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Transfer and incorporation of D-glucose across the wall of the gastric caecum, the stomach and the intestine of the echinoid *Echinocardium cordatum*

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Abstract: The present study investigates *in vitro* ^{14}C D-glucose transfer across the digestive wall of the irregular echinoid, *Echinocardium cordatum* (Echinodermata). It aims to characterize absorption ability of the gastric caecum. The experimental device used was the Ussing chamber system. It consists of two compartments clamped together, sandwiching the digestive wall. This allows orientated measurements of radiolabelled nutrient flux through the digestive wall i.e., from the digestive lumen to the perivisceral coelomic cavity. Incorporation of ^{14}C D-glucose by the digestive tube wall as well as the transmural flux and the total amount of ^{14}C D-glucose transferred to the coelomic compartment were quantified. The results show C^{14} D-glucose incorporation in the digestive wall did not differ significantly in the stomach, the gastric caecum and the intestine whereas the amount of ^{14}C D-glucose in the coelomic fluid was significantly higher in the intestine and in the gastric caecum than in the stomach. These observations indicate regional specialization of the digestive tube and suggest that the caecum like the intestine is an important site of nutrient absorption.

Résumé : *Transfert et incorporation de D-glucose à travers la paroi du caecum gastrique, de l'estomac et de l'intestin chez l'oursin* Echinocardium cordatum. Il s'agit de vérifier si le caecum gastrique chez l'échinide fouisseur *Echinocardium cordatum* se démarque des autres régions digestives (estomac et intestin) par son rôle absorbant. Ce rôle est caractérisé par le transfert *in vitro* de ^{14}C D-glucose à travers la paroi digestive en utilisant le dispositif expérimental des chambres de Ussing, Ce dispositif permet d'orienter un fragment de paroi digestive entre deux compartiments (digestif et cœlomique) et de réaliser des mesures de flux de ^{14}C D-glucose de la lumière digestive vers la cavité coelomique. L'incorporation de ^{14}C D-glucose par la paroi digestive ainsi que la quantité totale de ^{14}C D-glucose transférée dans le compartiment coelomique ont été mesurées. Les résultats montrent que l'incorporation de ^{14}C D-glucose par la paroi digestive ne diffère pas significativement dans le caecum gastrique, l'estomac et l'intestin alors que la quantité totale de ^{14}C D-glucose transférée dans le compartiment coelomique est significativement plus élevée dans l'intestin et dans le caecum gastrique que dans l'estomac. Ces observations supportent l'idée d'une régionalisation fonctionnelle du tube digestif et suggèrent que le caecum gastrique tout comme l'intestin participe au transfert du glucose vers la cavité cœlomique.

Keywords: Echinocardium cordatum ● Echinoids ● Digestive tube ● Gastric caecum ● D-glucose absorption ● Ussing chamber

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Introduction

Acquisition of nutrients follows two main pathways in echinoids: epidermal (transtegumental) absorption of dissolved organic matter (DOM), and absorption of soluble digestive products across the digestive epithelium (e.g. Péquignat, 1966a & b; Bamford, 1982). In epidermal absorption, nutrients are immediately available from the surrounding seawater and directly supply body wall tissues and organs (Lawrence, 1987). Absorption of nutrients by the digestive epithelium involves ingestion and digestion of large food items. The digestive products are transferred to the coelomic and haemal fluids before allocation to the body parts (e.g., gonads) (Lawrence, 1987; Lawrence & Mc Clintock, 1994; Marsh & Watts, 2007).

Diet and food packaging are variable in echinoids (De Ridder & Lawrence, 1982; De Ridder & Jangoux, 1982). Most regular echinoids are herbivorous and pack their food into mucus-coated pellets that remain intact throughout the entire digestive transfer (Farmanfarmaian & Phillips, 1962; Buchanan, 1969). In contrast, the irregular echinoids are deposit feeders that ingest sediments and never form pellets. The source of nutrition in irregular echinoids like for most deposit feeders still remains incompletely understood (e.g. Self et al., 1995). The spatangoid species investigated here, *Echinocardium cordatum* (Pennant, 1777), lives deeply burrowed in sediments (*ca*. 15 cm deep). Its gut content consists of sediments mixed with detritus that are digested (De Ridder et al., 1985). The burrow is connected to the sediment-water interface through a vertical tube (the "chimney") that allows seawater circulation (respiratory currents) and gives access to detritus (Nichols, 1959; De Ridder et al., 1985). The trajectory of water along the echinoid body surface has been well described and indicates that some of the water enters the mouth while the remainder is directed backwards in the burrow (in the so-called "sanitary drain") (Nichols, 1959; Forster-Smith, 1978) (Fig. 1).

