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ARTICLE *in* JOURNAL OF EXPERIMENTAL ZOOLOGY · APRIL 1998

DOI: 10.1002/(SICI)1097-010X(19980415)280:6<375::AID-JEZ1>3.0.CO;2-K

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Biochemical Composition and Energy Sources During Embryo Development and in Early Juveniles of the Snail *Pomacea canaliculata* (Mollusca: Gastropoda)

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ABSTRACT Energy partitioning within the developing eggs and early juveniles of the gastropod *Pomacea canaliculata* was investigated from the time of fertilization to newly hatched snails. The forms and locations of the energy stored as well as their changes while development proceeded were studied in six stages (from morula to 3-day hatchlings). The rates of utilization of different lipid classes, protein classes, and total carbohydrates were measured for the first time in embryos and perivitelline fluid.

Perivitelline fluid is the primary energy store. Its biochemical composition at stage I is represented by carbohydrates, proteins, and lipids with 34.8%, 13.0%, and 1.5% dry weight, respectively. Dry weight of eggs showed no significant increase during development, indicating there was no important mass exchange with the medium.

Carbohydrates and proteins were identified as the major energy-providing components, and lipids as the minor one, contributing 142, 56, and 12 cal/100 mg egg, dry weight, respectively. The overall conversion efficiency (CE) was 32.8% (calculated as percentage of perivitellus energy transformed into embryonic tissues). Carbohydrates were the most important energy reserve supplying virtually all the catabolic demand (CE 14.6%).

Protein electrophoretic profiles during embryogenesis showed three distinct phases: An accumulation period (up to stage IV); a more active accumulation and selective utilization phase (stage V), and a selective consumption and protein synthesis period (hatchlings). Structural lipids from perivitellus (phospholipids and free sterols) were selectively conserved in embryos and juveniles, whereas triacylglycerols, hydrocarbons, and esterified sterols were preferentially depleted by hatchlings.

Therefore, protein and lipid reserves in *P. canaliculata* perivitellus provide structural precursors during embryogenesis, while they partially contribute to the energy supplied by carbohydrates. *J. Exp. Zool. 280:375–383, 1998.*

Physiological energetics deals with the study of gains and losses of energy and the efficiency of energy transformation from the standpoint of the whole organism. Studies on energetics during embryogenesis in invertebrates are difficult to compare, as authors employ different methodologies using either direct calorimetry or quantitating of the different egg biochemical components and their energetic equivalents (Gabbott, '75; Holland, '78; Beningher, '84; Beningher and Lucas, '84; McEdward et al., '88; Whyte et al., '92).

Conversion efficiency (CE), calculated here as the percentage of perivitellus energy transformed into embryonic tissues, gives a rough estimate of the amount of each constituent used by the embryos for growth, energy reserve, or metabolism. In many cases, CE is significantly higher for proteins than for lipids. This finding is consistent with the assumption that during development amino acids are conserved to build the structure of embryos, whereas lipids serve as fuel for active cell division and differentiation (Holland, '78).

Among molluscs, bivalves are the group that has been more extensively studied in terms of consumption profiles for different energy sources. It has been found that eggs accumulated high levels of proteins and lipids compared to carbohydrates,

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Received 12 December 1996; Accepted 11 November 1997

clearly showing that carbohydrate is not the major reserve for egg development (for reviews, see Holland, '78; Sastry, '79; Gabbott, '83). In cephalopods, Heras ('90) found that, during vitellogenesis, ovocytes of *Octopus tehuelchus* store mainly lipids and proteins and only a minor amount of carbohydrates. Energy stores in four species of the gastropod *Littorina* were studied by Holland et al. ('75), who determined that neutral lipids are the main energy source during development and metamorphosis. They did not find any evidence of either protein or carbohydrate consumption. Unlike the above mentioned examples for *Littorina* and other molluscs, many gastropods store carbohydrates for embryo development (Pandian, '69; Goudsmit, '72). In general, galactogen seems to be an important carbohydrate reserve in pulmonate and some prosobranch snail eggs, comprising 29.7% dry weight in *Helix pomatia* (Raven, '72) and 36.1% dry weight in *Lymnaea stagnalis* (Horstmann, '56).

