J. Mar. Biol. Ass. U.K. (2000), **80**, 885–890
Printed in the United Kingdom

Cytochemical and physiological studies of the energetic metabolism and osmotrophy in *Sagitta friderici* (chaetognath)

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Different glucidic labelled compounds were used for carbohydrate metabolism exploration, tissular glycogen storage localization and amino acid uptake response to osmotic variation in the adult of *Sagitta friderici*.

After [1-^{14}C] glucose or [6-^{14}C] glucose (osmotrophic) assimilation, the relative activity of the different metabolic pathways (glycolysis–citric acid cycle and pentose cycle) have been estimated by means of a micro-radiorespirometric method. The kinetics of expired $^{14}CO_2$ showed that adult *Sagitta* mainly used the glycolytic–Krebs cycle rather than the pentose cycle in glucose catabolism.

After 2-deoxy-D-[1-^{14}C] glucose (osmotrophic) assimilation, tissular glycogen storages were localized. It was shown that glycogen deposits are abundant in several tissues except the locomotor muscles, which probably represent the main glucose consumers in these very active animals. The problem of glucose transport from the storage localizations to the sites of utilization has been discussed.

The L-^{3H}-leucine uptake variations into the *Sagitta* body after an osmotic shock have been estimated.

INTRODUCTION

Chaetognaths are active marine planktonic predators (Furnestin, 1957; Reeve, 1964; Ghirardelli, 1968; Newbury, 1972), detecting prey by sensing movement and feeding principally on copepods (Feigenbaum, 1991). Ultrastructural studies, together with observations on feeding behaviour, have been published on the chaetognath digestive system (Duvert et al., 1980; Duvert & Salat, 1995; Arnaud et al., 1996; Perez et al., 1999). They attack their prey and continually act to maintain themselves at given levels inside a more or less definite depth range in the water column. All these locomotor activities need a regular supply of energy. Preliminary observations suggest that the locomotor muscle does not contain any glycogen or lipid reserve (Duvert & Salat, 1980; Duvert, 1989). Concerning the chaetognaths respiratory metabolism, a few studies had been carried out. Reeve (1966, 1970) showed a relatively high oxygen consumption by dry weight unit, with large variations (5–50 $\mu l^{-1} h^{-1} mg^{-1}$ dry weight according to the papers). This high oxygen consumption is in good accordance with ultrastructural data: the great development of the extracellular compartment in their large locomotory muscle together with the large mitochondrial volume, in at least one fibre type (B-fibre; Dress & Duvert, 1994). The main difficulties concerning physiological studies on chaetognaths consist in their small size and the impossibility of reproducing natural living conditions in an experimental apparatus. Collected animals live a few hours and it is practically impossible to get animals of the same age and stage of sexual maturity in the same batch. Duvert et al. (2000) have reported the existence of living beheaded chaetognaths in the plankton. These animals have no mouth but do have a gut; as in other soft-bodied marine animals, they probably absorb nutrients as dissolved organic substances across their body wall (Eckert et al., 1988; Wright & Manahan, 1989; Manahan, 1990). This hypothesis was tested in *Sagitta friderici* and the results suggest integumental nutrient transport in this phylum. To increase our knowledge of nutrient uptake, carbohydrate storage and energy metabolism of chaetognaths, glycogen localization was examined, and its possible turn over with labelled molecules. Also estimated was the relative contribution of the glycolytic–Krebs cycle and the pentose cycle to glucose metabolism by means of *in vivo* micro-radiorespirometry (Gourdoux et al., 1985). Finally, the integumentary amino acid uptake was modified by hypo-osmotic shock, such that these euryhaline animals meet in their natural biotope.

MATERIALS AND METHODS

Chemicals

Labelled D-[1^{14}C] 2-deoxyglucose (specific activity 1.92 GBq $mmol^{-1}$) D[1^{14}C] glucose (specific activity 1.87 GBq $mmol^{-1}$) and D [6-^{14}C] glucose (specific activity 1.89 GBq $mmol^{-1}$) (Commissariat à l'Energie Atomique, Saclay, France) was diluted in distilled water to give an activity of 37,000 Bq (1 μCi) in 10 μl. Each assay with 30 *Sagitta friderici* was executed in 1 ml sea water containing 9250 Bq (0.25 μCi) of labelled glucose.