The morphofunctional organization of the digestive tube is rather consistent throughout the Echinoidea (De Ridder & Jangoux, 1982). It has two long and wide superimposed loops, respectively named stomach and intestine. Two uncoiled segments, the esophagus and the rectum, respectively connect the stomach to the pharynx (plus mouth cavity) and the intestine to the anus. A narrow tube, the siphon, runs parallel to the first digestive loop (in cidarids and diadematids that lack a siphon, there is a siphonal groove along the inner stomach wall). The siphon bypasses the stomach by connecting the esophageal junction to the intestine (De Ridder & Jangoux, 1982; Lawrence & Klinger, 2001). Only a few echinoids depart from this general organization (Holland & Ghiselin, 1970). Although the proximal segments (oral cavity, pharynx and esophagus) and the stomach have specific functions, ensuring respectively food conditioning and digestion, absorption has been reported to occur along the entire digestive tube (Stott, 1955; Buchanan, 1969; De Ridder & Jangoux, 1982; Lawrence & Klinger, 2001). Data on absorption mostly concern herbivorous (regular) echinoids (see the reviews of Bamford, 1982 and Ahearn, 1988). They indicate that the ability to absorb nutrients varies regionally along the digestive tube according to the nature of the involved nutrient. D-glucose occurs in the digestive tube as a primary product of the digestion of polysaccharides and also as DOM in the ingested seawater (McDonald et al., 1973; Gorham, 1990). In regular echinoids, D-glucose absorption has been reported to occur in the stomach and in the intestine (Stott, 1955; Farmanfarmaian, 1969; Bamford & James, 1972; Bamford et al., 1972; West & Jeal, 1973; Bamford, 1982).

Some irregular echinoids (e.g., Spatangoida, Holasteroida) have a gastric caecum. This large, elongated pouch opens into the stomach, a little past its junction with the esophagus (Ziegler et al., 2010). An absorptive function of the gastric caecum is supported by several observations made with the spatangoid *Echinocardium cordatum*. First, cytological features indicate that the caecum enterocytes undergo a complex cycle of absorption and storage activities (De Ridder & Jangoux, 1993). Second, the caecum lumen contains fermenting microorganisms that produce short-chained fatty acids thought to be important for echinoid metabolism (Thorsen, 1998). Third, dissolved

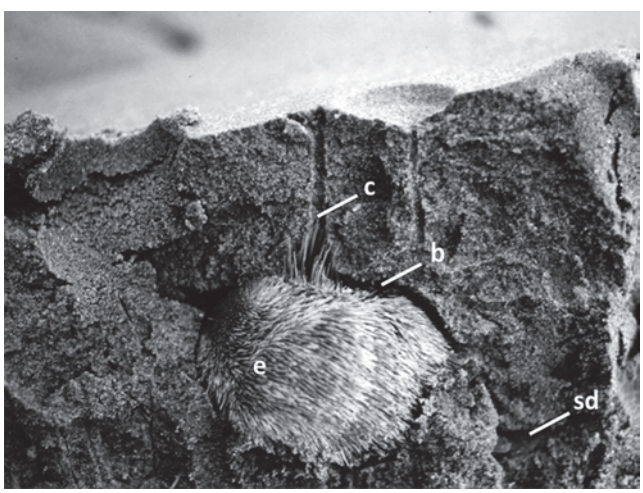

Figure 1. *Echinocardium cordatum*. The picture (adapted from De Amaral P. Nunes & Jangoux, 2004) shows the echinoid (e), the burrow (b) with its two extensions: the vertical chimney (c) and the horizontal sanitary drain (sd).