Gastropod eggs also differ from others in that vitellus is not an important energy source, this role being assumed by perivitelline fluid. The proteinaceous yolk in snails is mainly composed of proteolytic enzymes (primary lysosomes), which would be activated during embryo development to cope with the uptake and utilization of perivitelline fluid (de Jong-Brink et al., '83). Perivitelline fluid is mainly synthesized in the albumen gland and represents the major source of nutrients for the embryo (Wijsman and van-Wijck-Batenburg, '87). Studies in some pulmonate snails showed that the perivitelline fluid was composed of calcium, proteins, and polysaccharides, but lipids were not detected (Hortsmann, '56; Raven, '72; de Jong-Brink et al., '83). A more detailed analysis of perivitelline carbohydrates in the snail *Pomacea canaliculata* showed that they are composed of galactose and fucose units (Raven, '72). Bayne ('66, '67) studied the composition of the perivitelline fluid in the slug *Agriolimax reticulatus.* He found galactose, glucosamine, and free amino acids, but he did not detect either lipids or proteins.

Other perivitelline fluid components have been less studied. Morrill et al. ('64), reported 11 protein bands in an electrophoretic study of the perivitellus in *Lymnaea,* and they also showed protein consumption in *L. palustris* embryos during development. There are few data about the presence of lipids in this fluid in gastropods, among them the presence of a carotenoprotein in *P. canaliculata* (Cheesman, '58), some histochemical studies (e.g., Kessel, '82), and our report of three lipoproteins in *P. canaliculata* (Garin et al., '96).

In the present work, we studied energy partitioning within the developing eggs and embryos of *P. canaliculata* by determining under what forms and where energy is stored, and the subsequent changes as development proceeds.

MATERIALS AND METHODS

Sample collection

Egg masses from *P. canaliculata* were collected from females raised in our laboratory during summer and early fall (December to March). Clutches cemented to plants above water level were collected and kept in the lab at 25°C until hatching. At this temperature, hatching lasted 15 to 16 days. Embryo size and developing stage were checked daily in each egg mass using a stereoscopic microscope (Nikon SMZ-10, Tokyo, Japan). Embryos were suspended in NaCl 75 mM to remove the perivitelline fluid without affecting cell tonicity for microscopic observation. Five developing stages were defined using major developmental events. Stage I comprises embryos from four-cell morula to gastrula (less than 250 μm size) with a highly viscous perivitelline fluid; Stage II (250 to 900 μm): embryos from gastrula until endodermal mass is visible and the shell becomes apparent; Stage III (900 to 1,500 μm): Endodermal mass is divided into two lobules and the shell is bigger, without visible operculum; Stage IV (1,500–2,000 μm): Embryo is enclosed by the shell, which bears an operculum; Stage V (2,000–3,000 μm): Pigmentation of shell appears; Stage VI comprises juveniles with fewer than 3 days of free life. In general, perivitelline fluid decreases its viscosity during development.

Wet weight and moisture were obtained from egg masses in each developing stage. Moisture was determined by drying samples at 100°C for 24 h (IAFMM, '79). Samples were analyzed in triplicate, and each replica was composed of pools from three egg masses.

Isolation of embryos and perivitelline fluid

Eggs were weighed, then egg shells, perivitelline fluid, and embryos were separated from each other. Because of the high viscosity of perivitellus and the size of the morula, embryos from stage I were almost impossible to isolate free of perivitelline fluid. Therefore, at this stage, we studied whole egg homogenates without egg shells; shells were separated by mild centrifugation.

Stages II, III, and IV: Embryo, egg shell and perivitellus were separated using a Percoll® discontinuous density gradient (Pharmacia LKB,

Uppsala, Sweden) with solutions of 100%, 50%, and 25% Percoll diluted with NaCl 75 mM. Samples were loaded onto the gradient and then centrifuged at 400*g* for 10 min. Thus, a shell-containing pellet was formed while the embryos were located in the interphase of the two Percoll solutions, and the perivitelline fluid floated on top of the gradient. For stage II, 50% and 25% solutions were used; 100% and 50% Percoll solutions were used for stages III and IV. Stage V embryos were manually isolated from perivitellus under a stereoscopic microscope and washed repeatedly with a solution of NaCl 75 mM. Stage VI juvenile snails were collected manually in their first 3 days of free life.