Labelled L-^{3}H-leucine (specific activity 2.55 GBq $mmol^{-1}$) (Amersham) was diluted in 500 μl seawater natural or diluted with 1/4 or 1/3 distilled water to give an activity of 1,110,000 Bq (30 μCi).

Animals and sampling area

Sagitta friderici Ritter-Zahony, 1911, were collected in the autumn from plankton within the Bassin d'Arcachon

https://doi.org/10.1017/S0025315400002861 Published online by Cambridge University Press

Table 1. *Percentage of L-^{3}H-leucine radioactivity found after 1 h inside* Sagitta, *when animals underwent incubation in seawater (NSW), 3/4 seawater + 1/4 distilled water (DW) or 2/3 NSW + 1/3 DW, in the presence of 30 μCi/500 μl in each batch.*

	NSW	3/4 NSW 1/4 DW	2/3 NSW 1/3 DC
% injected dose	0.74	0.65	0.40

(oceanic waters). They were caught in the neritic external zone 2–3 m deep by an horizontal tract with a net of WP2 type, 180 μm mesh, towed at about 1 kn, in 25 m deep waters of around 15°C.

Histoautoradiographic studies

Animals were kept in natural seawater containing 2-deoxy-D-[1-^{14}C] glucose, for 2 h at room temperature (18–20°C). After washes in seawater, they were fixed and processed for cytochemical studies. Fixation was done in 5% glutaraldehyde, 0.1 M Na–Na_2 phosphate buffer pH 7, polyvinylpyrrolidone (PVP) 2%, saccharose 7%. After brief washes, they were postfixed in OsO_4 1%, 0.1 M phosphate buffer, then they were dehydrated and embedded in Epon. Semi-thin sections of about 1–2 μm thick were collected on glass-slides, coated with Ilford K5 nuclear emulsion (diluted 1/2 with distilled water at 40°C). Radioautographs were exposed for 22–30 d, treated in Kodak microdol. Slides were lightly stained with diluted Unna's stain (RAL reactive) before observation in the light microscope.

Glycan cytochemistry

Animals were fixed in glutaraldehyde 2.5% in 0.2 M Na–cacodylate buffer pH 7.5, several hours at 4°C. After washing in the cacodylate buffer, they were embedded in Epon. Thin sections were collected and processed for the PATAg reaction (Thiéry, 1967); controls were carried out without periodate oxidation or with silver proteinate alone.

Micro-radiorespirometric method

Following the protocol found in Moreau (1973) and Gourdoux (1979) groups of 30 animals, about 1 cm length (representative of the adults *S. friderici*) received a respiratory gaseous mixture composed of 70% N_2 and 30% O_2. Animals were labelled with ^{14}C compounds in an artificial seawater bath (Duvert & Savineau, 1986), and expired CO_2 and expired $^{14}CO_2$ were measured at 20°C (owing to the low activity of the animals due to confinement) for 60–80 min. Measurements began 10 min after introducing the animals to the reactive flask filled with 1 ml of seawater, and 2 ml of gas flow mixture. The delay time was necessary to stabilize the respiratory CO_2 efflux. The labelled compound was then injected through a septum into the seawater. The labelled compounds were never used combined. The *Sagitta* could assimilate the diluted labelled substance without any apparent difficulties. Data were detected with a Binos infrared analyser CO_2 (Liebold-France), and an argon methane (90–10%) radiation counter (Berthold-France). A IBM recorder with an adapted program (Darac-France) registered total CO_2, and $^{14}CO_2$, continuously.

