mals. One group was given vehicle and immediately decapitated for the measurement of intestinal calcium transport by the everted sac method (*17*). The remaining groups were injected intraperitoneally with prolactin (250 μg) (*18*) and killed 4, 8, 10, 12, or 24 hours later. Serum was collected for the measurement of serum calcium concentration by atomic absorption spectrometry on a sample diluted with 0.1 percent $LaCl_3$ (Fig. 1). Within 4 hours after prolactin injection intestinal calcium transport increased significantly, reaching a maximum after 8 hours and then falling to preinjection levels after 12 to 24 hours. Since no 25-OH-D_3 or 1,25-$(OH)_2D_3$ could be detected in the plasma of these rats [the detection limit of 1,25-$(OH)_2D_3$ is 2 pg/ml], the increase in intestinal calcium transport could not have been caused by increased levels of residual metabolite. A 10-pmole dose of 1,25-$(OH)_2D_3$ would be required for the intestinal response to prolactin observed in this experiment (*19*). Such a dose would increase plasma 1,25-$(OH)_2D_3$ to 30 to 40 pg/ml (*20*).

Other groups of rats were then tested to determine the optimal dose of prolactin required for maximum intestinal calcium transport within 8 hours. This dose was found to be 100 to 250 μg per 250 g of body weight. Serum calcium concentration also increased significantly after 8 hours (Fig. 1). The question then arose as to whether the elevation in serum calcium was the result of the improved intestinal calcium absorption or due to the liberation of calcium from bone. An experiment was therefore carried out in which vitamin D–deficient rats, 10 weeks after weaning, were placed for an additional 2 weeks on a diet low in either calcium or phosphorus. The rats were then given a single intraperitoneal injection of prolactin (300 μg). Eight hours later blood calcium and phosphorus were measured (Table 1). Serum calcium was increased in both groups, and serum phosphorus was significantly increased in rats on the diet low in phosphorus.

These results suggest that prolactin can act independently of vitamin D and parathyroid hormone in stimulating intestinal calcium transport and the liberation of calcium and phosphorus from bone (*21*). Thus, in pregnant mammals prolactin may have a secondary role in liberating calcium to support fetal calcification and the lactation process. If the effect of prolactin can be confirmed in the pregnant or lactating vitamin D–deficient rat, prolactin would have to be considered as an important regulator of calcium metabolism under some circumstances. These results may aid in the elucidation of certain disease states [it has, however, been reported that hyperprolactinemia in man does not appear to be associated with abnormal calcium metabolism (*22*)]. The results are sufficient to warrant additional detailed investigation into the possible role of this hormone in the regulation of calcium, especially during pregnancy and lactation.

Table 1. Effect of prolactin on bone mineral liberation in vitamin D–deficient rats fed diets low in calcium or phosphorus. Diet A contained 0.02 percent calcium and 0.3 percent phosphorus and diet B contained 1.2 percent calcium and 0.1 percent phosphorus. Values are means ± standard deviations.

Diet	Prolactin treatment	Serum calcium (milligrams per 100 ml)	Serum phosphorus (milligrams per 100 ml)
A	No	3.4 ± 0.1	8.4 ± 0.8
	Yes	3.8 ± 0.3*	9.1 ± 1.0
B	No	7.9 ± 0.2	2.4 ± 0.4
	Yes	8.2 ± 0.2†	3.4 ± 0.5*

*Significantly different from corresponding control value at $P < .01$ (Student's t-test). †$P < .025$.

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Active Ion Transport in Dog Tongue: A Possible Role in Taste

Abstract. *An in vitro preparation of the dorsal epithelium of the dog tongue actively transports ions, producing a transepithelial potential difference characteristic of the ions and their concentration. Hypertonic sodium chloride solutions generally cause increased potentials and short-circuit currents and reduced resistances when placed on the mucosal surface. This hypertonic flux is eliminated by ouabain and is not found in ventral lingual epithelia. When either sodium acetate or tetramethylammonium chloride is substituted for sodium chloride in the mucosal medium, the currents are diminished but their sum at a given concentration approximates that for sodium chloride at the same concentration. This result suggests a current composed of inward sodium ion movement and outward chloride ion movement. Actively regulated potentials and currents, whether generated in the taste buds or in supporting cells, may be important in both normal chemotransduction and in taste responses evoked by currents passing through the tongue.*

The dorsal lingual epithelium has been generally regarded as a tissue of low permeability (*1*). Although this conclusion seems justified for a variety of nonelectrolytes diffusing across nonmetabolizing tissue, we show here that it is invalid for ionic species in metabolizing epithelia. This result is particularly important in the area of taste reception because in current theories of salt taste reception it has been assumed that the lingual epithelium is virtually impermeable to ion transport (*2*). This has resulted in models of taste reception in which ion transport across the lingual epithelium has been largely ignored as a factor in receptor activation. There has been rather an emphasis on events at the interface between the oral cavity fluid and the receptor cell membrane. Ion transport

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either has been considered passive (*3*) or has been thought to be restricted to include only receptor cells and their extracellular fluid, a consequence of the assumed impermeability of the mucosal cell layer (*2*).