Embryos were homogenized in a Potter type homogenizer (Thomas Sci., Swedesvoro, NJ) using Tris-HCl buffer 0.02 M, pH 7.5 containing 2 mg/ml aprotinin (Trasylol, Mobay Chemical Co., New York). The buffer: sample ratio was kept at 3:1 v/v. All samples obtained were frozen at –70°C until analysis.

Protein, lipid, and carbohydrate determination

Proteins were determined by the method of Markwell et al. ('78), using bovine serum albumin (BSA) as standard. Samples and BSA were previously treated with NaOH 1N 5:1 v/v, vortexed, and incubated overnight at 37°C.

Lipids were extracted with a chloroform-methanol mixture following the method of Bligh and Dyer ('59), and total lipid concentration was determined gravimetrically.

Carbohydrates were determined following the spectrophotometric procedure of van Handel ('65). Carbohydrates were purified from snail eggs to be employed as standard to avoid errors caused by the differential response of the anthrone reagent to different carbohydrates, following the procedure of Rendina ('74) with some modifications. In short, eggs were homogenized and digested with 33% KOH at 100°C for 20 min, and carbohydrates were precipitated with saturated Na_2SO_4 and cold ethanol. Samples were then heated at 90°C for 5 min, cooled, and centrifuged at 2,000*g* for 10 min. Supernatant was discarded, and pellet was redissolved in cold TCA 10%. After centrifugation at 2,000*g* for 10 min, the pellet containing precipitated proteins was discarded. Supernatant was dissolved in water, and the process was repeated twice. Finally, carbohydrates were suspended in absolute ethanol, centrifuged, and the carbohydrate pellet dried at 40°C until a constant weight was obtained.

Gel electrophoresis

Perivitelline fluid and homogenized embryos from each stage were analyzed by native and dissociating electrophoresis. Nondissociating electrophoresis analysis was done by using a 4%–20% acrylamide gradient gel electrophoresis (PAGGE) (Davis, '64). Apoproteins from each fraction were analyzed by SDS-PAGGE using a gradient of 4%–23% acrylamide (Laemmli, '70). Proteins were visualized with Coomassie Brilliant Blue R-250 staining (Sigma Chemical Co., St. Louis, MO) and checked for minor bands by silver staining (Merril, '90). Molecular weight standards (Pharmacia, Uppsala, Sweden) were run in the same gel slabs. Molecular weight of native proteins was calculated by the method of Felgenhauer ('74).

Lipid analysis

Ninety percent of each sample was used for lipid analysis. Lipid class analysis was performed by thin layer chromatography (TLC) on silica gel Chromarods (type S-III) with quantitation by flame ionization detection using an Iatroscan TH-10, Mark III (Iatron Laboratories Inc., Tokyo, Japan) as described by Parrish and Ackman ('85). The separation was conducted with a sequence of three different solvent systems. The first development was carried out for 45 min in hexane:diethyl ether:ethyl acetate:formic acid (91:6:3:1 v/v/v/v). Chromarods were dried, partially scanned to analyze neutral lipids, and then developed in acetone for 15 min to quantify a carotenoid peak. Finally, the Chromarods were developed in chloroform: methanol: formic acid: water (50:30:4:2 v/v/v/v) for 60 min and completely scanned to reveal the different phospholipids. The set of Chromarods was divided into lots containing five rods. Each lot was considered one analytical unit and calibrated independently. Tetracosanol was used as an internal standard, and quantitation was performed by obtaining calibration curves of authentic standards run under the same conditions. Lipids were also identified on HP-TLC plates (Merck, Darmstadt, Germany) developed with hexane:diethyl ether: acetic acid (80:20:1.5 v/v/v) for neutral lipids and chloroform:methanol:acetic acid:water (65:25:4:4 v/v/v/v) for polar lipids. Standard lipids, iodine vapors, and specific reagents were used to identify lipid classes. Preparative HP-TLC developed for neutral lipids as above was also used to isolate an egg carotenoid pigment, which was then employed as a standard for Chromarod calibration.

