J. exp. Biol. 111, 75–80 (1984)
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ANALYSIS OF CONTACT-REHYDRATION IN TERRESTRIAL GASTROPODS: ABSORPTION OF ^{14}C-INULIN THROUGH THE EPITHELIUM OF THE FOOT

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Accepted 13 December 1983

SUMMARY

1. Contact-rehydration in terrestrial slugs involves a specific drinking behaviour during which water is rapidly absorbed through the integument of the foot.

2. When dehydrated slugs were placed on wet filter paper containing ^{14}C-inulin, they displayed the characteristic drinking posture and absorbed both water and ^{14}C-inulin. Samples of haemolymph from dehydrated slugs after 12 min of contact-rehydration contained about 6 μg of ^{14}C-inulin 100 mg^{-1} of haemolymph (0·24 mmol l^{-1} ^{14}C-inulin in the substrate). The haemolymph of hydrated slugs however contained no detectable radioactivity after 12 min on the filter paper.

3. Electron microscopy revealed that the intercellular spaces between the epithelial cells of the foot were reduced in dehydrated slugs, but were rapidly enlarged during contact-rehydration.

4. It is concluded that contact-rehydration in terrestrial slugs is mediated by bulk flow of water through an epithelial paracellular pathway in the integument of the foot.

INTRODUCTION

Terrestrial slugs can recover from dehydration by absorbing water through the integument of the foot while in contact with a moist surface (Dainton, 1954; Prior, 1982, 1984). This process of contact-rehydration has been shown to involve a characteristic behavioural sequence known as drinking (Prior, 1982). Dehydrated slugs move onto a moist surface and assume a flattened posture which they maintain while water is absorbed through the foot. They remain in this posture until they have absorbed enough water to reach their rehydration set-point (see Table 1, Prior, 1984).

The rate of water uptake during the initial minutes of contact-rehydration has been shown to be as high as 20 μl cm^{-2} min^{-1} (see Fig. 3, Prior, 1984; based on the 2–3 min period with a 1·0 g slug). This rate is greater than that predicted from the increase in haemolymph osmotic pressure that occurs during dehydration. This suggested that the permeability of the foot may increase during contact-rehydration. The present

Key words: Terrestrial slug, water and inulin uptake, paracellular pathway.

experiments were designed to determine if water absorption during contact rehydration is due to water flow through an epithelial paracellular pathway. Support for this view is provided by our demonstration that during contact-rehydration there is absorption of the extracellular marker, ^{14}C-inulin, into the haemolymph. A preliminary report of these results has appeared in abstract form (Prior, 1982).

MATERIALS AND METHODS

Specimens of *Limax maximus* Linnaeus, 1758 and *Lehmannia valentiana* (Férussac, 1823) were maintained in the laboratory and fasted as previously described (Prior, Hume, Varga & Hess, 1983). Fully hydrated (i.e. 100 % of initial body weight or IBW) slugs or dehydrated (i.e. 60–70 % IBW) slugs weighing 1·0–1·5 g were placed on wet filter paper (Whatman No. 1 circles, 5·5 cm diameter). The filter paper was saturated with distilled water containing 0·05 mmol l^{-1} inulin-carboxyl, [carboxyl-^{14}C] (i.e. ^{14}C-inulin; 10 μCi ml^{-1}; 5000–5500 Da; New England Nuclear). The test period was started once the slug's foot made contact with the wet filter paper (usually less than 10 s). After varying periods of time (10–240 s at 18–22 °C) on the wet filter paper, the slugs were rinsed in three changes of water and extracted in 10 ml of 70 % ethanol for 48 h. The slugs were cut into three or four pieces to facilitate extraction of the isotope. Preliminary experiments had shown that the absorbed isotope was readily extractable by this procedure. The slugs were removed from the ethanol, dried for 48 h (95 °C) and weighed (±0·1 mg). Radioactivity in the ethanol extracts was determined using liquid scintillation spectrometry. Appropriate corrections for quench were made. Absorption rates were calculated from the corrected isotope contents of the ethanol extracts and are expressed as μg ^{14}C-inulin g^{-1} ethanol-extracted dry weight per unit time (where indicated).