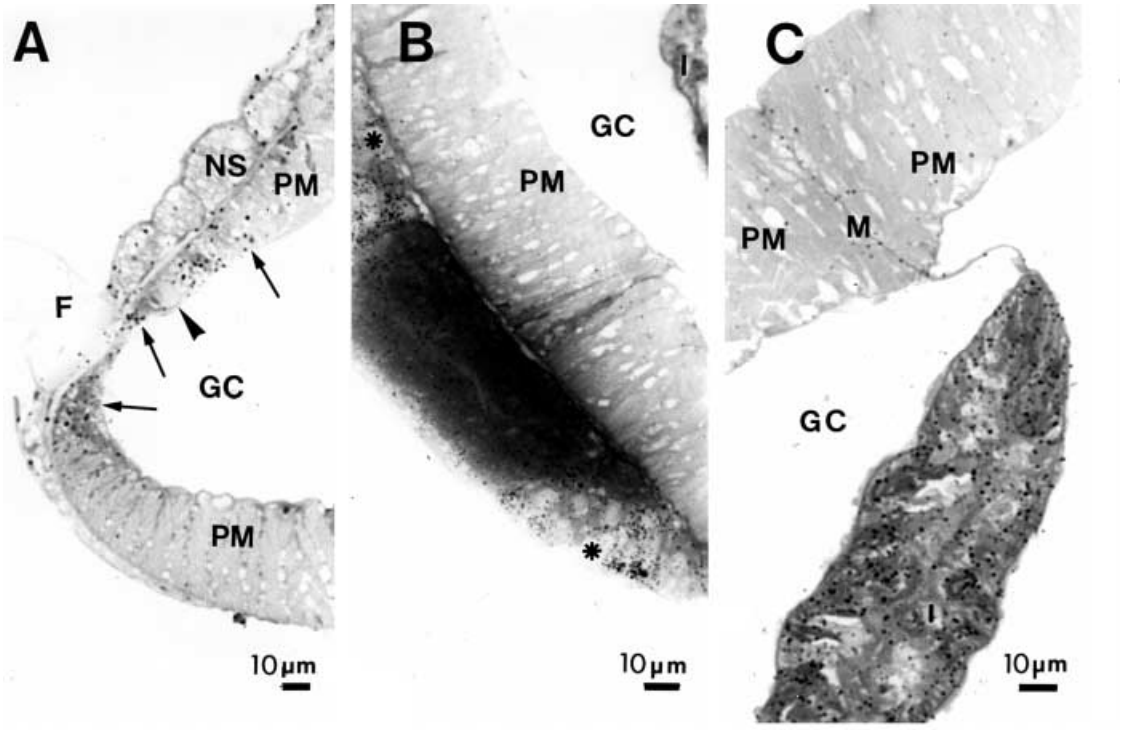

Figure 1. Radioautographic studies, transverse semi-thin sections, Unna's stain (×400). (A) Lateral field level: deposits are seen in some nerves, in lateral field cells and cells associated with the secondary muscle (arrows). Arrowhead shows secondary muscle, which, as the primary muscle, contains very few or no deposits. (B) Ventral ganglion level; silver grains are mainly shown on nervous cells and in the epidermis (asterisks). (C) Medioventral level; silver grains are densely distributed over the intestine and are also found along the mesentery. F, fin; GC, general cavity; I, intestine; M, mesentery; NS, nervous system; PM, primary muscle.

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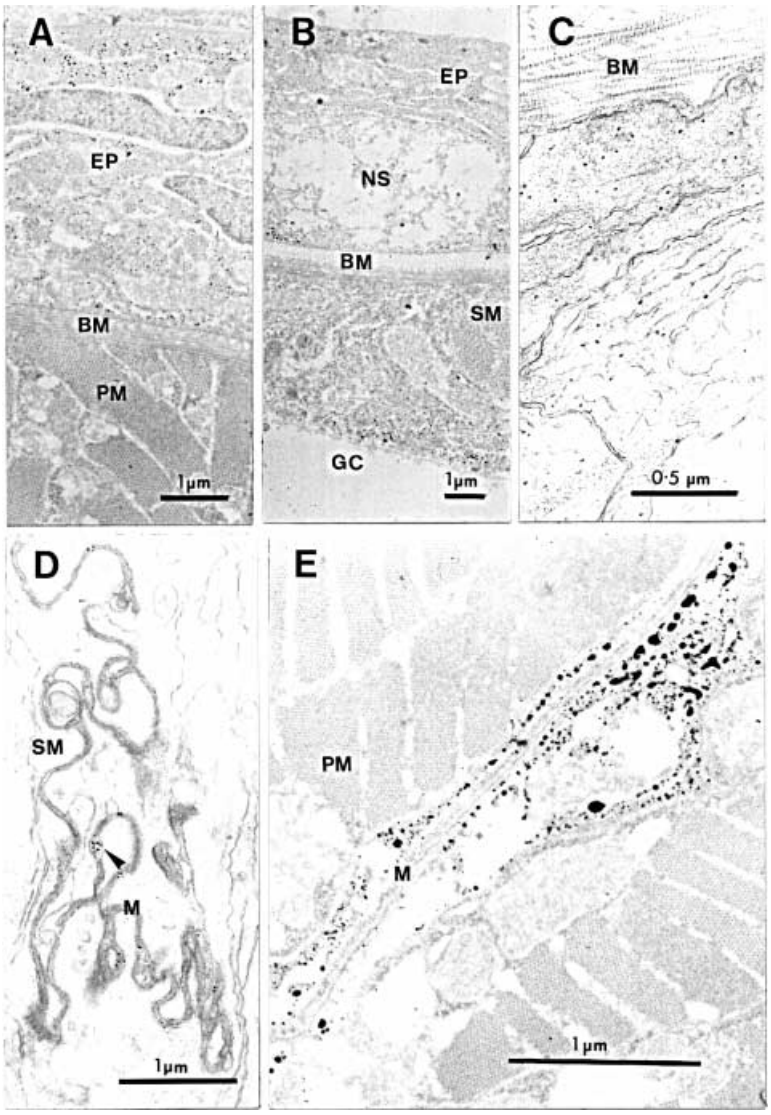