Energy conversion factors

We employed the energy conversion factors described by Beningher ('84), which were calculated for aquatic invertebrates: Carbohydrates: 4.1 kcal/g or 17.2 kJ/g; proteins: 4.3 kcal/g or 17.9 kJ/g, and lipids: 7.9 kcal/g or 33.0 kJ/g.

Statistical analyses

Data collected from all experiments were analyzed by ANOVA using Instat v. 2.0. Where significant differences between samples occurred, a post-hoc Tukey's HSD test was performed to identify the differing means when significant differences were found among samples. Results were considered significant at the 5% level.

RESULTS

The use of Percoll gradients allowed us to separate the perivitelline fluid from embryos and egg shells of *Pomacea canaliculata,* in order to analyze them separately for the first time in this species. Dry weight of eggs showed a slight increase during development from 24.5% (stage I) to 30.3% (stage V), which was not statistically significant, indicating there was no important mass exchange with the medium.

Energetic changes during development

The initial composition of perivitelline fluid showed that the major nutrients were carbohydrates (34.8% dry weight) followed by proteins (13.0% dry weight) and lipids (1.5% dry weight) (Fig. 1). From the biochemical composition of the developing eggs, it was possible to calculate the equivalent calories depicted in Figure 2. The overall conversion efficiency was 32.8%. The formula used for that calculation was (final weight/initial weight) × 100 (Holland, '78). All reserves displayed significant changes from stages IV to V (Table 1), evidencing a net fall in the perivitelline fluid and an increase in the embryo content. The conversion efficiency for carbohydrates was 14.6%, 29.0% for proteins, and 268.8% for lipids.

Changes in protein electrophoretic profiles during egg development

Figure 3 shows the electrophoretic pattern during development. Perivitelline fluid from stage I to V (Fig. 3A) shows the lipoprotein bands corresponding to the previously reported perivitellins PV1 (300 kD), PV2 (400 kD), and PV3 (100, 64, and 26 kD) (Garin et al., '96). PV1 is the most abundant lipoprotein, followed by PV2. PV3 is a minor component better identified in Figure 3B by its 21 kD apoproteic band. Embryos from stage II to IV absorb PV1 from perivitellus (Fig. 3A) and to a lesser extent, PV2 (67 kD band of Fig. 3B). In the embryo, new proteins are synthesized such as one of 560 kD, which becomes quantitatively more important at the end of development. Embryos at stage V displayed a pattern characterized by a higher concentration of PV2 and PV3 (Figs. 3A and B, respectively). After hatching, only PV1 and the 560-kD protein were found to be the major proteins. On the other hand, PV2 (Fig. 3A) and PV3 were almost absent, probably having been used as an endogenous source of energy and structural molecules during the transition to free life (Fig. 3B).