Large slugs (2–3 g) were used when samples of haemolymph were needed for analysis. The slugs were placed on filter paper containing 0·24 mmol l^{-1} ^{14}C-inulin (50 μCi ml^{-1}) and after 12 min were rinsed in three changes of water. A superior tentacle of each slug was cut and samples of haemolymph (5–30 mg) were collected, weighed (±0·1 mg) and immediately analysed for ^{14}C content as described above. These slugs were then extracted in 15 ml of 70 % ethanol as described above to determine total ^{14}C-inulin absorbed. Results are expressed as the mean ± S.E. of three to five replicates. Differences were analysed using a Student's t test and a P value of less than 0·05 was considered significant.

RESULTS

Epithelial paracellular pathway

It was initially necessary to determine if during contact-rehydration the slug foot

Fig. 1. (A). The time course of water uptake by two species of slugs (*Limax maximus* ●——● and *Lehmannia valentiana* O- - -O) during contact-rehydration. Each point is the mean of from five to nine measurements with only one measurement from each slug. The data on *Limax* is redrawn from Prior, 1984. The rates of absorption of ^{14}C-inulin by dehydrated *Lehmannia* (B) and *Limax* (C) parallel that of water absorption during the 4-min test period (compare A with B and C). There was negligible ^{14}C-inulin uptake by hydrated control slugs of either species (O——O in B and C). Each point in B and C is the mean ± S.E. of three to five replicates.

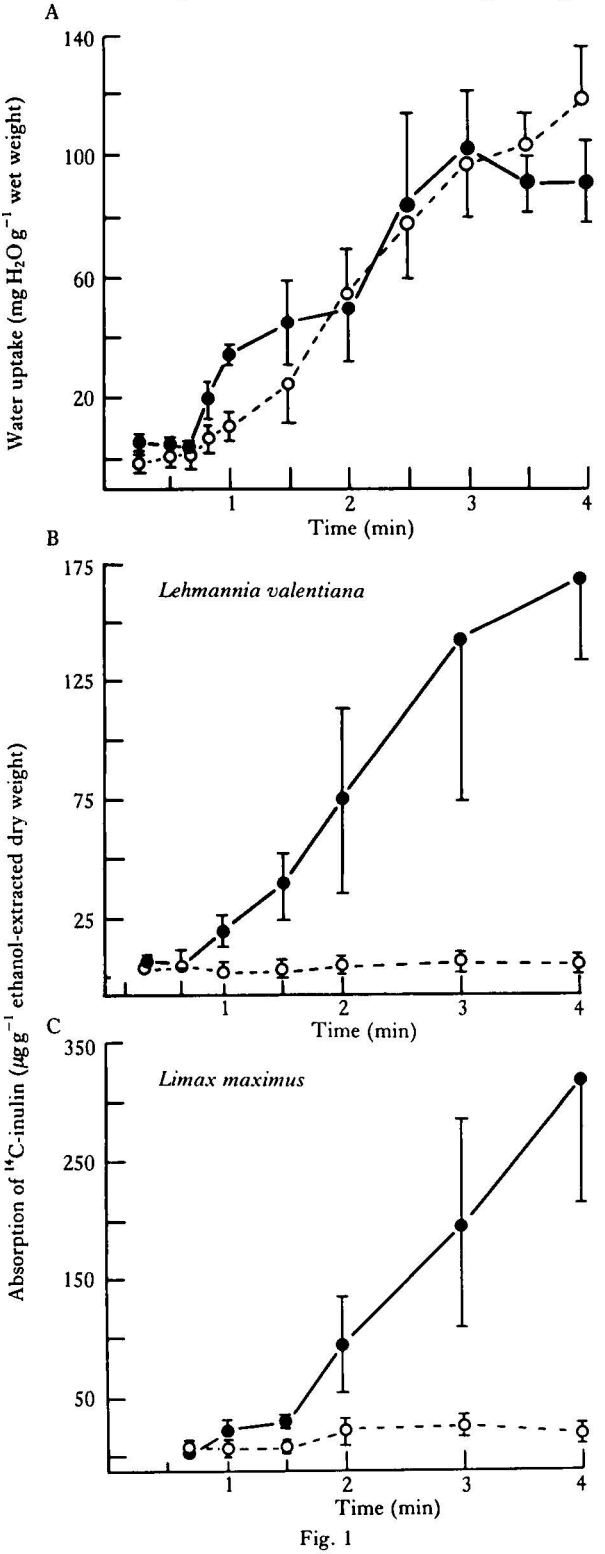
A
140
100
60
20
Water uptake (mg H_2O g^{-1} wet weight)
1
2
3
4
Time (min)
B
175
125
75
25
Lehmannia valentiana
Absorption of ^{14}C-inulin (μg g^{-1} ethanol-extracted dry weight)
1
2
3
4
Time (min)
C
350
250
150
50
Limax maximus
1
2
3
4
Time (min)