Figure 2. PA.T.Ag (periodic acid 30 min; T.S.C.: 40 min; P.Ag. 30 min); unstained thin sections. (A) At the trunk level, only the epidermis shows a positive reaction. (×9600). (B) At the secondary muscle and at the lateral field levels; deposits are also found in some nerves, in epithelial cells, and in cells which are associated with the secondary muscle and with the lateral field (×6000). (C) Lateral field level; note the positivity of the collagen fibrils in the 'basement membrane' (×23,200). (D) In the thin mesentery cells (×6000). (E) In mesentery cells which are on each side of the extracellular matrix extending from the 'basement membrane' towards the intestine (×43,000). BM, basement membrane; EP, epidermis; F, fin; GC, general cavity; M, mesentery; NS, nervous system; PM, primary muscle; SM, secondary muscle.

Hypo-osmotic shock and amino acid uptake

Batches of 65 *S. friderici* were placed in the following solutions for a period of 1 h. The first batch was composed of 500 μl of seawater containing 30 μCi L-^{3}H-leucine, the second and third contained the same labelled compound, but with 500 μl medium composed of 3/4 seawater, 1/4 distilled water and 2/3 seawater, 1/3 distilled water, respectively. After incubation the animals were taken off the medium, rinsed twice in distilled water, sonicated in the presence of 100 μl distilled water, then after addition of 1900 μl scintillant liquid (scintillation cocktail, s 609581, Beckmann Instruments, Fullerton, USA) the samples were read in an $\alpha\,\beta$ Beckmann–France counter to estimate L-^{3}H leucine uptake (Saclay, France).

Data expression

The specific radioactivity of $^{14}CO_2$ was recorded as dpm per unit volume CO_2 contained in the expired gaseous mixture (ppm), at regular times (min) after addition of [1-^{14}C] glucose or [6-^{14}C] glucose (respectively C1 and C6). Each datum represents the average of n measurements ±SEM. The C6/C1 ratio of the specific radioactivities of the $^{14}CO_2$ released was calculated at four different times after introduction of ^{14}C-labelled compounds. The C6/C1 ratio was taken as an index of the activity of the pentose cycle. A C6/C1 ratio very close to the unit indicated a weak contribution of the pentose phosphate pathway in glucose degradation, while a C6/C1 ratio lower to the unit indicated a more active utilization of the pentose phosphate cycle (Moreau, 1973).