The evidence we present here not only demonstrates that there is active ion transport across the lingual epithelium but also that the dorsal surface, which contains the taste organs, responds to hypertonic NaCl with increasing potential and short-circuit current. This behavior is not observed in the ventral lingual tissue and can be largely eliminated by ouabain or with the tissue under nitrogen. The hypertonic stimulation of the potential and the short-circuit current also occurs with sodium acetate and tetramethylammonium chloride, but to a lesser extent.

Tongues were obtained from freshly killed dogs, and the dorsal epithelium was dissected free from the underlying muscle. A section of middle to anterior tissue was mounted in an Ussing chamber (*4*). Care was taken to exclude circumvallate tissue in order to avoid submucosal glands. Histological examination of the sections chosen revealed stratified squamous cells comprising the filiform and fungiform papillae, the latter often bearing taste buds. Oxygenated Krebs-Henseleit buffer (*5*) was placed in the mucosal and serosal compartments. Each compartment was well stirred, and the temperature was maintained at 34°C. The tissue developed an open-circuit potential (ΔV_{oc}) (serosa positive), which reached a pseudo steady state within an hour. At intervals an external current was briefly ramped across the tissue in order to obtain the short-circuit current (I_{sc}) and the tissue resistance (R). Mean values from 16 tissues are as follows: ΔV_{oc} = 17.2 ± 2.4 mV, I_{sc} = −31.4 ± 6.7 μA/cm^2, and R = 576 ± 127 ohm-cm^2 (*6*). Two preparations, which were left for 4 hours, gave the following values: ΔV_{oc} = 22.2 ± 3.1 mV, I_{sc} = −35.3 ± 12.0 μA/cm^2, and R = 617 ± 129 ohm-cm^2. The potentials and currents are markedly reduced under nitrogen; in one experiment ΔV_{oc} and I_{sc} were reduced by 79 and 75 percent, respectively, after 30 minutes. Upon return to oxygen, ΔV_{oc} and I_{sc} recovered to 80 percent of their control values in 12 minutes (*7*). If the mucosal solution is replaced in sequence by a series of NaCl solutions, the ΔV_{oc} and I_{sc} curves shown in Figs. 1 and 2 are obtained (*8*). The unusual feature of the curves for the dorsal surface is the increase in ΔV_{oc} (up to 0.75*M*) and the marked increase in I_{sc} as the mucosal bath becomes hypertonic. In contrast, the response to NaCl of the ventral surface of the same tongue shows declining potentials and saturating low levels of current at concentrations beyond 0.5*M*. At 1*M* NaCl, I_{sc} has only 13 percent of the dorsal surface value. The diminished potentials and resistances observed in the ventral surface more closely parallel the hypertonic response of other transporting epithelia such as frog skin (*9*). The magnitude of the current density produced in the dorsal tissue suggests that it is a general property of the surface and not confined to individual taste cells.

When ouabain is placed in the serosal bathing medium, ΔV_{oc} and I_{sc} decay to zero when the chambers are symmetrical in Krebs-Henseleit buffer. The current also remains low when the mucosal solution is hypertonic in NaCl (Table 1). At 1*M* NaCl the current is reduced by 92 percent. After ouabain treatment, the current under hypertonic conditions increases linearly with concentration whereas the control values tend toward saturation. The saturable hypertonic response can also be eliminated with the tissue under nitrogen, and it is substantially reduced by amiloride (*7*). These findings suggest that, even under hypertonic conditions, specific mechanisms

Table 1. The effect of ouabain on the I_{sc} of the dorsal surface. After the control data were obtained, the system was returned to symmetrical conditions in Krebs-Henseleit buffer; 9×10^{-4}*M* ouabain in buffer was placed in the serosal bath. After about 1 hour, the potential and current had decayed to zero. The I_{sc} values were then obtained at the indicated NaCl concentrations in the mucosal bath.

