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Fluid Transport: Concentration of the Intercellular Compartment

Abstract. *Intercellular spaces of* Periplaneta *rectal pads are visible at a magnification of* × *100 and distend during fluid uptake. Samples (0.025 to 0.1 nanoliter) obtained by micropuncture from the spaces were consistently more concentrated than the fluid in the rectal lumen. This observation supports the hypothesis of "local" osmosis in epithelial fluid transport.*

Water "transport" across epithelia is a passive consequence of active transport of solutes (*1*). Thus a basic problem is to explain how solute movement generates a flow of water. One suggestion is that the long, narrow intercellular spaces, which are characteristic of absorptive epithelia, have a functional role in transport by acting as the sites of osmotic gradients (*2*). According to this hypothesis, solutes are pumped into the spaces, making them hyperosmotic to the cell and lumen, creating the osmotic gradients that bring about water absorption. This concept, which has been termed "local" osmosis (*3*) may have general application for epithelia such as gall bladder, intestine, and kidney, which have intercellular spaces that are closed off from the lumen but open in the direction of fluid transport (*2*, *4*). However, there has been no demonstration of an osmotic gradient between the intercellular spaces and the lumen, from which the fluid is absorbed. Indirect evidence has come from study of rabbit gall bladder, in which measurements of diffusion potential led to the calculation that the NaCl concentration in the intercellular spaces is about 10 m*M* higher than in the bathing medium (*5*). More direct evidence can only be obtained by methods that allow precise determination of the concentration of intercellular fluids. Insect rectal pads offer good opportunities for a direct approach because they have intercellular spaces that are large enough to see with a dissecting microscope, and fluid samples can be collected from them by means of micropuncture. Samples collected from the spaces were consistently hyperosmotic to the rectal lumen during fluid uptake.

Insect rectal pads and papillae are thickenings of the rectal epithelium, composed of tall columnar cells. They function to absorb water and solutes from the fecal material prior to excretion (*6*). In dehydrated animals absorption of water occurs against an increasing osmotic gradient, and very concentrated fecal material is excreted (*6*, *7*). Rectal pads and papillae have complex systems of intercellular spaces that drain in the direction of fluid uptake, that is, into the hemocoel of the insect (*8*, *9*), and it has been suggested (*7*, *9*, *10*) that the spaces are the sites of local osmotic gradients.

Adult male *Periplaneta americana* L. were dehydrated for 4 to 7 days and then anesthetized with CO_2. The rectum was exposed by dissection, cannulated through the anus, and covered with paraffin oil. The cannula was attached to a syringe containing either a fluid resembling colon fluid (*11*) or a more concentrated fluid (*12*). The rectal contents were forced into the colon by injecting fluid into the rectum. The junction between colon and rectum was then ligated. The artificial colon fluid was injected in order to simulate the most active absorptive condition in vivo. This occurs after a dry fecal pellet is excreted and dilute colon fluid moves into the rectal lumen. A non-permeant dye (Nigrosine, 1 mg/ml) was added to the injected fluid to provide a dark background, facilitating observations of the intercellular spaces. The prepara-

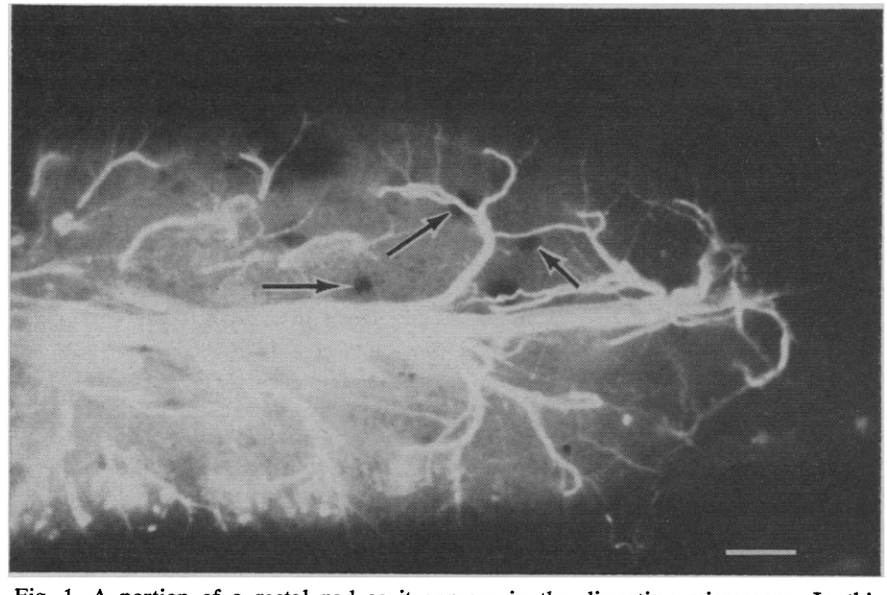

Fig. 1. A portion of a rectal pad as it appears in the dissecting microscope. In this preparation intercellular spaces (arrows) distended within 2 minutes after artificial colon fluid was injected into the lumen. The dark appearance of the spaces is due to the presence of Nigrosine dye in the injected fluid. The rectal pads are richly supplied with tracheae that appear as highly branched tubes. Marker is 0.1 mm.