Figure 1. *Echinocardium cordatum*. (d'après De Amaral P. Nunes & Jangoux, 2004) (e), le terrier (b) avec ses deux extensions : l'une verticale, la cheminée (c) et l'autre horizontale, le drain sanitaire (sd).

organic matter (DOM) could enter the gastric caecum and be absorbed as seawater regularly fills this organ (Rolet et al., 2011).

The present study investigates *in vitro* ^{14}C D-glucose transfer across the digestive wall of *Echinocardium cordatum* to verify if the gastric caecum has special absorption abilities compared to those observed for the stomach and intestine. We used the Ussing chamber system (Ussing & Zerhan, 1950; Ahearn et al., 1992). This device consists of two compartments clamped together and sandwiching the digestive wall. It allows oriented measurements of radiolabelled nutrient flux through the digestive wall, i.e., from the digestive lumen to the perivisceral coelomic cavity.

Materials and Methods

Echinocardium cordatum is mainly distributed subtidally but it is locally accessible during lower low tides (e.g., Ursin, 1960; Buchanan, 1966). Twenty specimens were collected intertidally in September 2007 at Penhir, Brittany, France. They were placed into tanks containing sediment and aerated seawater for transport to the laboratory (IAEA) of Monaco where they were held in an aquarium (salinity: 33; temperature: 17°C) until dissected. The experiments were performed within 3-5 d after collection on healthy individuals (N = 10). Some were eliminated because of the presence of a red zone without spines on their tests. Five specimens were used to test the Ussing chamber system.

Experimental device

For each individual, the stomach, intestine and gastric caecum were dissected and placed into Petri dishes containing a physiological Ringer's solution (Lawrence et al., 1967). The digestive segments were treated separately. The contents of each segment were gently removed and the segment was cut longitudinally. A section of 2 cm was cut from each segment and placed onto a fenestrated plastic insert. The section (composed of three layers: the luminal epithelium, the connective tissue and the mesothelium) was sandwiched between a second fenestrated insert and fixed on the support bar of the Ussing chamber between the two chamber compartments (Fig. 2). The compartment facing the luminal epithelium corresponds to the gut lumen ("digestive compartment"). The compartment facing the mesothelium corresponds to the coelomic cavity ("coelomic compartment"). The two half chambers were filled with equal volumes of aerated Ringer's solution. This was done to avoid chemical, mechanical or electrical driving forces between the two compartments. The experiment began by replacing the unlabelled Ringer's solution of the digestive compartment with a labeled solution of Ringer's fluid with 0.5 mM ^{14}C D-glucose. The rate of appearance of ^{14}C D-glucose in the coelomic compartment was then followed for 1 h by removing 1 ml of Ringer's solution every 15 min. One milliter of Ringer's solution was added to the coelomic compartment after each sampling to keep the volume constant. The samples were placed in PolyEthylene (PE) tubes to which 9 ml of UltimaGold XR scintillation liquid was added for subsequent radioactivity measurement. The specific activity of the 0.5 mM ^{14}C-labelled D-glucose Ringer's solution was determined at the end of the 1 h experiment by sampling 50 µl of Ringer's solution in the digestive compartment. This 50 µl sample was placed into a PE tube containing 1 ml of Ringer's solution and 9 ml of UltimaGold XR Scintillation liquid. The radioactivity background was determined by adding 9 ml of UltimaGold XR Scintillation liquid to 1 ml unlabelled Ringer's solution in a PE tube. To measure the assimilation of ^{14}C-labelled D-glucose in the digestive fragment after the 1 h experiment, the tissue fragment was removed from the Ussing half chambers, rinsed with