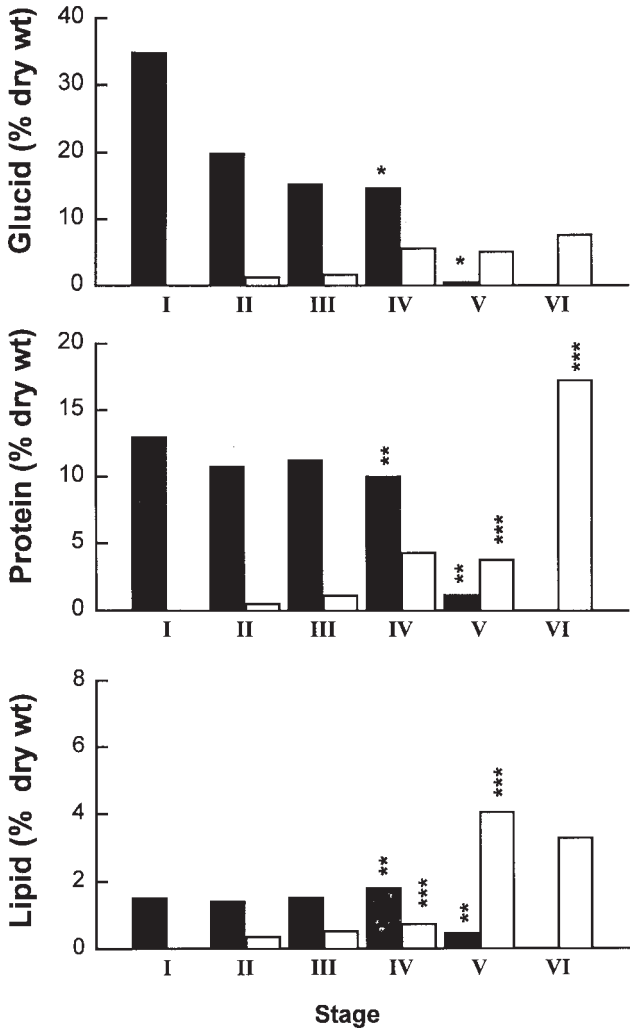

Fig. 1. Changes in glucid, protein and lipid content in embryos (open bars) and perivitelline fluid (solid bars) during development. Values are expressed as % dry weight. Bars with different characters on top are significantly different from the next bar. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

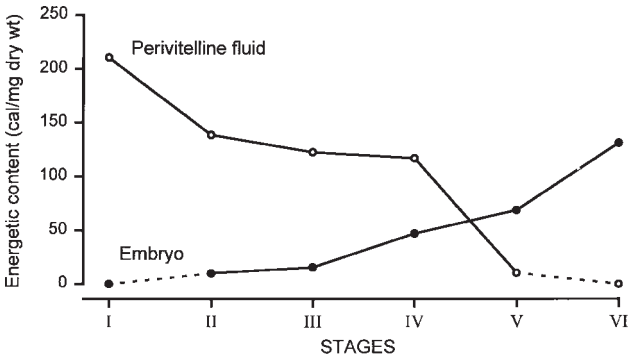

Fig. 2. Total caloric changes in embryos and perivitelline fluid during development. Values are expressed as total cal/100 mg eggs (dry weight) for each stage, and were calculated by adding protein, carbohydrate, and lipid calories.

Changes in lipid class composition during development

In order to know about lipid dynamics during development, each lipid class was studied separately in embryos and perivitelline fluid. Phospholipids and free sterols were the most important lipids in perivitelline fluid, representing more than 86% w/w at stage I. Both of them showed significant changes between stages IV and V, when they decreased more than 30% in the perivitelline fluid total lipid (Table 2). This decrease was accompanied by a rise of free sterols and phospholipids in embryos from 58% (Stage IV) to 84% w/w (Stage V) (Table 3). The carotenoid pigment was also taken up by embryos at stage V. Free fatty acids were not an important lipid in perivitellus except at stage V (9.4% w/w), while embryos showed generally higher values decreasing from 16% to 6% of total lipids during development. Triacylglycerols did not change significantly in perivitellus during development, but they showed a steady increase in embryos up to stage V, followed by a 400-fold reduction after hatching. Esterified sterols co-chromatographed with hydrocarbons and did not show any changes either in embryos or in perivitelline fluid during development, although they decreased after hatching.

TABLE 1. Caloric changes in embryos and perivitelline fluid lipids, carbohydrates and proteins during development

	Perivitelline fluid caloric content (cal)			Embryo caloric content (cal)		
Stage	Protein	Carbohydrate	Lipid	Protein	Carbohydrate	Lipid
I	56	143	12	nd	nd	nd
II	46	81	11	2	5	3
III	48	62	12	5	7	4
IV	43	60	14	18	23	6
V	5	2	4	16	21	32
VI	nd	nd	nd	74	31	26

Values are expressed as cal/100 mg eggs (dry weight) for each stage. nd, not determined.