Table 1. Concentrations of fluids collected from the rectal lumen and intercellular spaces. In experiments 1 through 5 colon fluid (*11*) was injected into the lumen at the beginning of the experiment. In experiments 6 through 9 a 500-milliosmolal fluid (*12*) was injected, and in experiments 10 through 12 a 600-milliosmolal fluid (*12*) was used. In experiments 5, 8, 11, and 12 the animals defecated when anesthetized; osmolalities of the excreted material were 902, 849, 583, and 370 milliosmoles respectively. Hemolymph samples were obtained from animals used in experiments 4 through 12 prior to the beginning of the experiment. These samples ranged in concentration from 317 to 442 milliosmoles, average 410 milliosmoles.

Experiment (No.)	Concentration (milliosmoles/kg H_2O)	
	In lumen	In space
1	383	414
2	502	612
3	389	415
4	665	713
5	595	795
6	598	676
7	588	703
8	572	717
9	640	731
10	675	975
11	617	730
12	520	805

tion was observed with a dissecting microscope (× 100). Within 1 to 10 minutes after injection of fluid into the rectal lumen, the spaces became distended to a diameter of 10 to 30 μ (Fig. 1). Capillaries with beveled tips 4 to 5 μ outer diameter were used to collect samples from the intercellular spaces, by means of standard micropuncture techniques. The tip of the capillary could be seen in the intercellular space. The procedure was considered successful when application of suction to the capillary caused a gradual collapse of the intercellular space as the fluid was withdrawn. Samples ranged from 0.025 to 0.1 nl in volume. Immediately after the sample was obtained, another capillary was used to collect a sample from the rectal lumen. Shortly after collection, both samples were analyzed for concentration by determination of the freezing-point depression (*13*).

The fluid in the intercellular spaces was from 31 to 300 milliosmoles more concentrated than that in the rectal lumen (Table 1). The average difference was 130 milliosmoles. Variations in the final concentration of the fluid in the rectal lumen occur, probably because it is not possible to wash all of the fecal material out of the lumen at the beginning of the experiment. Because of this it was not possible to determine if there was a change in concentration in the lumen during the short time between injection of fluid and collection of the sample from the space. However, as in previous studies (*14*), the volume of fluid in the rectal lumen decreased for at least 1½ hours after the experiment began (*15*), an indication that a net uptake of fluid was occurring at the time the samples were collected from the intercellular spaces. Deterioration of these preparations *in situ* is slow because the rectal pads are protected by a muscle sheath from direct contact with the paraffin oil. There is a narrow sinus between the pad cells and the muscle layer, so that the basal surfaces of the pads are actually bathed in the absorbed fluid (*9*).

These results are consistent with the concept of local osmosis, since they show that intercellular spaces of rectal pads contain fluid that is hyperosmotic to the lumen when fluid uptake is occurring. Presumably the local osmotic gradient is created by solute transport into the spaces, and the resulting osmotic flow of water creates a hydrostatic pressure that distends the spaces and brings about the flow of fluid into the hemolymph.

Studies on other absorptive epithelia have shown that intercellular spaces distend when fluid uptake occurs and collapse when transport is stopped, suggesting that the spaces are the routes of fluid flow (*10*, *16*). However, recent studies on toad bladder (*17*) show that the spaces may also distend when no net transport is occurring; in insect salivary glands narrow channels of the intracellular canaliculi have constant dimensions over a 60-fold range in transport rate (*18*). All of these studies were based on microscopic examination of sections of fixed material. More direct observations have been made on living isolated kidney collecting ducts, in which water flow induced with vasopressin brings about a widening of intercellular spaces that can be observed by phase-contrast microscopy (*19*). Again the interpretation was that fluid flows through the intercellular spaces. In the case of the rectal pads of *Periplaneta*, we were unable to make any correlation between the size of the intercellular spaces, as observed in sectioned material, and the condition of the animals from which the recta were obtained (*20*). However, from our study it is clear that the spaces distend rapidly during water absorption and that the intercellular fluid is hyperosmotic to the fluid in the lumen. This evidence is consistent with the hypothesis that "uphill" transport of water from the rectal lumen to the hemolymph results from passive water flow down a local osmotic gradient between the lumen and the intercellular spaces.

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