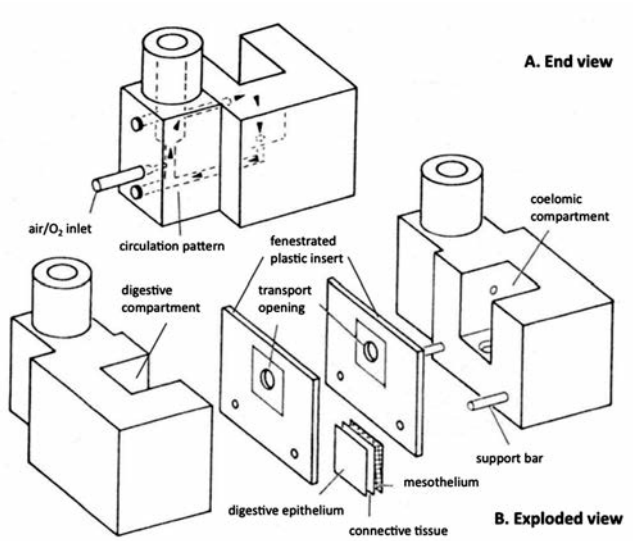

Figure 2. *Echinocardium cordatum*. Ussing chamber system used to determine movement of ^{14}C D-glucose across the wall of the digestive tube. The digestive compartment is filled with Ringer's solution containing 0.5 mM ^{14}C D-glucose while the coelomic one is filled with unlabeled Ringer's solution. Arrows indicate direction of Ringer's solution circulation, stirred by aeration (adapted from Ahearn et al., 1992)

Figure 2. *Echinocardium cordatum*. Chambres de Ussing permettant de mesurer le transfert de ^{14}C D-glucose à travers la paroi du tube digestif. Le compartiment digestif est rempli d'une solution de Ringer radiomarqué 0,5 mM ^{14}C D-glucose alors que le compartiment coelomique est rempli d'une solution de Ringer non-marquée. Les flèches indiquent la direction de la circulation de la solution de Ringer, assurée par l'aération (adapté de Ahearn et al., 1992).

unlabelled Ringer's solution, drained on filter paper and weighed. The tissue fragment was then homogenized in a PE tube containing 1 ml of Ringer's solution and 9 ml of UltimaGold XR Scintillation liquid. Radioactivity of all the samples was measured with a Packard 1600T Liquid Scintillation Analyzer.

Theory/calculation

The radioactivity of the samples was expressed in disintegrations per minute (dpm) and converted to micromoles. Using the specific activity (µmol.dpm^{-1}) for the radioactive Ringer's solution and the surface area (in centimeter squared) of the exposed digestive fragment, the flux of D-glucose across the digestive wall (from the gut lumen to the coelomic cavity) was then converted in to micromoles per centimeter squared. The results were plotted against time (Fig. 3) to calculate the flux (expressed in micromoles per centimeter squared per hour). After the 1 h experiment, the amount of D-glucose incorporated into the tissue was calculated taking into account the calculated specific activity and the wet weight of the tissue fragment. The results are expressed in micromoles per gram per hour. Since the data do not satisfy the assumption of normality, non-parametric tests were used to identify significant differences in the data set. Wilcoxon rank tests were used-for comparisons. All these analyses were done with SAS JMP software (5.0.1.2).

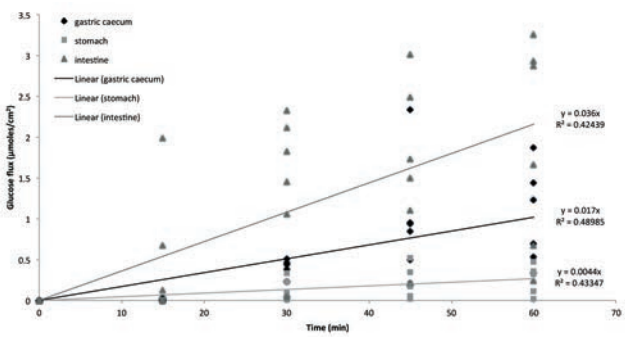

Figure 3. *Echinocardium cordatum.* Transfer of ^{14}C D-glucose across the stomach, gastric caecum and intestine walls (N = 10), using the Ussing chamber system to measure flux from the digestive lumen to the coelomic cavity

Figure 3. *Echinocardium cordatum.* Transfert de ^{14}C D-glucose à travers la paroi digestive de l'estomac, du caecum gastrique et de l'intestin (N = 10), en utilisant les chambres de Ussing pour mesurer le flux de la lumière digestive vers la cavité coelomique.

