate and subcellular fractions demonstrated little or no ability to incorporate acetate-1-C^{14} into fatty acids. The small amount of subcellular incorporation was greatest in the mitochondria. Experiments with an ATPase inhibitor (KF) and employing acetyl-1-C^{14} CoA, to eliminate the need for ATP, did not increase the rate of fatty acid synthesis. Incubation of protein fractions obtained by ammonium sulfate precipitation of the soluble cytoplasm failed to mediate the synthesis of fatty acids in the experimental system.

The low rate of incorporation of acetate-1-C^{14} by homogenates and subcellular fractions suggests the loss of lipid synthesis ability as the hepatopancreas is broken down. When the hepatopancreas is removed from the snail, disruption undoubtedly causes the release of digestive enzymes. As the hepatopancreas is minced or homogenized, more digestive enzymes would be released. The release of digestive enzymes may be destroying the enzymes needed for the synthesis of fatty acids.

This possibility finds support in earlier investigations where hepatopancreas homogenates were demonstrated to possess the enzymes necessary to carry out the Krebs cycle, but oxidative phosphorylation

could not be demonstrated (Rees, 1953). Further, Bryant (1965) found that homogenates of the whole snail metabolized glucose-1-C^{14} but hepatopancreas homogenates did not.

Since little acetate-1-C^{14} was incorporated into non-volatile fatty acids by subcellular fractions, the determination of the mechanism of fatty acid synthesis was approached in two other ways. These involved gas chromatographic separation or decarboxylation of fatty acids obtained from the hepatopancreas after _in vivo_ incubation with acetate-1-C^{14}.

Gas chromatographic separations showed that the 14, 16, 18, 20 and 22 carbon fatty acids contained radioactivity. Palmitic acid contained 26 per cent and fatty acids with 18 carbons contained 43 per cent of the radioactivity. When the ratio of the per cent radioactivity to mole per cent composition is considered, myristic and palmitic acid are much higher in specific activity than other fatty acids. As has been previously reviewed, mitochondria do not incorporate acetate into fatty acids shorter than 16 carbons. Therefore, the detection of radioactivity in the 14 and 16 carbon fatty acids suggests a _de novo_ synthesis. Appreciable radioactivity in the 18 carbon fatty acids suggests the presence of an elongation system(s).

Decarboxylation of _de novo_ synthesized fatty acids (palmitic) would remove approximately one-eighth of the radioactivity while decarboxylation of mitochondrally elongated fatty acids (mainly 18 carbon fatty acids would remove almost 100 per cent of the incorporated radioactivity. Decarboxylation of radioactive fatty acids extracted from the hepatopancreas of the snails utilized in the _in vivo_ experiments removed one-fifth of the radioactivity. Removal of only one-fifth of the radioactivity upon decarboxylation further implies the existence of

de novo and elongation incorporation of acetate-1-C^{14} into fatty acids by the terrestrial snail *Otala lactea*.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Experiments were carried out to determine the lipid content of the hepatopancreas, albumen gland, reproductive tissue and foot tissue in the edible snail Otala lactea. Incorporation of acetate-1-C^{14} into lipids of these same tissues was studied in vivo and in vitro. The following results were obtained.

1. The lipid content of the tissues was low, varying from 1.4 to 2.2 per cent of wet weight. The lipids were 40 to 73 per cent phospholipids. Palmitic acid and fatty acids with 18 carbons were the predominant fatty acids.

2. Acetate-1-C^{14} incorporated in vivo was found mainly in the phospholipids of the various tissues, except in the hepatopancreas extracts. All tissue minces studied incorporated acetate-1-C^{14} into lipids. Hepatopancreas homogenates and subcellular fractions incorporated little or no acetate-1-C^{14} into fatty acids.

3. Hepatopancreas fatty acids obtained from in vivo incubations incorporated acetate-1-C^{14} into even numbered fatty acids with 14 to 22 carbons. Decarboxylation of these same fatty acids removed one-fifth of the total radioactivity.

It appears that the lipid content as well as lipid biosynthesis is quite low in Otala lactea. Phospholipids were the main lipids produced from incorporated acetate, suggesting a primary use as structural lipids.

Hepatopancreas from *in vivo* incubations had the greatest amount of acetate incorporation per gram of wet weight. It also had a majority of its incorporated acetate located in the neutral lipids. Dissection of the hepatopancreas resulted in decreasing lipid synthesis, presumably as a result of released digestive enzymes.

Analysis of the fatty acids isolated from experiments with intact snails suggest *de novo* and chain elongation pathways for fatty acid synthesis. This in turn suggests that one of the physiological roles of fatty acid metabolism in the snail is the oxidation of reduced pyridine nucleotides.

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