RESULTS

Radioautographic studies

Labelled D2-deoxyglucose was found in some organs and tissues where it was unevenly distributed. It was

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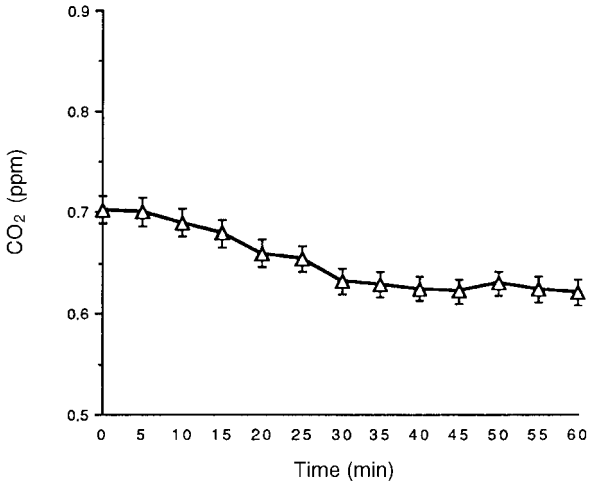

Figure 3. CO_2 expired by *Sagitta* (N=30). The flow rate of the respired gaseous mixture (30% O_2, 70% N_2) was 3 l h^{-1}. Results are expressed as the mean of four measures ±SE.

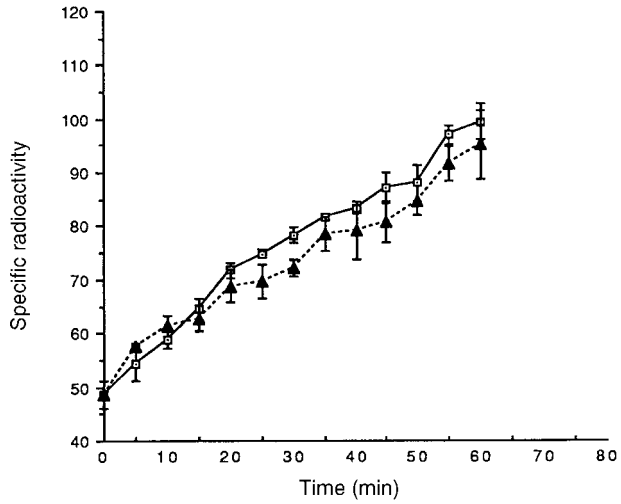

Figure 4. Specific radioactivity of expired $^{14}CO_2$ in *Sagitta* (N=30), after assimilation of labelled [1-^{14}C] (——⊡——) or [6-^{14}C] glucose (---▲---). Results are expressed as the mean of four measures ±SE. Data were analysed using the Student's *t*-test.

largely absent from the primary and the secondary muscles where an inconstant positive reaction seemed to be at a back ground noise level. Constant labelling was found: (1) in the intraepidermic nervous plexus, more particularly at some lateral nerve trunk levels (Figure 1A); in nerve cells of the brain and various ganglia, and in ventral ganglion cells (Figure 1B); (2) in the epidermis where silver grains are unevenly dispersed (Figure 1A); (3) in some tissues lining the general cavity, as in the lateral fields and in the mesentery (Figure 1A–C); (4) all the digestive system was heavily labelled (Figure 1C) from the mouth to the anus; (5) gonads and reproductive cells were also labelled.

Glycan cytochemistry

Ultrastructural cytochemical data were in accordance with PAS data already reported. Irregular deposits, which represent glycogen particles are abundant in three main trunk tissues: (1) the epidermis showed many glycogen deposits, scattered in the cells (Figure 2A,B).

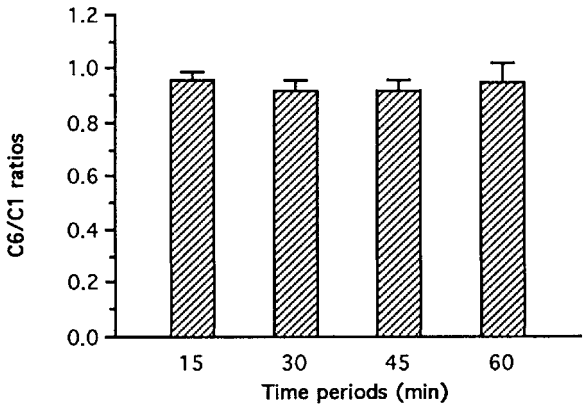

Figure 5. C6/C1 ratios of the radioactivity expired by *Sagitta* (N=30), after assimilation of labelled [1-^{14}C] or [6-^{14}C] glucose. Results are expressed as the mean of four measures±SE. Data were analysed using the Student's *t*-test.