Results

Flux of ^{14}C D-glucose across the digestive wall

The kinetics of 0.5 mM labeled D-glucose flux across the digestive wall reached 0.0044 µmol.cm^{-2}.min^{-1} for the stomach, 0.017 µmol.cm^{-2}.min^{-1} for the gastric caecum and 0.036 µmol.cm^{-2}.min^{-1} for the intestine (Fig. 3). The amounts of ^{14}C D-glucose measured in the coelomic compartment after the 1 h experiment are similar for the gastric caecum (median: 0.97 µmol.cm^{-2}.h^{-1}; minimum: 0.33 µmol.cm^{-2}.h^{-1}; maximum: 1.86 µmol.cm^{-2}.h^{-1}) and the intestine (median: 2.26 µmol.cm^{-2}.h^{-1}; minimum: 0.24 µmol.cm^{-2}.h^{-1}; maximum: 3.26 µmol.cm^{-2}.h^{-1}) and did not differ significantly ($p > 0.05$). The amounts of ^{14}C D-glucose measured in the coelomic compartment after the 1 h experiment were significantly lower for the stomach (median: 0.34 µmol.cm^{-2}.h^{-1}; minimum: 0.014 µmol.cm^{-2}.h^{-1}; maximum: 0.48 µmol.cm^{-2}.h^{-1}; $p = 0.0195$) (Fig. 4).

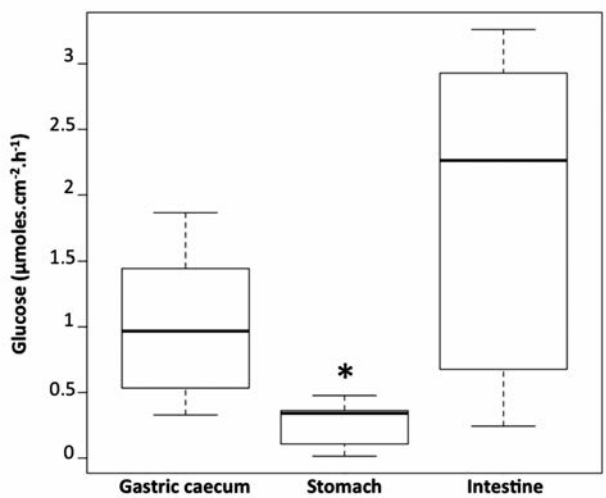

Figure 4. *Echinocardium cordatum*. Total amount of ^{14}C D-glucose measured in the coelomic compartment after 1 h experiment (Ussing chamber system) across the gastric caecum, the stomach and the intestine walls (N = 10). Middle bars are the medians: boxes and whiskers depict the interquartile and extreme (minimum and maximum) ranges. Significance difference after Wilcoxon rank test is indicated by asterisk ($p = 0.0195$).

Figure 4. *Echinocardium cordatum*. Quantité totale de ^{14}C D-glucose mesurée dans le compartiment cœlomique après 1 h d'expérience et ayant traversé la paroi digestive du caecum gastrique, de l'estomac et de l'intestin (N = 10). Les barres centrales représentent les médianes, les boîtes et les moustaches représentent les interquartiles et les valeurs extrêmes (minimum et maximum). La différence significative d'après le test de rang de Wilcoxon est indiquée par un astérisque ($p = 0,0195$).

Incorporation of 14C D-glucose into the digestive wall

The calculated amount of ^{14}C D-glucose incorporated into the digestive walls after 1h incubation for the stomach (median: 0.20 µmol.g^{-1}.h^{-1}; minimum: 0.045 µmol.g^{-1}.h^{-1}; maximum: 0.45 µmol.g^{-1}.h^{-1}), the gastric caecum (median: 0.18 µmol.g^{-1}.h^{-1}; minimum: 0.068 µmol.g^{-1}.h^{-1}; maximum: 0.41 µmol.g^{-1}.h^{-1}) and the intestine (median: 0.29 µmol.g^{-1}.h^{-1}; minimum: 0.021 µmol.g^{-1}.h^{-1}; maximum: 0.62 µmol.g^{-1}.h^{-1}) (Fig.5) did not differ significantly ($p > 0.05$).