Fig. 1

Table 1. *Measurement of ^{14}C-inulin in* Limax maximus *haemolymph during contact-rehydration*

Wet weight (g) of dehydrated slugs before contact-rehydration	^{14}C-inulin (μg) extracted from whole animal	Weight (mg) of haemolymph samples	^{14}C-inulin (μg) in haemolymph samples	^{14}C-inulin (μg 100 mg^{-1} haemolymph) ($\bar{x} = 6{\cdot}1 \pm 1{\cdot}3$)
2·46	23·2	24·1	1·6	6·7
2·64	23·2	46·5	2·3	5·0
2·21	72·5	23·5	1·2	5·3
2·01	21·1	31·2	1·0	3·0
2·40	27·2	4·2	0·4	10·7

Haemolymph samples from hydrated control slugs ($N = 5$) that were kept in contact with the ^{14}C-inulin pads for the same period of time contained no measurable isotope.

was permeable to an extracellular marker molecule. Slugs that were fully hydrated, or dehydrated to 60–70 % IBW, were placed on filter paper pads saturated with water that contained ^{14}C-inulin. The dehydrated slugs assumed the characteristic drinking posture and partially rehydrated themselves during the 12 min test period. Haemolymph samples from these slugs contained $6{\cdot}1 \pm 1{\cdot}3$ μg of ^{14}C-inulin 100 mg^{-1} of haemolymph (Table 1). The remaining ^{14}C-inulin that was absorbed during rehydration was readily extracted with 70 % ethanol. There was negligible additional isotope recovered following complete solubilization of the tissues. In contrast, there was no detectable radioactivity in the haemolymph samples from fully hydrated slugs after they had been on the filter paper for 12 min. These results indicate that during contact-rehydration ^{14}C-inulin can move through the epithelium of the foot into the haemocoel *via* a paracellular pathway.

Rate of ^{14}C-inulin absorption

The absorption of ^{14}C-inulin during contact-rehydration was compared to that of the uptake of water over a 4-min period (Fig. 1). The absorption rates of ^{14}C-inulin by hydrated and dehydrated slugs at 40 s were not significantly different. By 60 s, however, dehydrated slugs had absorbed significantly more ^{14}C-inulin than the hydrated control slugs. The progressive absorption of ^{14}C-inulin by dehydrated slugs closely paralleled the absorption of water over the same period (compare Fig. 1A with 1B and 1C). In contrast, the absorption of ^{14}C-inulin by hydrated slugs did not increase over the 40–240 s period.