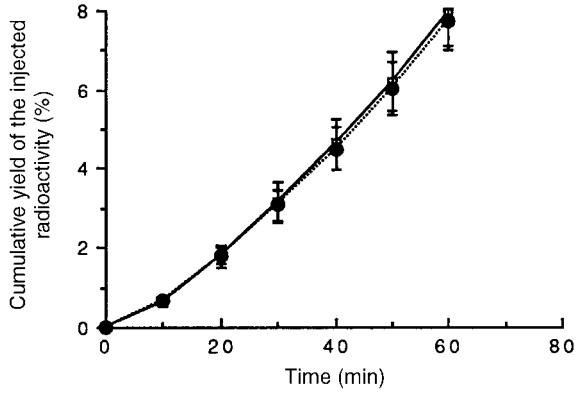

Figure 6. Cumulative yields of the percentage of radioactivity in $^{14}CO_2$ from labelled assimilated compounds by *Sagitta* in the expired gaseous mixture (N=30). The radioactivity of the expired $^{14}CO_2$ came from [1-^{14}C] (——⊡——) or [6-^{14}C] glucose (---●---). Results are expressed as the mean of four measures ±SE.

Deposits were small particles, about 10 nm in diameter, which could be assembled in larger edifices. (2) Glycogen particles were abundant in lateral field cells and in myoepithelial cells related to the secondary muscle (Figure 2B,C); they were also abundant along mesenteries (Figure 2D,E). In these myoepithelial cells, on each side of the connective tissue or the extracellular matrix, there were occasionally large deposits. Among the large homogenous deposits, small particles could be seen: in the lateral field cells, in the mesentery axis and their lateral cells. (3) Deposits were scarce in nerve fibres or in larger trunks (Figure 2B).

No deposits were found in the primary muscle (Figure 2A,E) nor in the secondary muscle (Figure 2B), intestine, ovaries and in the ventral nervous ganglion.

All controls were negative. No lipid droplets are present in the muscular tissue.

Expired CO_2

The expired CO_2 of 30 *Sagitta friderici* was continuously recorded after a 10 min latent period. The confined animals showed a relatively regular production of expired CO_2 with very small variations (Figure 3). Only a slight

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but regular decrease from 0.720 to 0.618 ppm was observed during the recording time; suggesting that the animals' activity steadily decreased. At the end of the experiment, at least 90% of the *Sagitta* remained alive (they are able to move and, in the tail cavity, the sperm cells are kept in continual movement). The dry weight (DW) of 30 adults (estimated by lyophilization towards constant dry weight) was 0.52 ± 0.16 mg (N = 5). The flow rate of the respired gaseous mixture was 3 l h^{-1} and the mean of CO_2 produced was 0.635 ppm. The average CO_2 expired volume by dry weight unit was:

$$\frac{0.635 \times 3}{0.52} = 3.721\ \mu l\ h^{-1}\ mg^{-1}\ DW \qquad (1)$$

Metabolic pathways

Just after adding the labelled glucose to the reaction flask, $^{14}CO_2$ could be observed in the expired gaseous mixture. This shows that the animals could assimilate metabolites introduced. When the animals received [1-^{14}C] or [6-^{14}C] glucose via the medium, radioactivity data was recorded to produce the specific radioactivity curves presented in Figure 4. No significant differences could be observed between the kinetic curves of specific radioactivity obtained after the utilization of [1-^{14}C] or [6-^{14}C] glucose.

When the C6/Cl ratio was measured 15, 30, 45, 60 min after the beginning of the recording time (Figure 5), it was observed that the C6/Cl ratio was always close to the unit values.

Cumulative yields of $^{14}CO_2$

The cumulative yields of the percentage radioactivity found in the expired $^{14}CO_2$ from [1-^{14}C] or [6-^{14}C] glucose showed no significant differences and were comparable in the two cases of labelled glucose molecules used (Figure 6).

Table 1 shows that L-^{3}H-leucine uptake by *S. friderici* was significant in seawater, 1% of the injected dose. This uptake was significantly decreased by hypo-osmotic shock.

DISCUSSION

Very few studies concern dynamic aspects of chaetognath metabolism. Some authors have observed metabolic parameters concerning some aspects of the alimentary canal physiology (Parry, 1944), feeding behaviour (Reeve, 1964; Øresland, 1987; Newbury, 1972; Thuesen & Kogure, 1989), biochemical composition and energy sources (Reeve, 1970), some cytochemical data on the intestine (Welsch & Storch, 1983), and digestive function (Perez et al., 1999).