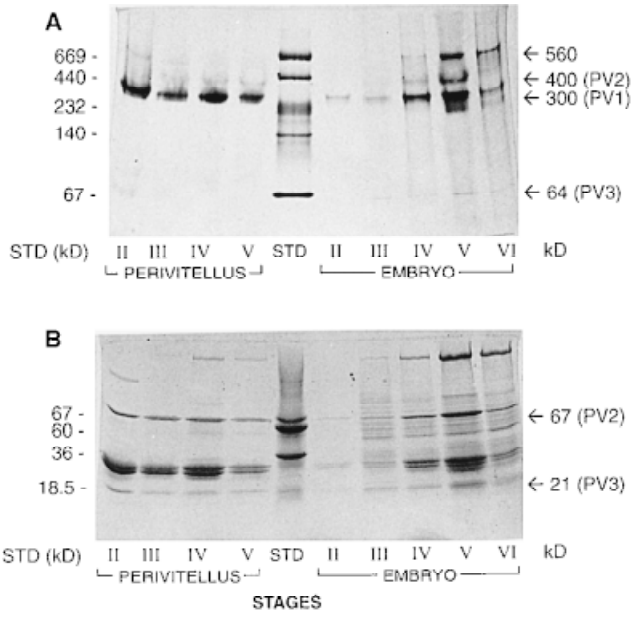

Fig. 3. Native (**A**) and dissociating gel electrophoresis (**B**) of embryo and perivitelline fluid samples from each developing stage. Gel A was prepared using an acrylamide gradient of 4–20% w/v; gel B was prepared using an acrylamide gradient of 4–23% w/v. Proteins were revealed by Coomassie blue staining. STD: High Molecular Weight standards (Pharmacia). Native proteins: Thyroglobulin (MW 669,000), Ferritin (MW 440,000), Catalase (MW 232,000), Lactate Dehydrogenase (MW 140,000) and Albumin (MW 67,000). Standards under dissociating conditions: Albumin (MW 67,000), Catalase (MW 60,000), Lactate Dehydrogenase (MW 36,000), and Ferritin (MW 18,500).

DISCUSSION

During development, *Pomacea canaliculata* egg masses, cemented to plants and other substrates above water level, are exposed for about 15 days to sunlight, air, and changing temperatures. It is interesting to note that even under these desiccating conditions, no significant changes in the water content during development were observed. It is probable that ovorubin, here called PV1, is responsible for keeping optimal conditions for the embryos (see below).

The composition of perivitelline fluid at stage I showed that carbohydrates (34.8% dry weight) were the major component followed by proteins (13.0%), but, unlike other aquatic invertebrates,

TABLE 2. Lipid class composition of P. canaliculata *perivitelline fluid during development*

Lipid classes	mg lipid/g egg wet weight (% w/w)[1]				
	Stage I	Stage II	Stage III	Stage IV	Stage V
HC+ES	95 ± 6 (2.5)	74 ± 13 (2.1)	76 ± 7 (2.0)	207 ± 100 (4.5)	181 ± 38 (13.2)
TG	60 ± 3 (1.6)	102 ± 16 (2.8)	92 ± 16 (2.4)	161 ± 44 (3.5)	174 ± 19 (12.7)
FFA	21 ± 7 (0.6)	28 ± 5 (0.8)	17 ± 3 (0.4)	118 ± 95 (2.6)	129 ± 72 (9.4)
ST	1432 ± 112 (38.2)	1192 ± 115 (33.1)	1595 ± 30 (41.0)	887 ± 143 (19.3)	119 ± 7 (8.7)
PIGM	346 ± 31 (9.2)	530 ± 72 (14.7)	358 ± 61 (9.2)	607 ± 116 (13.2)	259 ± 5 (18.9)
PE	1101 ± 63 (29.4)	733 ± 75 (20.4)	890 ± 109 (22.9)	861 ± 300 (18.8)	180 ± 48 (13.1)
PC	490 ± 94 (13.1)	601 ± 109 (16.7)	505 ± 106 (13.0)	1396 ± 121 (30.4)	189 ± 49 (13.8)
SM	205 ± 10 (5.5)	339 ± 10 (9.4)	356 ± 74 (9.2)	352 ± 120 (7.7)	138 ± 7 (10.1)
TOTAL	3750 (100%)	3600 (100%)	3890 (100%)	4590 (100%)	1370 (100%)

C + ES, hydrocarbons + esterified sterols; TG, triacylglycerols; FFA, free fatty acids; ST, free sterols; PIGM, pigment; PE, phosphatidylethanolamine; PC, phosphatidylcholine; SM, sphingomyelin.
[1]Values are the mean of triplicate analysis ± 1 SD. Values in parentheses correspond to the percentage of each lipid class w/w.