Ultrastructure of the foot epithelium

The foot epithelium of slugs is composed of a single layer of pyramidal cells which are joined by septate junctions near their microvillar borders (Fig. 2A–D). There are numerous mitochondria near these contacts and on the peripheral aspects of the nuclei. The extracellular spaces between the epithelial cells are continuous with the haemocoel (Fig. 2B, C). While the extracellular spaces are quite apparent in hydrated slugs (Fig. 2B), in dehydrated slugs they are greatly reduced (Fig. 2A). Even though the spaces in dehydrated slugs are difficult to see, after only 4 min of contact-rehydration they ar

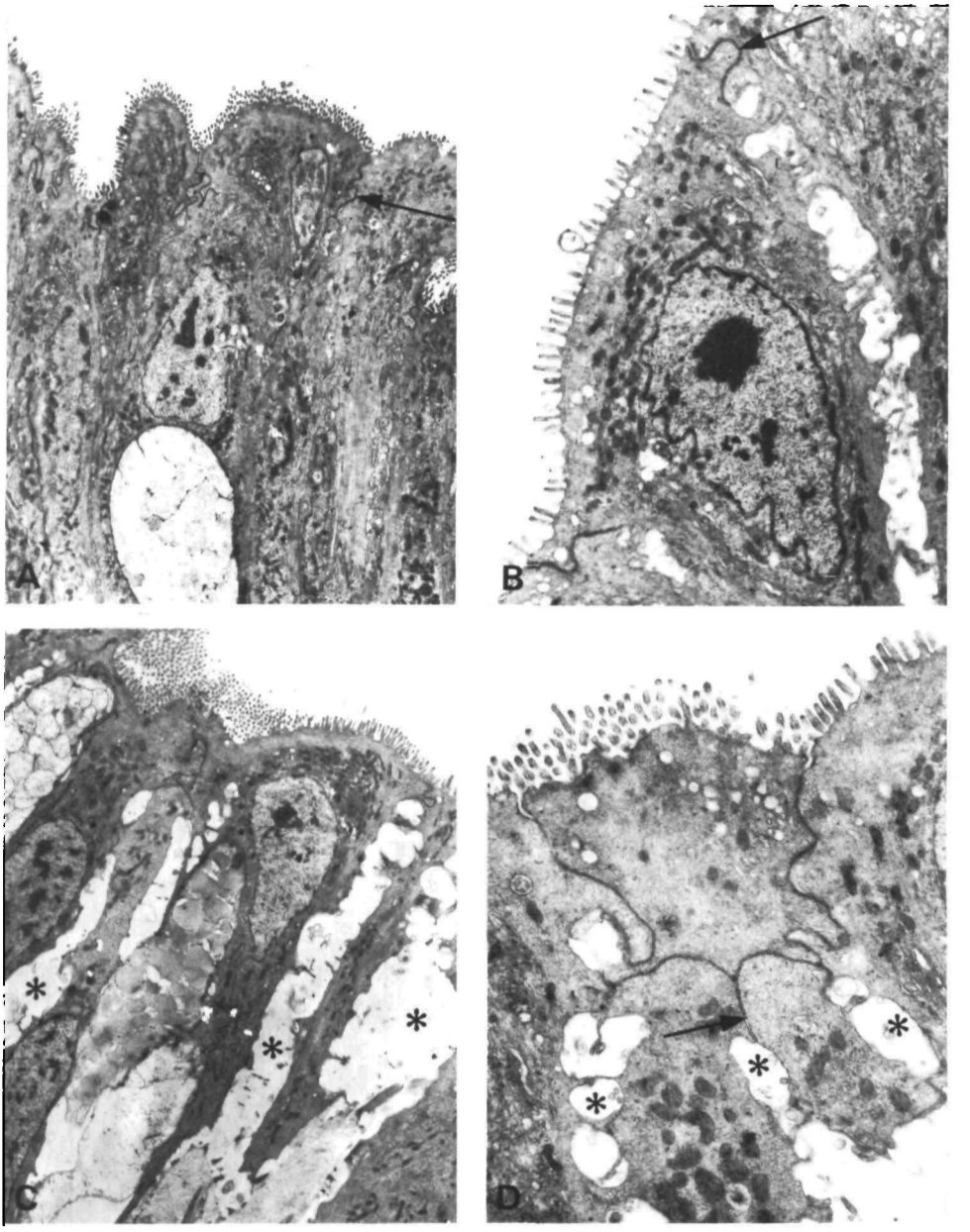

Fig. 2. Electron micrographs of foot epithelia of *Limax maximus* at various states of body hydration. The variation in the intercellular spaces (asterisks) as a function of body hydration is illustrated. The pyramidal epithelial cells are joined by septate junctions (arrows) at their microvillar borders. (A) A section from a slug dehydrated to 65 % IBW shows the reduced intercellular spaces (×2500). (B) In a fully hydrated slug (100 % IBW) the intercellular spaces are partly enlarged (×5100). (C) A section from a slug after 4 min of contact-rehydration reveals the considerable enlargement of the intercellular spaces (×3000). (D) A higher magnification of a section from a slug during contact-rehydration shows that the enlarged intercellular spaces are continuous with the spaces between the epithelial cells in the region of the septate junctions (arrow; ×8500).

swollen and appear to be continuous with the spaces between the cells in the region of the septate junctions (Fig. 2C, D).

DISCUSSION

It is well established that lateral or basolateral intercellular spaces represent major pathways for transepithelial ionic diffusion (Schultz & Fromm, 1982). In some epithelia these pathways may also represent a route for water flow in response to osmotic gradients (Schultz, 1977). In rabbit gallbladder, the distension of these spaces is correlated with experimentally induced fluid transport (Tormey & Diamond, 1967; Diamond & Bossert, 1967).

The present results support the hypothesis that in terrestrial slugs water absorption during contact-rehydration occurs through an epithelial paracellular pathway in the foot. It was shown in the preceding paper that contact-rehydration is mediated by integumental absorption of water (Prior, 1984). Because ^{14}C-inulin and water rapidly accumulate in the haemolymph during contact-rehydration, we are led to conclude that there exists a paracellular pathway through which both water and relatively large molecules can pass.