This work is the first to record expired CO_2, the incorporation and the localization of glycans and more precisely glycogen, in chaetognath trunk tissues. However, since the animals were closely confined in the experimental reaction flasks, rather than moving freely in the sea, it is not surprising that on any weight basis, our results for expired O_2 are lower that for other active animals (e.g. insects; Moreau & Gourdoux, 1971). Reeve (1966, 1970) measured O_2 uptake of chaetognaths in similar conditions to those used here and obtained comparable results.

The steady slow decline in CO_2 expired during experiments is probably the consequence of gradual decline in condition and activity. Chaetognaths survive poorly even in large containers after being caught in plankton and although most were alive after the experiment, they were likely to have been in poor condition.

Glycogen utilization and metabolism can be partially estimated by radioautographic data, they show the incorporation of [^{14}C] 2-deoxyglucose in the trunk wall, at sites where glycogen can be visualized by cytochemical methods: (1) in the mesenteries and in cells associated with the secondary muscle. All these zones regularly show myoepithelial cells; a tonic function was suspected for these myoepithelial cells, they perhaps help in the regulation of the body cavity fluid pressure (Duvert, 1989, 1991). One may suggest that glycogen could provide the energy source for this suggested mechanism. The same interpretation could be suggested for the rare glycogen deposits observed in the visceral muscle around ovaries. (2) In the lateral fields, cells are found related to a tissue which seem to play the central role in the exchanges between the intestine, the body cavity fluid and the trunk wall tissues (Duvert, 1989). Glycogen granules are stored here and glucose is probably delivered to various tissues, (as muscle fibres?) through the numerous gap junctions found between all the cells lining the general cavity (Duvert & Salat, 1980; Duvert, 1989, 1991).

Just as for lipid reserves, glycogen has not been found in other trunk tissues where a large incorporation of labelled 2-deoxyglucose was noted, as for instance in the intestine. These substances are also absent from muscular tissue where [^{14}C] 2-deoxyglucose incorporation is not evident.

It was previously observed that the intercellular spaces of the trunk wall tissues are accessible in seawater (Duvert & Savineau, 1986; Savineau & Duvert, 1986; Duvert, 1989). The present experiments suggest that *Sagitta* can achieve glucose as well as leucine uptake directly through its body wall; just after introduction of labelled compounds in the confined medium, the animals could incorporate [^{14}C] 2-deoxyglucose in their tissue or L-^{3}H-leucine. Similarly they breakdown labelled glucose molecules and reject labelled $^{14}CO_2$ in the experimental system. The permeability and epidermal transport of metabolites has been shown in other invertebrate groups (Schlichter, 1984; Gomme, 1984; Manahan, 1990). Although the main energetic sources in chaetognaths are obtained from the consumption of prey, osmotrophism may be significant, as in many invertebrate groups (Conover, 1978). This is suggested by another report (Duvert et al., 2000).

The present results showed that adult animals mainly used the glycolysis–Krebs cycle for glucose degradation, rather than the pentose cycle which in other species can be more important (as in insects; Gourdoux et al., 1985; Ben Khay et al., 1987). The energy used by these active animals is provided by oxidative breakdown of energetic molecules in the Krebs cycle. Together with the glucose degradation, animals store glycogen in some tissues but not in muscles.

Locomotory activity presumably requires oxygen consumption as suggested by structural and ultrastructural data (relatively thin trunk wall, large extracellular space in A and B fibres, importance of mitochondria in B fibres; Duvert, 1989), and as physiological data shown

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(Reeve, 1970). Glycogen is stored in some tissues but not in the intestine and muscles. Glucose probably diffuses from the intestine to the trunk wall tissues where it is transported via the thin endothelium surrounding the general cavity (Duvert, 1989), the role of the haemal system (Shinn, 1997) remains to be established. The glucose supply to target tissues like amino acid penetration or nutrient uptake provided by unknown ways and mechanisms, but it is suggested that gap junctions are of central importance in such a phenomenon.

The authors thank Mrs C. Salat for technical assistance and Dr Q. Bone for the critical reading of the manuscript and for help in correcting the English text.

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Submitted 28 June 1999. Accepted 6 June 2000.