[NaCl] (*M*)	I_{sc}, control (μA/cm^2)	I_{sc}, ouabain (μA/cm^2)
0.1	−9.2	5.0
0.15	−14.1	0
0.25	−32.5	−2.1
0.50	−70.6	−3.9
0.75	−89.0	
1.00	−104.5	−7.8

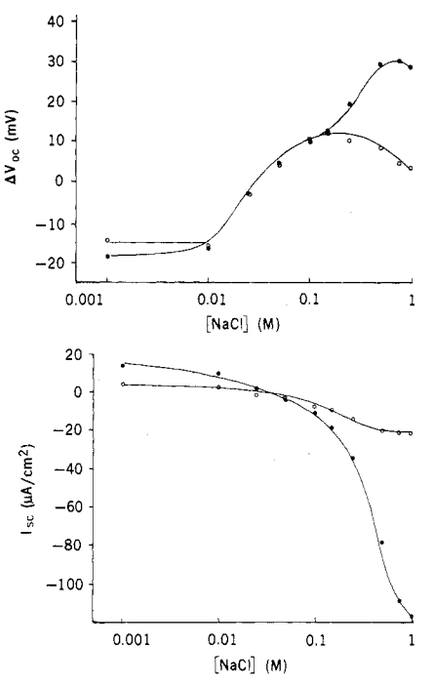

Fig. 1 (top). Open-circuit potential (ΔV_{oc}) as a function of the mucosal NaCl concentration for the dorsal lingual surface (●) and the ventral surface (○) of the same dog tongue; ΔV_{oc} was measured with 1.5*M* NaCl salt bridges between saturated calomel electrodes. In all cases Krebs-Henseleit buffer was the serosal medium, and mucosal solutions were changed in sequence from dilute to concentrated. The null potential occurs at about 0.035*M*. Fig. 2 (bottom). Short-circuit current (I_{sc}) as a function of NaCl concentration for the dorsal surface (●) and the ventral surface (○) of the same dog tongue. The ouabain-sensitive hypertonic response is found only in the dorsal epithelium.

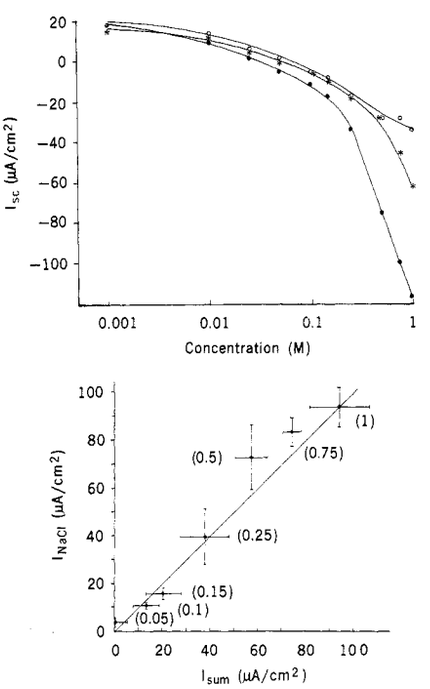

Fig. 3 (top). Mean short-circuit current (I_{sc}) as a function of the mucosal salt concentration: ●, NaCl (five experiments); *, sodium acetate (four experiments); ○, tetramethylammonium chloride (four experiments). Fig. 4 (bottom). The short-circuit current for NaCl (I_{NaCl}) versus the sum of the short-circuit currents for sodium acetate and tetramethylammonium chloride (I_{sum}) at the indicated mucosal salt concentration. For clarity, the absolute values of the currents are plotted. The bars are the standard deviations from the mean for four tissues in which each salt was studied. The line is the theoretical identity, which is substantially the same as the linear regression line (slope, 0.96; intercept, 1.2 μA/cm^2).

predominate over passive transport in determining ion movement. Sodium chloride produces higher values of I_{sc} than either sodium acetate or tetramethylammonium chloride (Fig. 3). However, at concentrations above 0.05*M* the sum of I_{sc} for tetramethylammonium chloride and sodium acetate approximates I_{sc} for NaCl at the same concentration. This is shown in Fig. 4, which is based on pooled data from four tongues where each salt was investigated. This near-identity suggests a current composed of an inward Na^+ and a smaller outward Cl^- component.

A NaCl concentration of 0.035*M* produces a null potential. This is about the Na^+ concentration in saliva secreted at basal flow rates (*10*). It is also approximately the recognition threshold for salt taste in humans adapted to saliva (*11*). It is conceivable that normal salivary secretions maintain a transepithelial potential close to zero and that the salt taste response coincides with positive potential changes. Such changes may be produced either by increasing salt concentration or by passing anodal current through a concentration that would be subthreshold as a tastant. In this manner positive potentials might elicit "electric taste" (*12*) as a consequence of externally driven Na^+ and Cl^- currents.