TABLE 3. Lipid class composition of P. canaliculata *embryos during development*

Lipid classes	mg lipid/g egg wet weight (% w/w)[1]				
	Stage I	Stage II	Stage III	Stage IV	Stage V
HC+ES	84 ± 30 (9.3)	142 ± 9 (10.5)	203 ± 66 (10.9)	328 ± 38 (2.7)	32 ± 20 (0.3)
TG	161 ± 51 (17.6)	186 ± 116 (13.8)	213 ± 65 (11.4)	367 ± 15 (3.0)	1 ± 0.7 (0.0)
FFA	142 ± 42 (15.6)	131 ± 22 (9.7)	154 ± 19 (8.2)	674 ± 260 (5.5)	612 ± 165 (5.6)
ST	70 ± 7 (7.7)	209 ± 36 (15.5)	334 ± 20 (17.9)	3569 ± 510 (29.1)	2543 ± 44 (23.4)
PIGM	157 ± 10 (17.3)	169 ± 17 (12.5)	214 ± 13 (11.5)	642 ± 5 (5.2)	1280 ± 90 (11.8)
PE	118 ± 36 (13.0)	265 ± 121 (19.6)	369 ± 92 (19.8)	4263 ± 113 (34.7)	2543 ± 71 (32.6)
PC	115 ± 35 (12.7)	181 ± 40 (13.4)	265 ± 11 (14.2)	1975 ± 453 (16.1)	1931 ± 152 (17.8)
SM	61 ± 6 (6.7)	67 ± 7 (5.0)	116 ± 2 (6.2)	467 ± 186 (3.8)	910 ± 77 (8.4)
TOTAL	910 (100%)	1350 (100%)	1870 (100%)	12290 (100%)	10850 (100%)

HC + ES, hydrocarbons + esterified sterols; TG, triacylglycerols; FFA, free fatty acids; ST, free sterols; PIGM, pigment; PE, phosphatidylethanolamine; PC, phosphatidylcholine; SM, sphingomyelin.
[1]Values are the mean of triplicate analysis ± 1 SD. Values in parentheses correspond to the percentage of each lipid class w/w.

only a small amount of lipids was detected (1.5%). Carbohydrate content was within the same range as that determined in other gastropods (Hortsmann, '56; Raven, '72). In a study carried out by Holland ('78) where he analyzed the biochemical composition of several bivalve eggs (*Mytilus edulis, M. californianus, Placopecten magellanicus, Spisula solidissima,* and *Mya truncata*), he found that all of them contained proteins as the major component (40% to 68% dry weight) followed by lipids (10% to 14%) and carbohydrates (1% to 6%). In the same study, crustaceans showed a more heterogeneous picture coincident with their varied life history, with protein prevailing in some cases while lipids were the most important in others, carbohydrates always being a minor component. We must bear in mind that bivalves and crustaceans store reserves as vitellus and also have different reproductive strategies from those of *P. canaliculata,* therefore only rough comparisons can be made.

Carbohydrates are the major source of energy available in perivitelline fluid for embryogenesis of *P. canaliculata.* Approximately 98% of the perivitelline fluid carbohydrate was absorbed by the embryos during development, particularly during organogenesis (stages IV and V), but only 22% was left in hatchlings, suggesting it was actively catabolized by embryos for their growth and maintenance. Adult snails store glycogen instead of galactogen as energy reserve, therefore, carbohydrates present in juveniles may be newly synthesized glycogen.

During development, protein levels undergo significant changes, decreasing in the perivitelline fluid while increasing in embryos. Morrill et al. ('64) also found that the perivitelline fluid proteins were used for the growth of *Lymnaea* embryos. Embryos took up 90% of perivitelline fluid proteins with a sharp decrease in perivitellus proteins between stages IV and V similar to that observed in carbohydrates.

Interestingly, there was no net increase in embryo protein content until hatching, suggesting that most proteins incorporated at stages IV and V must have been either consumed as an energy source or converted into other components. A trend to increase the protein level between stages III and IV was also observed. Some of these findings were clarified when the dynamics of individual protein behavior during development was studied by electrophoresis (Fig. 3). Perivitelline fluid at stage V is very poor in proteins, and it seems to conserve mostly the stored perivitellin PV1,

which is a thermostable protein. Its presence throughout development would serve as an osmotic regulator, and the pigment that PV1 contains would protect the embryo from sunlight as suggested by Cheesman ('58).