Discussion

The study shows that D-glucose crosses the digestive wall of *Echinocardium cordatum* into the coelomic cavity. This supports previous observations on the role of the coelomic fluid in nutrient translocation (Farmafarmaian & Philips, 1962; Ferguson, 1964). Our results also indicate (1) that the absorption of D-glucose follows similar kinetics in the gastric caecum and the intestine, with higher values for these two regions than for the stomach, and (2) similar amounts of D-glucose are incorporated into the wall of these three digestive tube regions. Comparison with other published data is difficult because experimental conditions differ or because results are expressed in other units. For example, using the "everted sac" method, keeping the everted digestive tube in a 0.5 mM D-glucose solution for one hour, Farmanfarmaian (1969) observed a total transport across the intestinal wall of *Thyone* (Holothuroidea) of 2 to 3 µmol.h^{-1}.These values are similar to those observed in our study. Ahearn et al. (1992) used the Ussing chamber to measure D-glucose transfer through an epithelial monolayer (prepared from primary culture of hepatopancreas cells of the lobster *Homarus americanus* H. Milne Edwards, 1837). After 45 min exposure to a 0.5 mM D-glucose solution, the flux from the digestive compartment to the coelomic compartment was of 3.57 ± 0.07 nmol.cm^{-2}.min^{-1}, i.e. 0.214 ± 0.0042 µmol.cm^{-2}.h^{-1}. This is a low flux compared to our values.

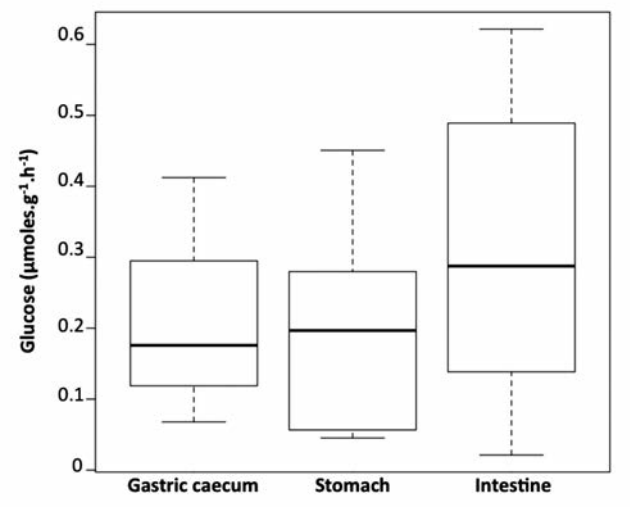

Figure 5. *Echinocardium cordatum*. Total amount of ^{14}C D-glucose incorporated into the digestive wall after 1 h experiment (Ussing chamber system). The gastric caecum, the stomach and the intestine are compared (N = 10). Middle bars are the medians: boxes and whiskers depict the interquartile and extreme (minimum and maximum) ranges. There is no significant difference based on the Wilcoxon rank test ($p > 0.05$).

Figure 5. *Echinocardium cordatum*. Quantité totale de ^{14}C D-glucose incorporée par la paroi digestive après 1 h d'expérience. Le caecum gastrique, l'estomac et l'intestin sont comparés (N = 10). Les barres centrales représentent les médianes, les boîtes et les moustaches représentent les interquartiles et les valeurs extrêmes (minimum et maximum). Le test de rang de Wilcoxon n'indique aucune différence significative ($p > 0,05$).

Several studies performed on echinoderms reported the "stomach" (i.e., the first digestive loop in echinoids, the midgut in holothuroids) is the main site of absorption of D-glucose (e.g., Farmanfarmaian, 1969; Bamford et al., 1972; Bamford, 1982; Self et al., 1995). However, the marked regionalization of D-glucose absorption within the digestive tube of *Echinocardium cordatum* (higher in the gastric caecum and in the intestine compared to the stomach) indicates that the gastric caecum and intestine are specialized for absorption. The cytological features of the caecum enterocytes support their absorptive role: they have high and ramified microvilli associated with abundant vesicles and mitochondria in their apical cytoplasm; the microvilli were typically more developed in this organ than elsewhere along the digestive tube (De Ridder & Jangoux, 1993). These observations corroborate the work of Ahearn et al. (1992) who compared nutrients transport in the pyloric caeca of asteroids, in the hepatopancreas of mollusks and crustaceans and concluded that gut diverticula in invertebrates are absorbing organs.