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6. The quantity I_{sc} is defined as the current required to maintain the potential difference at zero with or without a concentration difference across the tissue. We measured the contribution of the series solution resistance to the total in the chambers by substituting filter paper for the tissue. For symmetrical Krebs-Henseleit conditions, this is 44 ohm-cm^2, a correction of 7 percent at most. With 1 m*M* NaCl in the mucosal bath, the resistance is 7 ohm-cm^2, a correction of 5 percent at most. Under hypertonic conditions the correction amounts to about 7 percent.
7. A detailed account of these effects will appear elsewhere (J. A. DeSimone, G. L. Heck, S. K. DeSimone, in preparation).
8. The ΔV_{oc} and I_{sc} values analyzed herein are steady-state values. Their time course with changes in concentration is, however, an important matter from the standpoint of taste reception. In one experiment in which the mucosal bath was changed from Krebs-Henseleit buffer to 1*M* NaCl, I_{sc} approached its asymptotic value of −137 μA/cm^2 exponentially with a time constant of 74 seconds and an initial rate of increase of 1.4 μA/cm^2 per second. Restoration of the system to symmetrical conditions is slower, requiring about 20 minutes to reach steady state. A complete analysis of transients will be presented elsewhere (*7*).
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Honey Bee Orientation: A Backup System for Cloudy Days

Abstract. *On cloudy days, honey bees are known to navigate to familiar food sources and orient their dances accurately. This capacity could be based on a magnetic compass sense, an ability to perceive the sun or patterns of polarized light through the clouds, or on the bees' memory of the diurnal course of the sun with respect to local landmarks. Experiments pitting these alternatives against one another demonstrate that the navigational backup system of bees is based on memory.*

Forager honey bees use the sun as the primary reference in both navigation and their dance language. The dance communicates the direction of the food to bees in the hive by means of a waggling run performed at an angle with respect to vertical which represents the angle between the azimuths of the sun and food (*1*). A bee must know the sun's position to execute the dance. Under a partial cloud cover, the sun-linked patterns of polarized light in blue sky can replace the sun as an orientation cue (*2*), but even under complete overcast bees continue to orient their dances correctly (*1*), apparently having a second backup system for such sky conditions. Von Frisch *et al.* (*3*) originally proposed that bees might see the sun directly through the clouds in the ultraviolet, but recent sky measurements (*4*) and our preliminary behavioral observations cast doubt on this hypothesis. A second possible explanation is that bees might employ a magnetic compass such as homing pigeons use under overcast (*5*); a third is that bees might be able to remember from previous days the sun's position at each time of day relative to the flight direction or landmarks.

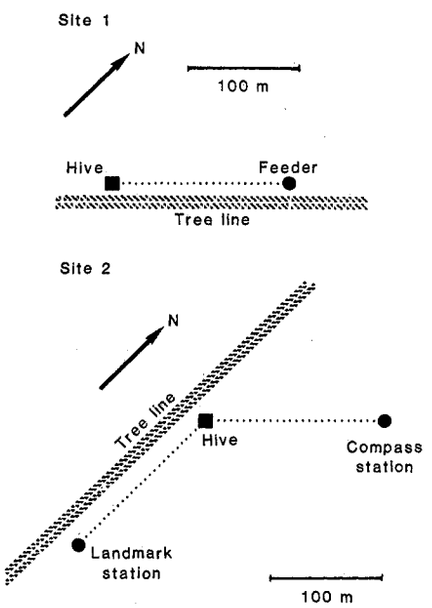

Fig. 1. Tree lines used in experiments. Bees were trained at site 1 under sunny skies, and the hive remained there at all times except on test days, when it was moved to site 2. Both sites are located on sod farms in Lawrence Township, New Jersey.

The third possibility was tested against the first two by exploiting the tendency of experienced bees to rely on landmarks for their flight navigation (*6*). When their hive is moved overnight to a site lacking conspicuous landmarks, bees trained to a food source ordinarily use the sun as a compass and search for food in the direction in which they have been flying previously (*7*). If landmarks are available along the line of flight, however, most experienced bees at a new site ignore the sun, even when it is clearly visible, and search for the food by using similar landmarks (*6*). In the present experiment foragers from an observation hive were individually marked and trained to a feeder containing a scented sucrose solution. The feeder was moved along a line of trees (site 1 in Fig. 1) to a distance of 160 m, and the trained bees were allowed to forage for several days under sunny skies. On test days, the hive was closed

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