The correlation between the time courses of absorption of ^{14}C-inulin and water over the 4-min period indicated that there was movement of the substrate into the slugs by solvent drag (Fig. 1). There was a 50–60 s delay before the onset of rapid absorption. This delay may be the time necessary for proper posturing of the slugs or could represent the time needed to initiate paracellular movement of substrate. The data in Fig. 1 do however indicate that ^{14}C-inulin and water were absorbed together.

Ryder & Bowen (1977) provided ultrastructural evidence for the existence of a paracellular pathway in the foot of the slug, *Agriolimax reticulatus*. Sections were made of foot tissue from slugs that had been in contact with a substrate containing the extracellular markers, peroxidase and ionic lanthanum. Both markers passed between the epithelial cells and appeared in the zonulae adhaerens, septate desmosomes and in the intercellular spaces central to the septate desmosomes. This extensive system of intercellular spaces was a major feature in Newell's (1977) description of the ultrastructure of the slug foot epithelium. Our observations have revealed a correlation between the hydration state of the slug and the width of the intercellular spaces (Fig. 2). Most notable was the considerable expansion of the spaces in dehydrated slugs during contact-rehydration. Without the use of histological markers we cannot, however, determine whether the spaces were expanded by redistribution of haemolymph due to the posturing that occurs during drinking behaviour or influx of water from the substrate.

In conclusion, contact-rehydration in slugs appears to be mediated by bulk flow of water through an epithelial paracellular pathway in the foot. Furthermore, the efficacy of the paracellular movement of solutes with water seems to be modulated by the state of hydration of the slug, the pathway being open only in dehydrated slugs. The characteristics of this paracellular pathway and the mechanisms underlying its control are presently being investigated.

This research was supported by grants from NSF (PCM 78-16088) to GLU, the Whitehall Foundation (DJP) and an NIH-RCDA to DJP. We are pleased to acknowledge the facilities made available to us by Alan Stein and his associates at 803 South. We thank Dr Kook J. Lee for his technical assistance with the electron microscopy.

This is contribution no. 202 from the Tallahassee, Sopchoppy and Gulf Coast Marine Biological Association.

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