In *Echinocardium cordatum,* like other echinoids, seawater flows through the digestive tube. The ingested seawater follows three trajectories: it can be conveyed to the gastric caecum (Rolet et al., 2011) and to the intestine via the siphon that bypasses the stomach or via the stomach (Stott, 1955; Buchanan, 1969; De Ridder & Jangoux, 1993; Lawrence & Klinger, 2001). Functionally, this circulation together with the fact the digestive tube is absorptive along its entire length suggests that dissolved organic matter (DOM) originating from seawater could be a source of nutrients to the echinoid. D-glucose is abundant in seawater (*ca*. 50 mg C.l^{-1}), and could constitute a nutritional source for marine invertebrates (Gohram, 1990). A nutritional role of DOM in seawater by absorption by enterocytes is not

well documented in echinoids although several authors since Stott (1955) have mentioned the occurrence of circulating seawater in the digestive tube and suggested a nutritional role (Buchanan, 1969; De Ridder & Jangoux, 1993; Lawrence & Klinger, 2001; Rolet et al., 2011). Interestingly, the higher absorption rates of D-glucose measured along the digestive tube of *Echinocardium cordatum* occur in the two regions receiving freshly ingested seawater, i.e., seawater that is presumably rich in DOM as it originated from the sediment-water interface. In echinoids, regional variation in nutrient absorption along the digestive tube can be related to regional differences in nutrient availability (James & Bamford, 1974). Moreover, the few studies on sugar absorption by the digestive tube in echinoderms have led to the conclusion that absorption of D-glucose is mediated by a carrier system and that it is an active process (see reviews of Bamford, 1982 and Ahearn, 1988). Differences in substrate carrier densities among the gut regions are also involved in the regionalization of nutrient absorption (Self et al., 1995).

The digestive wall of *Echinocardium cordatum* incorporates D-glucose as has been reported for regular herbivorous echinoids (Farmanfarmaian & Philips, 1962). Similar amounts of D-glucose are incorporated into the stomach, the gastric caecum and the intestine walls although they have different structures (De Ridder & Jangoux, 1993). The digestive epithelium of the stomach and of the gastric caecum is highly developed, 80 and 100 µm in thickness, respectively. The connective layer is very thin, with a thickness of *ca* 2 µm in the caecum and up to 6 µm in the stomach). In contrast, the connective tissue forms the most developed layer in the intestine, reaching 50 µm in thickness, while the digestive epithelium height varies between 20 to 30 µm. These structural differences suggest that in the stomach and caecum walls the enterocytes could be the main site of D-glucose assimilation while in the intestine, the connective tissue and more particularly its haemal lacunae could also be involved if D-glucose is transferred from the enterocytes. Studies of the digestive tube of sea urchins indicate that it is a nutrient storage organ (De Ridder & Jangoux, 1982). In *Echinocardium cordatum*, lipid droplets but no glycogen occurs in the stomach and caecal enterocytes (De Ridder & Jangoux, 1993). According to Allen & Giese (1968), ^{14}C D-glucose can be incorporated into lipid fractions as observed in the pyloric caeca of the sea star *Pisaster ochraceus*.

The digestive tube of *Echinocardium cordatum* clearly displays a regionalized difference in ability to absorb D-glucose. This regionalization corresponds to the presence of freshly ingested seawater in particular organs and could reflect an adaptation to the use of DOM carried by seawater along the digestive lumen. Further observations using a wider set of nutrients are needed to understand how and where absorption preferentially occurs in the digestive tube of *Echinocardium cordatum* and to clarify the role of seawater DOM as a potential nutrient.

Acknowledgments

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