Although no net increase in the embryo protein content between stages IV and V was observed, new protein bands appeared corresponding to PV2, PV3 perivitellins, and the 560-kD protein, suggesting that the uptake systems along with the catabolic pathways of embryos are more active at these stages. It is interesting to note that these perivitellins are temporarily stored unaltered by the embryos. After hatching (stage VI), the perivitellins are rapidly depleted in juveniles. Hatchlings show an important increase in their total protein content, which is difficult to explain unless there is an external carbon source. Therefore, protein conversion efficiency was calculated between stages I and V, and the low value observed suggests they are not only used for embryo structure but also as an energy source.

As we have previously reported, lipids in the perivitelline fluid of fertilized eggs are found associated with the lipoproteins PV1, PV2, and PV3 (Garin et al., '96). In the present paper, we follow and study the lipid dynamic during development. Embryos incorporate lipids together with most of the nutrients, mainly between stages IV and V. This was reflected by a significant decrease in perivitelline fluid paralleled by a fivefold increase in the embryo lipid content at stage V. The high-energy conversion efficiency suggests an active lipid synthesis during development.

We could, therefore, suggest that the absorption of nutrients from the perivitelline fluid is divided into two phases. The first one shows a mild uptake up to stage IV followed by a second phase of very active uptake of all nutrients and selective consumption of proteins and carbohydrates from stage IV to V, where they would be utilized for organogenesis. After snail hatching, there would be a specific consumption of PV2 lipoprotein and triacylglycerols. This is merely a simplification of the real picture, as we are only considering interconversions among the different reserves. On the whole, perivitelline fluid seems to provide the embryo with both an adequate environment through development and a nourishing source particularly for late embryogenesis.

In general, the literature about energy metabolism is based on lipid consumption as a whole, without giving detailed data as to which lipid class is used as the energy source. This may cause misleading results. For example, Holland ('78) corrected the conversion efficiency values for *Balanus balanoides* and *B. balanus* (80% and 88%) and found they were 21% and 31%, respectively, considering that barnacles used triacylglycerols to a greater extent than phospholipids during egg development (Holland, '78). Similarly, in *P. canaliculata,* we might think a priori that lipids do not represent an energy source because there is an important synthesis of lipids throughout embryonic development. When we analyzed lipid class composition during development, we did find that the major lipid classes (free sterols and phospholipids) showed a significant de novo synthesis. These are mainly membrane lipids whose synthesis would result from using carbohydrates as energy and carbon sources. Other lipids such as free fatty acids, esterified sterols, and the carotenoid pigment also have high-conversion efficiency suggesting an active synthesis. On the contrary, about 98% of triacylglycerols were consumed between stages V and VI (hatchlings), where triacylglycerol concentration decreased about 400 times. This fact is also coincident with the fall of the triacylglycerol-rich PV2 lipoprotein after hatching. Triacylglycerols may be a particularly useful energy source for hatchlings during short fasting periods, but they could also provide the essential fatty acid pool used during embryo nervous system development.

Lipids in biological systems have a fundamental structural and metabolic role in biomembranes. Likewise, they have the highest energetic yield of all molecules, and they are a compact and concentrated form of energy storage. Most aquatic invertebrates have taken advantage of this latter property, and high lipid yield is a common feature in many marine species (Joseph, '89). This is the case with females that need a compacted energy store because egg volume imposes body-space restrictions. However, in the present study with *P. canaliculata* snails, space restrictions in females do not seem to be a strong selective pressure for energy compaction, and therefore they do not use lipids as a major energy reserve. On the other hand, carbohydrate stores provide them with the unique advantage of being able to produce energy either aerobically of anaerobically. Other species such as fish (Terner, '79) and *Drosophila* (Guzeit et al., '94) store carbohydrates in the form of glycogen in their eggs. Thus, the possibility of using glycolysis as an energy source during embryogenesis may endow the species with some advantages in the unpredictable freshwater environment.

ACKNOWLEDGMENTS

We thank Dr. C. Ituarte for his comments on the manuscript. This work was partially supported by grants from CONICET, Fundación Antorchas, Argentina, and Efamol Res. Institute, Canada. R.J.P. is a member of Carrera del Investigador, CIC (Bs. As.), Argentina.

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